THE CONTROL OF PULMONARY INFECTION IN PATIENTS WITH CYSTIC FIBROSIS

MILDRED DRAIN

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Department of Microbiology

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SUMMARY

Sixteen patients with cystic fibrosis were monitored for eighteen months by monthly bacteriological examination of nose and throat swabs and sputum in order to determine if better control of respiratory infection could be achieved by more rational usage of antibiotics.

The patients were divided into two groups; a Variable Antibiotic group and a Flucloxacillin group. The first group received only one antibiotic which was initially chosen and then changed as culture results dictated, while the second group was given continuous anti-staphylococcal chemoprophylaxis plus one other antibiotic if required, but the latter for a period of 10 days only.

Weight, height, and radiological data showed that in both groups of patients the clinical status did not alter significantly from the start of the investigation, although no deterioration was observed. However, the number of hospital admissions, which reflected exacerbation of pulmonary infection, was greatly reduced in both groups during the 18 months of the investigation.

The predominant organism isolated from both groups was Staphylococcus aureus. However, in patients with severe pulmonary involvement, both S. aureus and Pseudomonas aeruginosa occurred. This agrees with previous findings that the main organisms associated with respiratory problems in CF patients are S. aureus and P. aeruginosa, despite the presence of other bacterial species in the respiratory tract.

Control of S. aureus and Haemophilus influenzae infection was significantly better in the Flucloxacillin group, but

neither these organisms nor P. aeruginosa were completely eradicated.

Antibiotic sensitivity tests indicated that bacterial isolates from CF patients were, in general, more sensitive to antibiotics than the same species from non-CF patients. This greater incidence of antibiotic-susceptible bacterial isolates from CF patients, as compared with non-CF patients in a children's hospital, has not previously been reported. This lesser degree of antibiotic resistance was thought to be due to lower selection pressure on the organisms.

The mucoid strain of P. aeruginosa showed greater susceptibility to certain antibiotics than the rough strain - a point on which previous investigations have been divided.

Phage-typing of the S. aureus isolates from the CF patients revealed that the upper respiratory tract was not the source of this organism in pulmonary infection. No evidence was found to suggest the predominance of any single phage-type, although each patient usually harboured S. aureus from one phage-group.

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INTRODUCTION

History and Incidence of Cystic Fibrosis

Cystic fibrosis (CF) is the most common lethal genetic disorder of Caucasians and is the cause of much of the chronic progressive lung disease present in children and young adults. The incidence of CF in European countries is generally accepted as 1 in 2000 live births (di Sant' Agnese and Talamo, 1967).

CF was reported for the first time by Fanconi *et al* (1936). He described the relationship of "bronchiectasis" and congenital cystic pancreatic fibrosis.

However, cystic fibrosis was not recognised as a separate and distinct disorder until the extensive work of Andersen (1938) This was the first detailed pathological description of the disease. She termed the disorder "fibrocystic disease of the pancreas" because of the marked fibrosis and dilated ducts in the pancreas gland. Her work showed the clear association of pancreatic pathology with widespread pulmonary infection.

These findings were confirmed by Blackfan and May (1938) and the modified term "cystic fibrosis of the pancreas" came into use.

CF is also known as "mucoviscidosis". This term was coined by Farber (1945). He chose this name since he thought that it described the basic defect viz an abnormality of mucous secretions.

CF is expressed only in the homozygous state without X-linkage. Both sexes are therefore affected with equal frequency. Parents, who are normally heterozygous, are unaffected.

Until recently, CF was believed to be transmitted as an autosomal recessive trait. Schaap and Cohen (1976), however, have proposed that CF is determined by dominant alleles at two autosomal loci, with interaction between them.

CF is not genetically linked with ABH blood group substances (Virtanen, 1966) MNS blood groups (Steinberg and Morton, 1956) or HLA antigens (Polymenidis, Ludwig and Gotz, 1973).

The patient with CF presents with chronic chest disease, pancreatic insufficiency with steatorrhoea, azotorrhoea, malnutrition and growth failure, and abnormally high sweat sodium levels. Some or all of the symptoms are present from birth. The degree of progression of both pulmonary and gastrointestinal symptoms is very variable.

Until the advent of antibiotics and a greater understanding of the underlying effects of the defect of CF, life expectancy was short. In 1955 50 per cent of CF patients were expected to live until 4 years of age; whereas, in 1975, this age had increased to 17 years.

A recent report by Warwick and Pogue (1977) gives an even more optimistic prognosis for the future. The authors point out that although it is true that CF patients who reach 18 years of age have a shorter life span than their "normal" counterparts, this must be placed in perspective. Data from the CF registry of Warwick and Pogue show the median life expectancy for patients 15 years of age and older to be more than 11 years. This indicates a better survival than that for leukaemia.

Diagnosis

There are four criteria currently in use for the diagnosis of CF (1) Chronic obstructive pulmonary disease; (2) Exocrine pancreatic insufficiency; (3) Family history of CF; (4) A positive sweat test (i.e. > 60mEql^{-1} Na^{+}). Most clinics require at least 2 of the above criteria for a diagnosis. However, a diagnosis is rarely made in the absence of a positive sweat test.

Several methods of collecting body sweat have been used and discarded for various reasons. The most reliable, widely accepted, and safest method is by the induction of localized sweating by pilocarpine iontophoresis (Gibson and Cooke, 1959). At least 100mg of sweat must be collected for accurate determination using this method.

Pathophysiology of Cystic Fibrosis

Despite documentation of many of the characteristic symptoms of CF the basic defect is still unknown.

There is a general pattern to the condition. Much of the pathology results from the change in the nature of mucous and serous secretions which occurs throughout the body. The former are abnormally sticky or dry and the latter concentrated. The secretions are therefore hard to disperse and consequently block ducts or ductules. The blockage of ducts is apparent in a number of organs but is particularly important in the lungs and pancreas where it leads to the cardinal clinical features of the disease, namely a progressive obstructive suppurative chest disorder together with malabsorption due predominantly

to an insufficiency of the pancreatic digestive enzyme secretion.

The sweat is the most obviously abnormal of the serous secretions in that its sodium and chloride content is 3-4 times that of normal sweat.

In the pancreas, secretions precipitate within the lumen of the ducts causing blockage and duct dilatation. Destruction of exocrine secretory tissue and replacement with fibrous tissue then occurs. The alteration of function in most cases is maximal at birth and therefore malabsorption is usually present from that time.

Unlike the pancreas, the lungs are structurally normal at birth, but the bronchioles are soon obstructed by the rather viscous mucus. The static mucus in the bronchioles becomes infected. Examination of such a mucus plug will show it contains, in addition to bacteria, bacterial products, leucocytes, and deoxyribonucleic acid. The infected mucus is difficult to expectorate and becomes widespread throughout the bronchial tree producing an obstructive suppurative chest illness which, if untreated, becomes progressive and leads eventually to bronchiectasis, pulmonary fibrosis, and finally cor pulmonale, cardiac failure, and death.

Respiratory disease contributes with the nutritional deficiency caused by malabsorption in the typical manifestations of untreated CF viz chronic productive cough, dyspnoea, clubbing of fingers, signs of malnutrition, and abnormally large greasy offensive stools.

Chronic respiratory disease is, therefore, the major cause

of morbidity and mortality in CF. It is also the most difficult aspect of the condition to treat and control throughout the patient's life.

Microbiology of CF

Pathogenic bacteria play a dominant role in the pulmonary involvement of patients with CF. These patients usually harbour the same pathogenic bacteria for long periods even when signs of clinical improvement are evident. Such persistence of pathogenic bacteria in the respiratory tract is not generally found in patients suffering from chronic respiratory infections due to other causes.

As the state of the bronchial secretions plays an important role in pulmonary infection perhaps a definition would be appropriate at this point.

Bronchial secretion is the liquid lining the tracheobronchial tree; when this secretion reaches the larynx, mixes with upper respiratory tract secretion and saliva and is expectorated, it is known as sputum. The main bulk of sputum representing bronchial secretions can be considered to arise from :

i) Mucus-secreting cells of surface epithelium and submucosal glands.

ii) Plasma component.

iii) Other specialised cells (e.g. plasma cells, mast cells).

iv) Alveolar surfactant.

The organisms considered to be the main pathogens in CF are Staphylococcus aureus, Pseudomonas aeruginosa, and Haemophilus

influenzae (di Sant' Agnese and Andersen, 1946; Huang *et al.*, 1961; Iacocca *et al.*, 1963; di Sant' Agnese and Talamo, 1967; Lawson, 1970; May *et al.*, 1972). Other bacteria including *Streptococci*, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis* are also found in sputum of CF patients.

The three foremost pathogens in CF have been the cause of considerable controversy not only over which causes most lung damage but also the order of infection.

The following order was proposed by Burns and May (1968).

1. *S. aureus*
2. *H. influenzae*
3. *P. aeruginosa*

Their reasoning was that *S. aureus* caused the damage which rendered the lungs susceptible to infection by other species. Antibiotics used to control *S. aureus* allowed the influx of *H. influenzae*. Finally, continued chemotherapy leads to the introduction and establishment of *P. aeruginosa* which ultimately supplants the others to become the sole pathogen. This hypothesis has recently been supported by Kilbourn (1978).

A significant decrease in the frequency of isolation of *S. aureus* has been noted during the past ten to fifteen years with a concomitant increase in *P. aeruginosa* (Mearns *et al.*, 1972). Antibiotic therapy is thought to be one cause of change in flora. However, in other paediatric situations the incidence of infection due to staphylococci has lessened and *S. aureus* is now no longer the predominant organism in neonatal infections.

Mucus would appear to be a contributing factor in the pathogenicity of S. aureus (Iacocca et al., 1963). The mucus may act as a protective coating for the organisms, effectively preventing penetration of antibodies and inhibiting leucocytic invasion. On the other hand, if the bacteria are surrounded by a mucus envelope it may act as a barrier for the host by confining infection to the lungs.

There is no evidence that bronchial secretions in CF have a growth promoting factor for S. aureus (Eichenwald, 1960).

P. aeruginosa has probably aroused most interest of the 3 organisms. There are 2 strains of this organism; a rough strain and a mucoid strain which is peculiar to CF. The non-mucoid strain of the organism always preceeds the mucoid form (Doggett et al., 1966). The latter is commonly the predominant and usually only organism found in the lungs at post mortem.

A carrier state was found exclusively in patients with CF (Laraya-Cuasay et al., 1976). Although most of the patients studied by these workers were persistant pharyngeal carriers of P. aeruginosa the organism was not isolated from nasal cultures.

There are 3 factors which are believed to predispose patients with CF to infection by P. aeruginosa :-

1. Antibiotic therapy. The frequent and prolonged use of antibiotics in these patients favours the persistence of Pseudomonas. However, in some cases P. aeruginosa is the initial pathogen.
2. Hospitalization. Equipment contaminated with P. aeruginosa e.g. inhalation equipment.

3. Intrinsic factors. Obstruction of bronchioles by tenacious mucus, and impairment of mucociliary clearance.

This latter factor, equally applicable to S. aureus, raises the question of an impaired immune system rendering CF patients susceptible to infection.

Extra-pulmonary infection is rare and CF patients are not more prone to infections outside the respiratory tract than normal subjects of the same age. This suggests a defect in local rather than systemic defence mechanisms. Local defence mechanisms in the lung include phagocytosis, mucociliary transport, and immunoglobulin secretion.

Impaired phagocytosis by the alveolar macrophage is the only inhibitory immune defect reported. This impairment has been attributed to defective opsonization (Biggar et al., 1971) or to a heat-labile inhibitor (Boxerbaum et al., 1973).

Biggar and co-workers observed during an assay of phagocytosis by rabbit alveolar macrophage that sera from CF patients failed to support normal phagocytosis of P. aeruginosa. Defective opsonization could be explained by a quantitative or functional defect of IgA antibodies specific for Pseudomonas.

Boxerbaum et al., (1973) found that inhibition of phagocytosis was specific for Pseudomonas since phagocytosis of Staphylococcus and Serratia was normal.

Phagocytic activity by blood leucocytes against Pseudomonas was reported to be unaffected (Biggar et al., 1971). However, it has recently been reported that there is a specific unresponsiveness to P. aeruginosa by blood lymphocytes

(Sorensen et al., 1977). This suggests that there is a defective clearing of P. aeruginosa from the lung since activation of pulmonary alveolar macrophage is partly achieved by lymphokines secreted by stimulated lymphocytes.

Local immunoglobulins probably play a greater role in CF than serum immunoglobulins as infection is localized. Accordingly several workers have sought a defect in locally synthesized secretory IgA, by studying immunoglobulin synthesis and levels in the exocrine glands.

Martinez-Tello, Braun, and Blanc (1968) examined post-mortem bronchial tissues and lymph nodes from patients with severe pulmonary diseases, for fluorescein-staining immunoglobulin-producing plasma cells. In the CF patients, the number of cells containing IgG, IgM, and IgA were normal as was the distribution of the secretory piece in IgA.

There is a relation between the severity of the pulmonary infections and the immunoglobulin content of sputum (Wallwork et al., 1974). This has been observed in other pulmonary infections. IgA levels are high in sputum from bronchiectactics and appreciable amounts of IgE is present in chronic bronchitis (Turnbull et al., 1977).

It is interesting that most precipitating antibodies in sputum are IgG rather than secretory IgA (McFarlane et al., 1975). This suggests a substantial transudation of serum antibody. McFarlane and co-workers also demonstrated the deposition of antigen-antibody complexes in the respiratory tract. Such complexes may lead to tissue damage.

Antibody function in CF is not defective. Thus there is evidence of serum precipitating antibodies to respiratory pathogens notably Pseudomonas (Høiby and Axelsen, 1973), Staphylococcus (Halbert, di Sant 'Agnese, and Kutek, 1960), H. influenzae (Halbert, 1967), and Aspergillus fumigatus (Schwartz et al., 1970). Mucoid strains of P. aeruginosa are associated with a strong and differentiated humoral immune response (Høiby, 1974b). This was indicated by a positive correlation between the number of precipitins and the titres of the strongest precipitins given by the mucoid strains. Non-mucoid strains gave significantly fewer precipitins.

Dissention still exists as to the role of P. aeruginosa as a pathogen in CF. This organism is regarded as an opportunistic pathogen in medical microbiology, and this may be the situation in CF.

An organism which is an opportunistic pathogen lacks such characteristics as invasiveness and virulence. Before it can become pathogenic to a host it has to surmount these deficiencies. This is achieved not so much by the organism itself but by a combination of factors. These factors are normally a susceptible host and the ability of the opportunistic pathogen to adapt and survive in environments hostile to many other bacteria.

The mucoid strain of P. aeruginosa is thought to be the result of such an adaptation by the naturally occurring rough strain.

The nature of the slime envelope produced by the mucoid strain is in itself unusual. It is secreted from the cell as an exopolysaccharide, a polymer of mannuronic and guluronic

acids. It resembles that of alginic acid, a seaweed polysaccharide (Doggett et al., 1964, 1965, 1966; Linker and Jones, 1964; Carlson and Matthews, 1966; Evans and Linker, 1973). The slime envelope differs chemically from the bronchial mucus of the lung.

There is no convincing evidence that the slime envelope produced by P. aeruginosa is toxic. It is more probable that it confers some form of protection on the organism against the action of antibiotics or phagocytes (Schwarzmann and Boring, 1971).

The trigger mechanism of the slime envelope production in P. aeruginosa is thought to be the environment of the CF lung. Williams and Govan (1973) have shown that mucoid and non-mucoid strains isolated from the same patient are of the same pyocine type. Thus, infection is not by naturally occurring mucoid strains.

Martin (1973) reported the induction of mucoid strains of P. aeruginosa by bacteriophage. She observed during routine phage typing of P. aeruginosa that in some plates the area of lysis was surrounded by a ring of wet, slimy growth. Subcultures from this slime grew mucoid colonies. The properties of the phage induced mucoid strains were the same as those from patients with CF. She concluded that if the mucoid strains in the CF lung arose as a result of phage the most likely source was other strains of P. aeruginosa.

Little is known of the incidence and survival of such viruses in the human lung.

The slime envelope of *P. aeruginosa* is not, however, stable. Mucoid *P. aeruginosa* rapidly reverts to the non-mucoid form when cultured *in vitro* (Govan, 1975). In the course of Govan's investigations he found that the slime envelope could be stabilised for approximately 50 subcultures when *P. aeruginosa* was grown in deoxycholate-citrate agar.

He also found that it could be stabilised when the organism was grown in the presence of surfactants similar to those found in normal lung e.g. dipalmitoyl lecithin. Dipalmitoyl lecithin is the major surfactant found in the human lung. The concentration at which maximum stabilisation occurred was approximately the same as that found in the lung. Thus dipalmitoyl lecithin may have a significant role in the production of mucoid strains.

Evidence suggests that *P. aeruginosa* probably has at least a secondary influence on the course of respiratory disease in CF. In most cases, CF patients tolerate chronic Pseudomonas infection since they have built up immunologic defences elsewhere in the body. This is in marked contrast to the usual fulminant course of Pseudomonas infections in immunosuppressed or burns patients.

H. influenzae has aroused interest recently as to its role as a pathogen in CF (McCrae and Raeburn, 1974). These authors found no evidence of *S. aureus* in their patients. *H. influenzae* was the commonest pathogen isolated in patients with cough and/or sputum. This was replaced in the more severe cases by *P. aeruginosa*.

Virus Infections in CF

The role of virus infections in CF has received little attention. The incidence of these infections appears to be no higher than in healthy children, and the prevalence of antibodies in serum no different from normal children (Huang *et al.*, 1961).

Viral respiratory infections may, however, exacerbate bacterial infection in CF. Measles and influenza can cause particularly severe pulmonary involvement. Because of this, some workers advocate immunization of CF children against these diseases (Wood *et al.*, 1976).

Fungus Infections in CF

Fungi such as *Candida albicans* and *Aspergillus fumigatus* are sometimes isolated from sputum cultures of CF patients. They are rarely implicated in clinical symptomatology.

Cell-mediated immunity against *A. fumigatus* has been found (Gibbons *et al.*, 1976; Mitchell-Heggs *et al.*, 1976). The latter group of workers also reported allergic aspergillosis in their patients.

Type I and type III hypersensitivity has been shown to *A. fumigatus* (Mearns, Longbottom and Batten, 1967) as well as to other antigens including food, bacteria, and human body tissue (McFarlane *et al.*, 1975). Type III hypersensitivity is probably responsible for the greater tissue damage, since this causes an Arthus reaction resulting in the deposition of immune complexes, possibly in the lung (McFarlane *et al.*, 1975).

Despite increased knowledge gained during the past 10 years, treatment of CF remains essentially empirical and symptomatic. Until the basic defect is known this situation seems likely to prevail.

OBJECT OF THE RESEARCH

Patients with CF are subject to chronic progressive infection of the respiratory tract. Why the CF child is so prone to respiratory infection is not known. The copious production of viscid mucus in the respiratory tract may be one factor only.

The objects of this investigation were :-

1. To find out if obtaining nose and throat swabs and sputum specimens at monthly intervals (which is considerably more frequent than usual) would lead to better control of infection.

2. To assess two methods of treatment in achieving control of infection. One method (Flucloxacillin group) involved the administering of continuous anti-staphylococcal antibiotics plus one other antibiotic if required. The other (Variable Antibiotic group) used only one antibiotic which was changed as culture results dictated.

3. To determine antibiotic sensitivities and resistances for all potential pathogens in CF and not only S. aureus, P. aeruginosa and H. influenzae and to see if those patterns changed with time in individual patients.

4. To assess the clinical improvement in the patients following therapy for 18 months.

MATERIALS AND METHODS

Specimens : Specimens of nose and throat swabs and sputum were taken at weekly intervals from the original six patients (AL, DS, IL, TK, YH, HW). When the number of patients under investigation increased to the final total of sixteen, specimens were taken thereafter at monthly intervals. Each specimen was collected by the author from the patients at home or at school. Nose and throat swabs were obtained using serum coated Hospiswabs (Medical Wire and Equipment Company Ltd.). Sputum was collected by aspiration into sterile plastic universal containers (Sterilin).

Culture Media : Horse Blood agar (Oxoid Columbia agar + 5% defibrinated horse blood, Gibco Ltd.) was used for the isolation of all pathogens; Chocolate Blood agar (Oxoid Blood agar Base No. 2, Gibco Ltd.) as the [illegible] for Haemophilus species, and MacConkey agar (Oxoid Code No. CM7b) for Enterobacteriaceae and Pseudomonas aeruginosa.

Culture Procedures : Each specimen was cultured immediately on return to the hospital laboratory, usually no more than 5 hours after the specimen was taken.

Nose and throat swabs were plated directly onto the culture media, the media was inoculated using the Hospiswabs in the following order :

1. Horse Blood agar
2. Chocolate Blood agar
3. MacConkey agar

The sputum specimens were extremely viscous. It was therefore very difficult to use a platinum loop to plate out this material such that a representative culture of the specimen was obtained. In order to overcome this problem a fresh, sterile Hospiswab was immersed in the most purulent area of the sputum. The specimen was cultured using the procedure for nose and throat swabs.

The Horse Blood agar was streaked with S. aureus to encourage the growth of H. influenzae due to the satellitism phenomenon.

The Horse Blood agar and Chocolate Blood agar plates were incubated at 37°C in an atmosphere of 5% CO_2. MacConkey plates were incubated aerobically at 37°C.

All plates were examined after incubation for 24 hours and colonies selected for identification. The primary plates were reincubated for a further 24 hours and any further colonies removed for identification.

Identification of Bacteria : S. aureus, H. influenzae, H. parainfluenzae, H. haemolyticus, H. parahaemolyticus, and Streptococcus faecalis were identified by the methods outlined by Cowan and Steel (1974).

Initial identification of the Enterobacteriaceae was by their ability to ferment lactose and, in the case of P. aeruginosa produce pigment. Final identification was confirmed by the API 20 Enterobacteriaceae (API 20E) System (API System S.A.).

Table 1

Arrangement of antibiotics on the Multodisks

MULTODISKS				
5789E	4798E	5675S	U2	5678S
Tobramycin	Neomycin	Cephaloridine	Cephaloridine	Tobramycin
Cephazolin	Kanamycin	Cephalexin	Colistin	Cephazolin
Streptomycin	Gentamicin	Cephradine	Nitrofurantoin	Cotrimoxazole
Cloxacillin	Cotrimoxazole	Cephazolin	Sulphonamide	Neomycin
Cephaloridine	Sulphonamide	Cephalothin	Kanamycin	Gentamicin
Tetracycline	Fusidic Acid		Nalidixic Acid	Carbenicillin
Ampicillin	Erythromycin		Tetracycline	
Benzylpenicillin	Lincomycin		Ampicillin	

Table 2

Multodisk antibiotic dose

ANTIBIOTIC	ABBREVIATION	STANDARD DOSE (μg)
Cotrimoxazole	SXT	25
Sulphonamide	SF	500 and 100*
Benzylpenicillin	P	1.5 units
Ampicillin	PN	25
Cloxacillin	OB	5
Carbenicillin	PY	25
Tetracycline	TE	50
Erythromycin	E	10
Cephazolin	KZ	30
Cephaloridine	CR	30
Cephradine	CE	30
Cephalothin	KF	30
Cephalexin	CL	30
Colistin	CT	10
Gentamicin	CN	10
Tobramycin	TOB	10
Neomycin	N	30
Kanamycin	K	30
Lincomycin	MY	2
Fusidic Acid	FD	10

*500μg used for gram negative organisms
100μg used for gram positive organisms

Definition of a Pathogen : For convenience of discussing results in this investigation, any organism isolated from the sputum of a patient was regarded as a pathogen or potential pathogen and is described as "a pathogen". The same organism, if isolated from a no-sputum producer, was treated similarly. All other organisms were regarded as non-pathogens or commensals.

Determination of Antibiotic Sensitivity : The antibiotic sensitivity of each isolate was determined using the disk diffusion technique. Commercially prepared disks (Multodisk, Oxoid) impregnated with various antibiotics (Table 1) at standard concentrations (Table 2) were placed on the centre of inoculated Diagnostic Sensitivity Test agar (DST, Oxoid)*.

The Haemophilus species with their more fastidious growth requirements were grown on Chocolate Blood agar. However, the diffusion of the sulphonamides is inhibited by this medium. They were tested on DST agar although actual growth of the organisms was poor.

Note, for brevity in this thesis, all antimicrobial drugs are referred to as "antibiotics". This includes sulphonamide and cotrimoxazole which are technically not antibiotics in the true sense of the word i.e. an antimicrobial agent of microbial origin. However, in this day of semi-synthetic antibiotics it was felt that to differentiate between the sulphonamides and "true" antibiotics would merely be pedantic.

* Inoculated plates were incubated for 24 hours at 37°C. Bacteria were considered to be sensitive to particular antibiotics if there was a zone of inhibition extending at least 5mm beyond the edge of the disk.

Table 3

Order of choice of Antibiotics

ORGANISM		
Staphylococcus aureus	Pseudomonas aeruginosa	Haemophilus influenzae AND OTHER Haemophilus species
Cloxacillin	Gentamicin }	Cotrimoxazole
Erythromycin	Tobramycin }	Ampicillin
Cotrimoxazole	Colistin	Erythromycin
Cephradine	Amikacin	
Clindamycin	Tetracycline	
Fusidic Acid		
Ampicillin		

Table 4

Antibiotic dosage and route of delivery

ANTIBIOTIC	DOSE	ROUTE
Cloxacillin Flucloxacillin	100mg/Kg/day	ORALLY
Cotrimoxazole (SEPTRIN)	1-2 mg/Kg/day of Trimethoprim 5-10mg/Kg/day of Sulphamethoxazole	ORALLY
Ampicillin	50mg/Kg/day	ORALLY
Erythromycin	30-50mg/Kg/day	ORALLY
Cephradine	50-100mg/Kg/day	ORALLY
Gentamicin	6-7.5mg/Kg/day	INTRAMUSCULARLY
Tobramycin	5mg/Kg/day	INTRAMUSCULARLY

S. aureus, H. influenzae, H. parainfluenzae, H. haemolyticus, and H. parahaemolyticus were tested using disks 5789E, 4798E and 5675S, P. aeruginosa, Pr. mirabilis, E. coli and St. faecalis using disks U2, 5678S and 5675S (See Table 1, page 18).

Selection and Dose of Antibiotics: The antibiotic for therapy was selected from the results of the antibiotic sensitivity tests, and in accordance with the pre-designed sequence (Table 3).

Antibiotic dose was determined following consultation with Dr. Hamilton and the hospital Pharmacist (Table 4).

The antibiotics actually used in therapy were :

Cloxacillin (Orbenin)	Beecham Pharmaceuticals Ltd.
Flucloxacillin (Floxapen)	Beecham Pharmaceuticals Ltd.
Ampicillin (Penbritin)	Beecham Pharmaceuticals Ltd.
Erythromycin (Erythrocin)	Abbott Laboratories Ltd.
Coxtrimoxazole (Septrin)	Burroughs Wellcome & Co.
Cephradine (Velosef)	E.R. Squibb & Sons Ltd.
Gentamicin (Genticin)	Nicholas Laboratories Ltd.
Tobramycin (Nebcin)	Lilly Eli & Co. Ltd.

Distribution of Antibiotics : The parents (usually the mother) of the patients were informed of the results of the specimen cultures either by telephone or by letter. They were also told which antibiotics to give their child and the dose (this was also printed on the label of the bottle).

The antibiotic was sent from the hospital pharmacy to the patients' home or the author telephoned the patient's general practitioner from whom the prescription was obtained.

Table 5

Age and sex distribution of cystic fibrosis patients

GROUP	PATIENT	AGE (years)	SEX
VARIABLE ANTIBIOTIC	TK	1.5	F
	LC	1.8	F
	AL	2.5	F
	HW	3.6	M
	IL	7.6	M
	JD	10.3	M
	ER	10.8	F
	EB	10.8	M
	DS	11.7	M
	JG	12.1	M
	MEDIAN	8.5	
FLUCLOXACILLIN	AT	3.8	M
	YH	4.8	F
	DB	6.4	M
	PA	8.2	M
	LM	10.3	F
	DA	16.3	M
	MEDIAN	7.3	

Phage-typing : The phage-typing of S. aureus was carried out by the phage-typing laboratory at Gartnavel General Hospital, Glasgow.

Patients : The patients in this study were chosen,at random, from the cystic fibrosis patients attending Wards 6a, 6b, 7a and 7b of the Royal Hospital for Sick Children, Glasgow. The total number of patients selected was 20.

Parental permission was sought prior to commencing therapy. This was refused in 4 cases.

The remaining 16 patients were divided into 2 groups (Table 5).

Variable Antibiotic Group : These patients were drawn from Wards 7a and 7b. When the results of the specimen cultures were known, an appropriate antibiotic was prescribed from the pre-designed sequence. The patients were kept on this antibiotic until either future cultures indicated a new pathogen was present which was resistant to the antibiotic, or, that the present pathogen, itself, had become resistant.

The exception to this procedure occurred if an amino-glycoside (tobramycin or gentamicin) was prescribed. As these drugs can be toxic, therapy was only given for 10 days, whereafter the patient was returned to the anti-staphylococcal antibiotic (Cloxacillin).

Flucloxacillin Group : The patients in this group were drawn from Wards 6a and 6b. These patients were always treated with an anti-staphylococcal antibiotic (Flucloxacillin). If culture indicated the presence of a pathogenic organism which was resistant to this drug, a second appropriate antibiotic was administered concurrently with the Flucloxacillin. This second antibiotic, however, was only given for a period of 10 days.

A record consisting of the type of specimen, the organisms isolated, the antibiotic sensitivities, and the antibiotic choice was kept for each patient in the 2 groups.

Measurement of Weight and Height : Weight and height were measured on each visit to the hospital clinic. The technique for both was that recommended by Tanner et al., (1966).

Statistical Methods : The Mann-Whitney test, Student's t-test, and the χ^2-test were used for the statistical analysis of the data presented in this thesis. The statistical tables of Finney et al., (1963) were also used.

RESULTS

Table 6

Range of sweat Na^+ levels in the CF patients in this investigation

Group	Individual values of Sweat Na^+ Levels ($mEql^{-1}$)	Median and(Range)
VARIABLE ANTIBIOTIC	79, 99, 102, 103, 122, 122, 137, 140, 151, 154	122 (79-154)
FLUCLOXACILLIN	84, 104, 107, 116, 122, 154	113 (84-154)

NORMAL LEVELS :- Children $< 40\ mEql^{-1}$

Adults $< 80\ mEql^{-1}$

Table 7

Medical and socio-economic history of the CF patients in the investigation

Group (No. of patients)	History of Pregnancy										Immunization							Previous Infections					Socio-economic class				
	Mean gestational period (weeks)	Spontaneous vertex delivery	Forceps	Caesarian section	Mean birthweight (lbs)	No. of patients breast fed	No. of patients bottle fed	Mean age at which diagnosis made (years)	Mean No. of hospital admissions	Mean duration of admissions (days)	Polio	Diphtheria	Tetanus	Pertussis	B.C.G.	Measles	Rubella	Pertussis	Measles	Rubella	Varicella	Mumps	Class I	Class II	Class III	Class IV	Class V
VARIABLE ANTIBIOTIC (10)	39	6	2	1	6	1	8	2	3	21	8	8	8	4	8	3	0	0	2	3	0	1	1	2	3	0	4
FLUCLOXACILLIN (6)	40	2	0	4	7	0	6	3	4	12	6	6	6	6	4	3	0	0	1	2	2	2	2	1	1	2	0

SECTION 1 : CLINICAL OBSERVATIONS ON CYSTIC FIBROSIS PATIENTS

Initial Status of the Patients

The patients in this investigation attended Wards 6a, 6b, 7a and 7b of the Royal Hospital for Sick Children, Glasgow, in the period from January, 1978 to June, 1979. They were all known CF cases, and no new CF patients were added to those selected.

Diagnosis of CF was based on presentation of pulmonary symptoms and pancreatic insufficiency, and was confirmed by a positive sweat test (Table 6) i.e. a level of Na^+ greater than $60mEql^{-1}$.

As detailed in the Materials and Methods, the patients were divided into 2 groups, the Variable Antibiotic group and the Flucloxacillin group, at the start of the investigation.

Medical and Socio-economic History

The medical and socio-economic history of the two groups of CF patients in this investigation are outlined in Table 7. There was no history of consanguinity in any of the families. Only one patient (DA) had a CF sibling who was still living. His brother (PA) was also one of the patients in the investigation.

The history of pregnancy in both groups showed that no children were born prematurely, and all the birthweights lay between the 10th and 25th centiles.

Table 8a

Initial relative underweight in the CF patients of this investigation

Group	Severely underweight	Moderately underweight	Slightly underweight
VARIABLE ANTIBIOTIC	ER, IL	TK, LC, HW, JD, DS	AL, EB, JG
FLUCLOXACILLIN**	LM	AT	YH, DB

Table 8b

Final relative underweight in the CF patients of this investigation

Group	Severely underweight	Moderately underweight	Slightly underweight
VARIABLE ANTIBIOTIC*	ER	TK, LC, HW, JD, DS, JG	EB
FLUCLOXACILLIN**	LM	YH	AT, DB

* Data not available for AL and IL

** Data not available for PA and DA

Both groups were diagnosed at about the same mean age. The mean ages at the start of the investigation for both groups were also approximately the same (7.3 and 8.3 years respectively). This indicated that any differences in the clinical status of the patients in each group was not due to the length of therapy alone as this was of the same mean duration in both groups.

Each group of patients had been mainly fully immunized against polio, diphtheria, tetanus, pertussis and tuberculosis.

The CF patients were drawn from all socio-economic classes (Registrar General's classification). There was no evidence to suggest that parental care of CF patients reflected the social class to which the patients belonged.

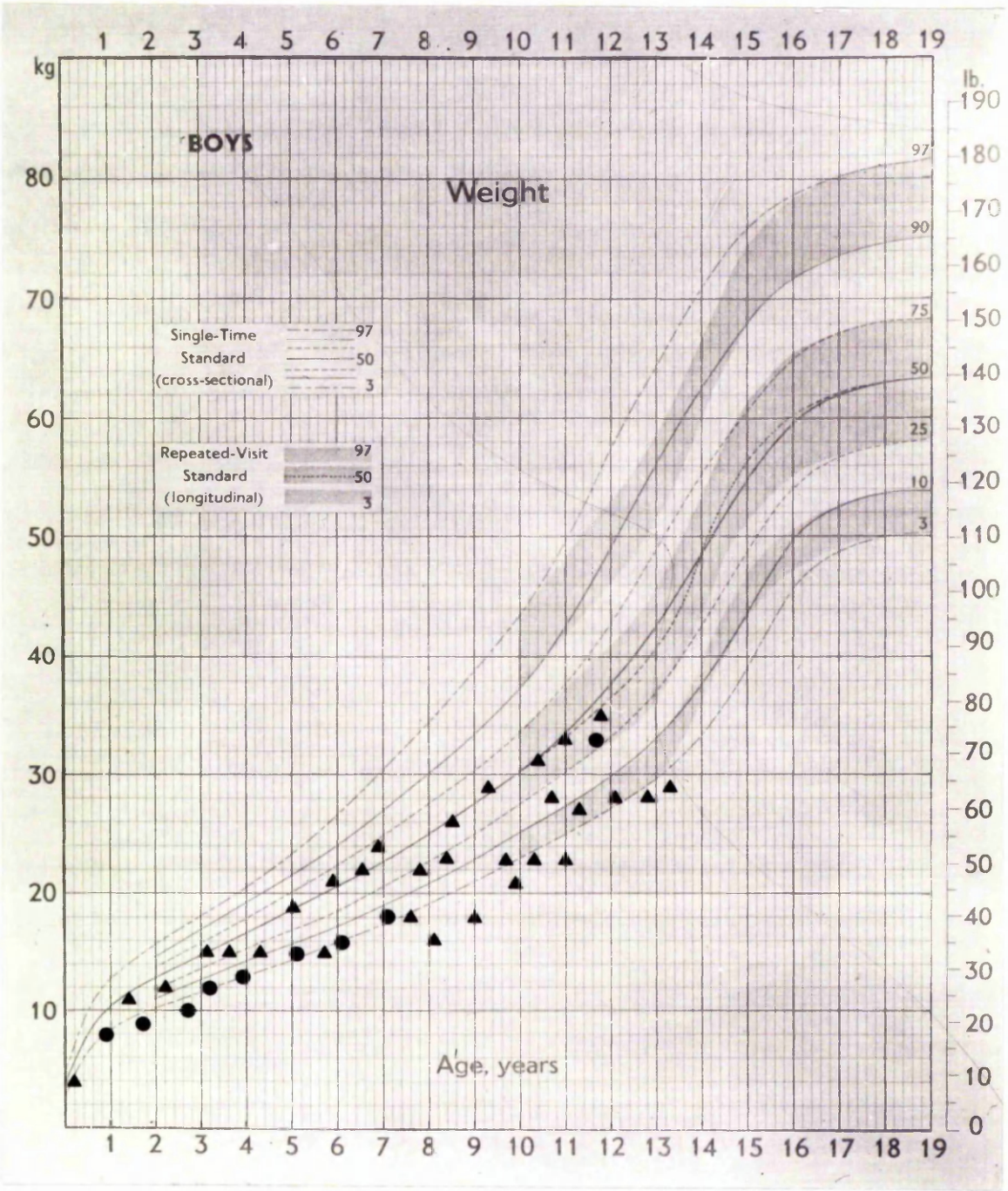
The initial status of the patients was also assessed in relation to their relative underweight(weight-deficit corrected for height) (Kraemer et al., 1978). The patients were divided into 3 categories depending on their degree of underweight (Tables 8a,b).It should be noted that those patients with respiratory involvement i.e. sputum producers (ER, IL, DS, LM, AT) fall into the highly and moderately underweight categories while those with little or no pulmonary involvement (no-sputum producers) fall into the slightly underweight category.

Clinical Progress

Effect of Therapy on Weight and Height of CF Patients

Cystic fibrosis, although affecting most of the secretory glands in the body, is largely discussed in relation to the

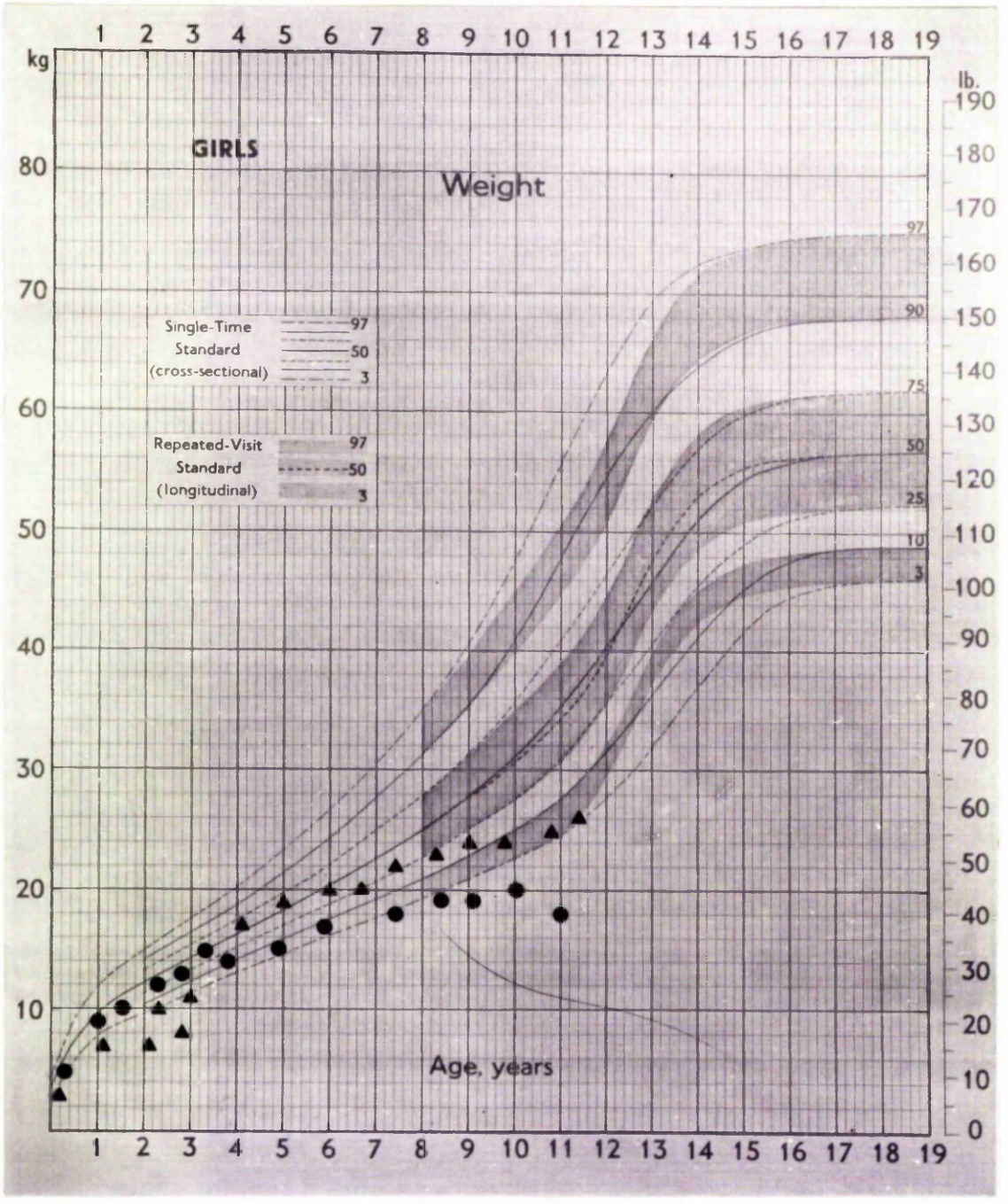
Fig. 1(a). Weight of CF patients (boys).



▲ = VARIABLE ANTIBIOTIC GROUP

● = FLUCLOXACILLIN GROUP

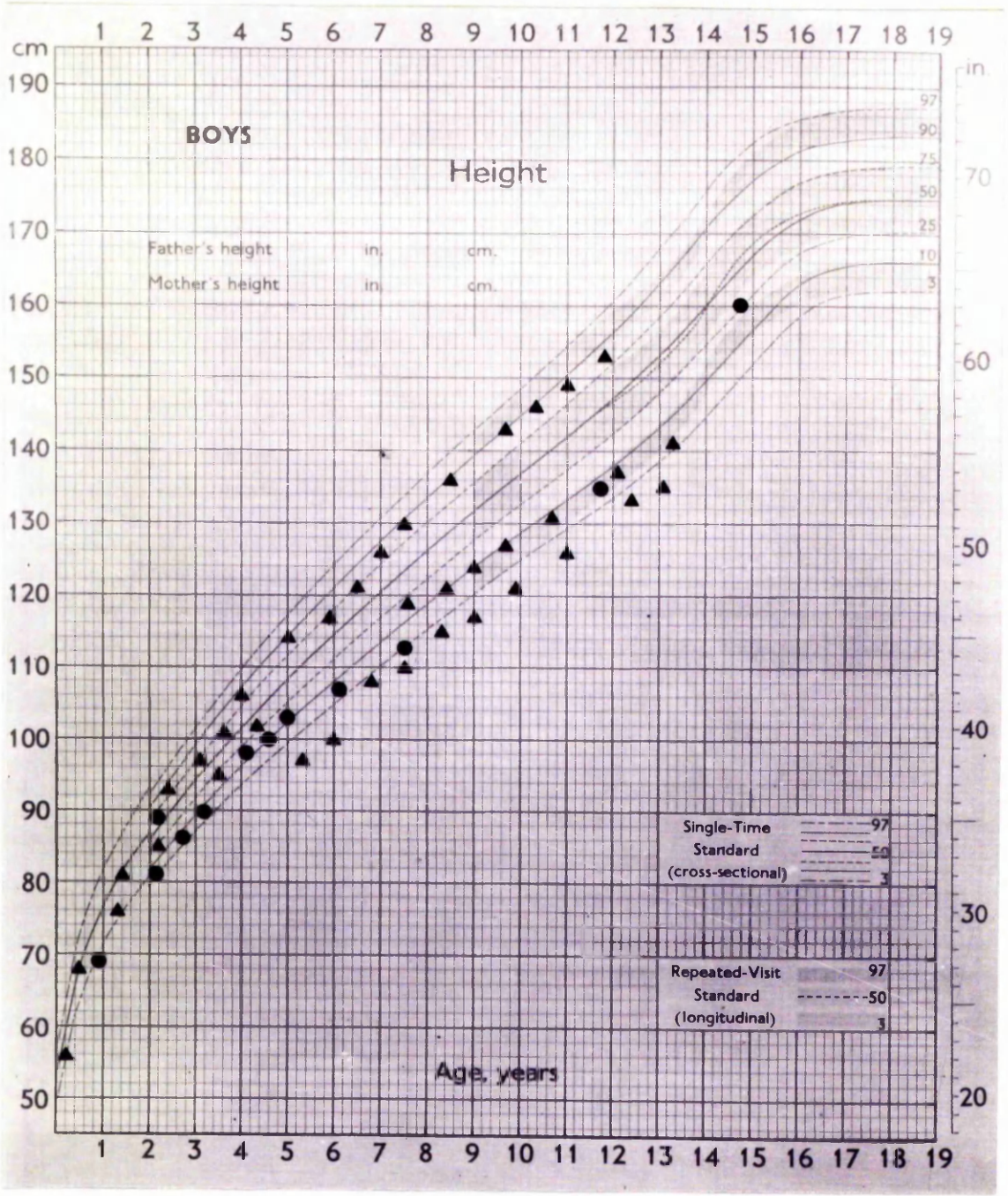
Fig. 1(b). Weight of CF patients (girls).



▲ =VARIABLE ANTIBIOTIC GROUP

● =FLUCLOXACILLIN GROUP

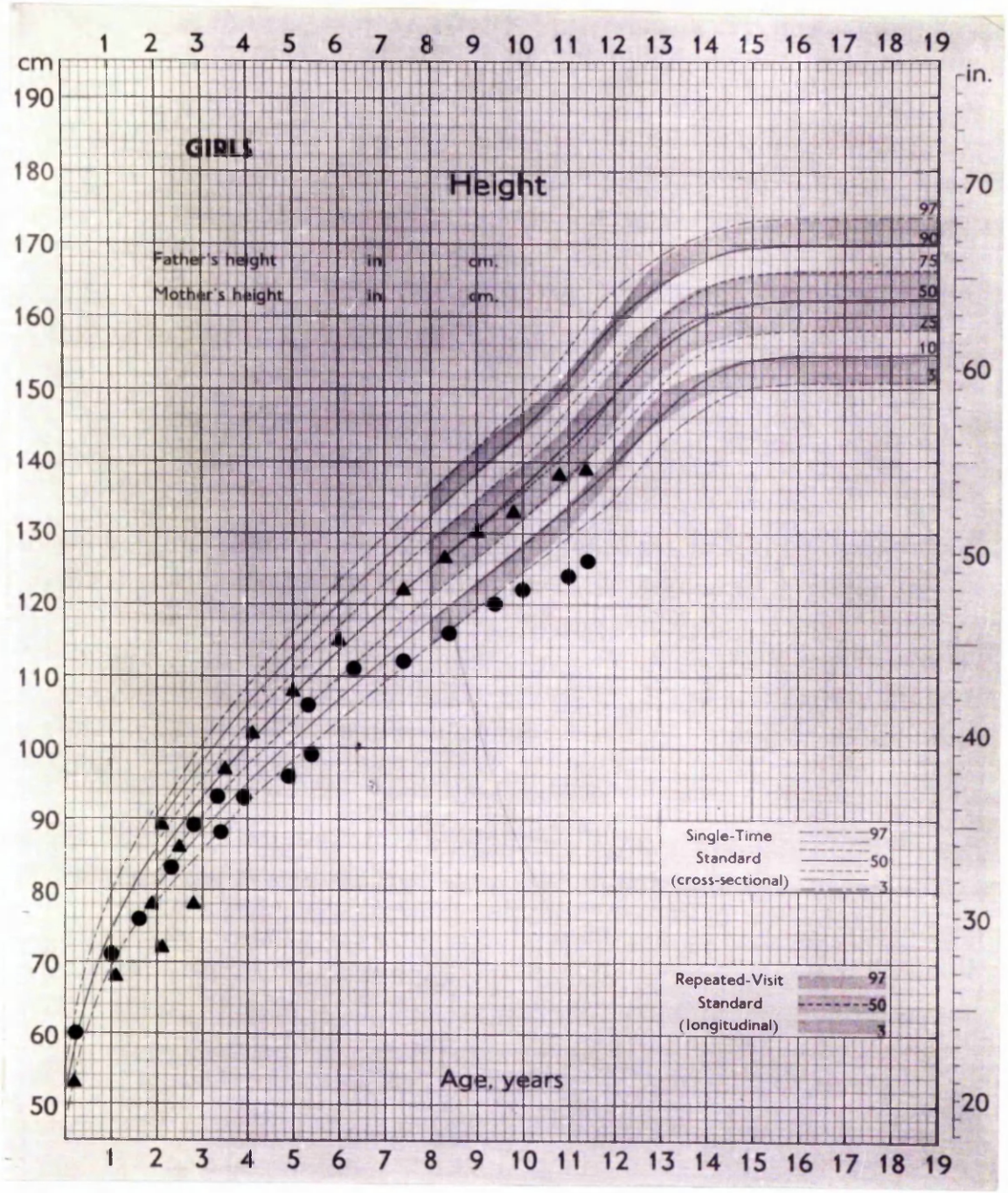
Fig. 2(a). Height of CF patients (boys).



▲ =VARIABLE ANTIBIOTIC GROUP

● =FLUCLOXACILLIN GROUP

Fig. 2(b). Height of CF patients (girls).



▲ =VARIABLE ANTIBIOTIC GROUP

● =FLUCLOXACILLIN GROUP

pulmonary and gastrointestinal involvement of the disease. This combination of pulmonary and gastrointestinal involvement frequently results in poor weight gain and short stature.

Weight and height are therefore very useful indicators of the clinical progress of the CF patient. Of the two parameters, weight is regarded as the most important (Sproul and Huang, 1964). They and other investigators (Doershuk et al., 1964) have shown that weight gain is not determined by the severity of the gastrointestinal symptoms as would be expected, but by the degree of pulmonary involvement. Thus, if infection of the respiratory tract could be well controlled one would expect the patients to gain weight normally.

In this investigation weight and height were considered jointly as a measure of improvement in clinical status.

Growth Records of CF Patients

Figs. 1(a), 1(b), 2(a), 2(b) show the growth records of the patients from birth to the end of the investigation. The records were grouped according to the patients' sex. The percentile markings are for a normal population.

From figs. 2(a) and 2(b) it can be seen that the heights of both boys and girls mainly lay between the 3rd and 25th centile. The data between the 75th and 90th centile (boys) and the 50th and 75th (girls) were due to single patients (EB and ER respectively).

The difference between growth data for CF patients and normal children became more apparent when weight rather than height was plotted on the charts. The charts also showed

Table 9

Weight gain in CF patients after 18 months

Group	Patient	Weight (Kg) Initial	Final	Gain
VARIABLE ANTIBIOTIC	TK	6.3	8.2	1.9
	LC	9.2	11.2	2.0
	AL	12.5	N/A	---
	HW	12.9	14.7	1.8
	IL	17.9	N/A	---
	JD	22.4	23.6	1.2
	ER	24.7	25.9	1.2
	EB	32.8	34.5	1.7
	DS	27.5	28.5	1.0
	JG	27.2	26.7	-0.5
	MEDIAN	20.2	24.8	1.5
FLUCLOXACILLIN	AT	13.2	15.9	2.7
	YH	15.5	17.2	1.7
	DB	16.9	19.0	2.1
	PA	N/A	N/A	---
	LM	20.00	18.0	-2.0
	DA	N/A	N/A	---
	MEDIAN	16.2	17.6	1.9

N/A = not available, --- = no result

Table 10

Height increase in CF patients after 18 months

Group	Patient	Height (cm) Initial	Final	Gain
VARIABLE ANTIBIOTIC	TK	70.0	77.8	7.8
	LC	79.5	88.0	8.5
	AL	86.2	N/A	---
	HW	97.5	103.4	5.9
	IL	118.5	N/A	---
	JD	123.1	128.7	5.6
	ER	137.6	138.8	1.2
	EB	147.7	151.5	3.8
	DS	134.0	138.5	4.5
	JG	127.5	135.4	7.9
	MEDIAN	120.8	132.1	5.8
FLUCLOXACILLIN	AT	96.0	102.5	6.5
	YH	102.0	111.0	9.0
	DB	107.3	113.0	5.7
	PA	N/A	N/A	---
	LM	123.0	127.0	4.0
	DA	N/A	N/A	---
	MEDIAN	104.7	112.0	6.1

N/A = not available ; --- = no result

Table 11

Weight retardation in CF patients. Deviation of actual weight from the expected weight (50th centile) for the chronological age of the patient

Group	Patient	Initial				Final			
		Age (years)	Actual weight (Kg)	Percentile Range	Expected Weight (kg) (50th centile)	Age (years)	Actual weight (Kg)	Percentile Range	Expected Weight (Kg) (50th centile)
VARIABLE ANTIBIOTIC	TK	1.5	6.3	< 3	11.1	2.8	8.2	< 3	13.7
	LC	1.8	9.2	< 3	11.7	3.0	11.2	3	14.3
	AL	2.5	12.5	25-50	13.3	N/A	N/A	N/A	N/A
	HW	3.6	12.9	3-10	15.8	4.6	14.7	3-10	17.7
	IL	7.6	17.9	< 3	24.2	N/A	N/A	N/A	N/A
	JD	10.3	22.4	< 3	31.3	11.4	23.6	< 3	35.9
	ER	10.8	24.7	3-10	34.3	11.4	25.9	3	37.2
	EB	10.8	32.8	50	32.8	11.6	34.5	25-50	35.9
	DS	11.7	27.5	3-10	36.4	12.9	28.5	< 3	42.1
	JG	12.1	27.2	3	38.2	13.1	26.7	< 3	43.2
	MEDIAN	9.0	20.2		27.8	11.4	24.8		35.9
FLUCLOXACILLIN	AT	3.8	13.2	3-10	16.2	5.0	15.9	10-25	18.5
	YH	4.8	15.5	10	17.9	6.1	17.2	3-10	20.6
	DB	6.3	16.9	3-10	21.2	7.4	19.0	3-10	23.5
	PA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	LM	10.3	20.0	< 3	32.2	11.3	18.0	< 3	36.7
	DA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	MEDIAN	5.6	16.2		19.6	6.8	17.6		22.1

N/A = not available

Table 12

Height retardation in CF patients. Deviation of actual height from expected height (50th centile) for the chronological age of the patient

Group	Patient	Initial				Final			
		Age (years)	Actual Height (cm)	Percentile Range	Expected Height(cm) (50th centile)	Age (years)	Actual Height (cm)	Percentile Range	Expected Height(cm) (50th centile)
VARIABLE ANTIBIOTIC	TK	1.5	70.0	< 3	80.5	2.8	77.8	< 3	91.3
	LC	1.8	79.5	10-25	83.2	3.0	88.0	10	93.0
	AL	2.5	86.2	10-25	88.9	N/A	N/A	N/A	N/A
	HW	3.6	97.5	25-50	98.7	4.6	103.4	10-25	105.7
	IL	7.6	118.5	10-25	123.9	N/A	N/A	N/A	N/A
	JD	10.3	123.1	< 3	138.3	11.4	128.7	< 3	143.1
	ER	10.8	137.6	25-50	141.4	11.4	138.8	10-25	145.4
	EB	10.8	147.7	75-90	140.8	11.6	151.5	75-90	145.2
	DS	11.7	134.0	3-10	145.7	12.9	138.5	3-10	152.8
	JG	12.1	127.5	< 3	147.9	13.1	135.4	< 3	154.1
	MEDIAN	9.0	120.8		131.1	11.4	132.1		144.2
FLUCLOXACILLIN	AT	3.8	96.0	10-25	100.1	5.0	102.5	10-25	108.3
	YH	4.8	102.0	10-25	105.8	6.1	111.0	25-50	114.0
	DB	6.3	107.3	3-10	119.3	7.4	113.0	3-10	122.8
	PA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	LM	10.3	123.0	< 3	138.3	11.3	127.0	< 3	144.7
	DA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	MEDIAN	5.6	104.7		112.6	6.8	112.0		118.4

N/A = not available

that the weight of the girls seemed to be more retarded as they approached puberty than the weight of the boys. After the age of 10 the weight range for girls was < 3rd to 10th centile. For boys this range was < 3rd to 50th centile. Again the data on the 50th centile were due to 1 patient (EB). There appeared to be no difference between the two investigated groups regarding the centiles for height and weight of patients of comparable age.

Assessment of Weight

The initial and final weight of each patient in the two groups was noted and the resulting gain or loss calculated (Table 9). The medians of each group were compared using the Mann-Whitney Test and found not significantly different.

Assessment of Height

The initial and final height was noted for each group and analysed as for weight (Table 10). The difference of the medians was again found to be not significant.

Comparison of CF Weight and Height with Normal Population

The actual weight and height for each patient was tabulated (Tables 11, 12) with the expected weight and height for their chronological age (50th centile). This was done for these parameters at the beginning and end of the investigation. The differences of the medians between the groups was analysed and once again, found not significant.

Fig. 3. Severity of cough in CF patients
(a) and (c) pre-trial; (b) and (d) post-trial
N/A = Data not available; - = no cough;
± = slight cough; + = intermittant cough;
++ = moderate cough; +++ = severe cough.

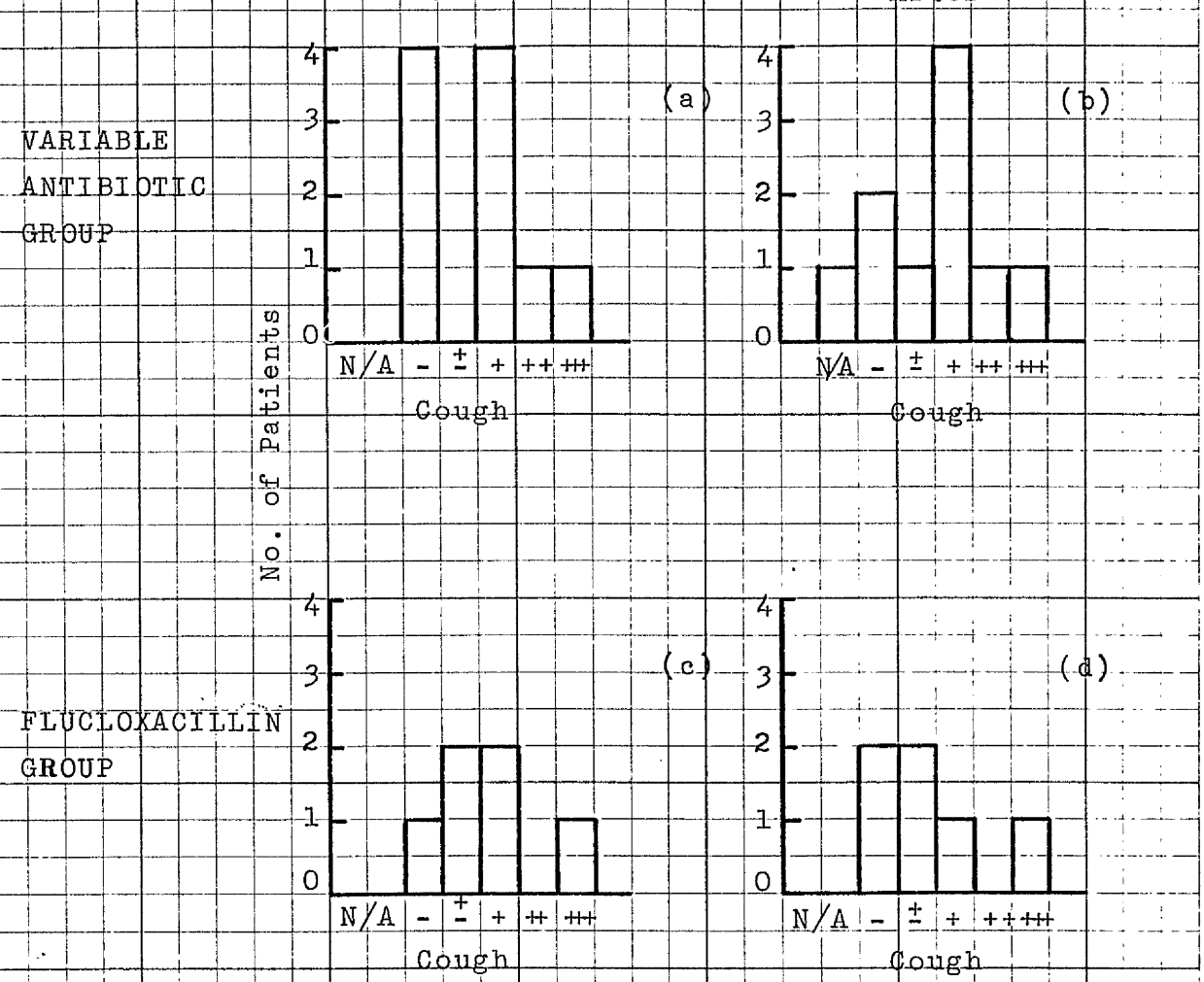
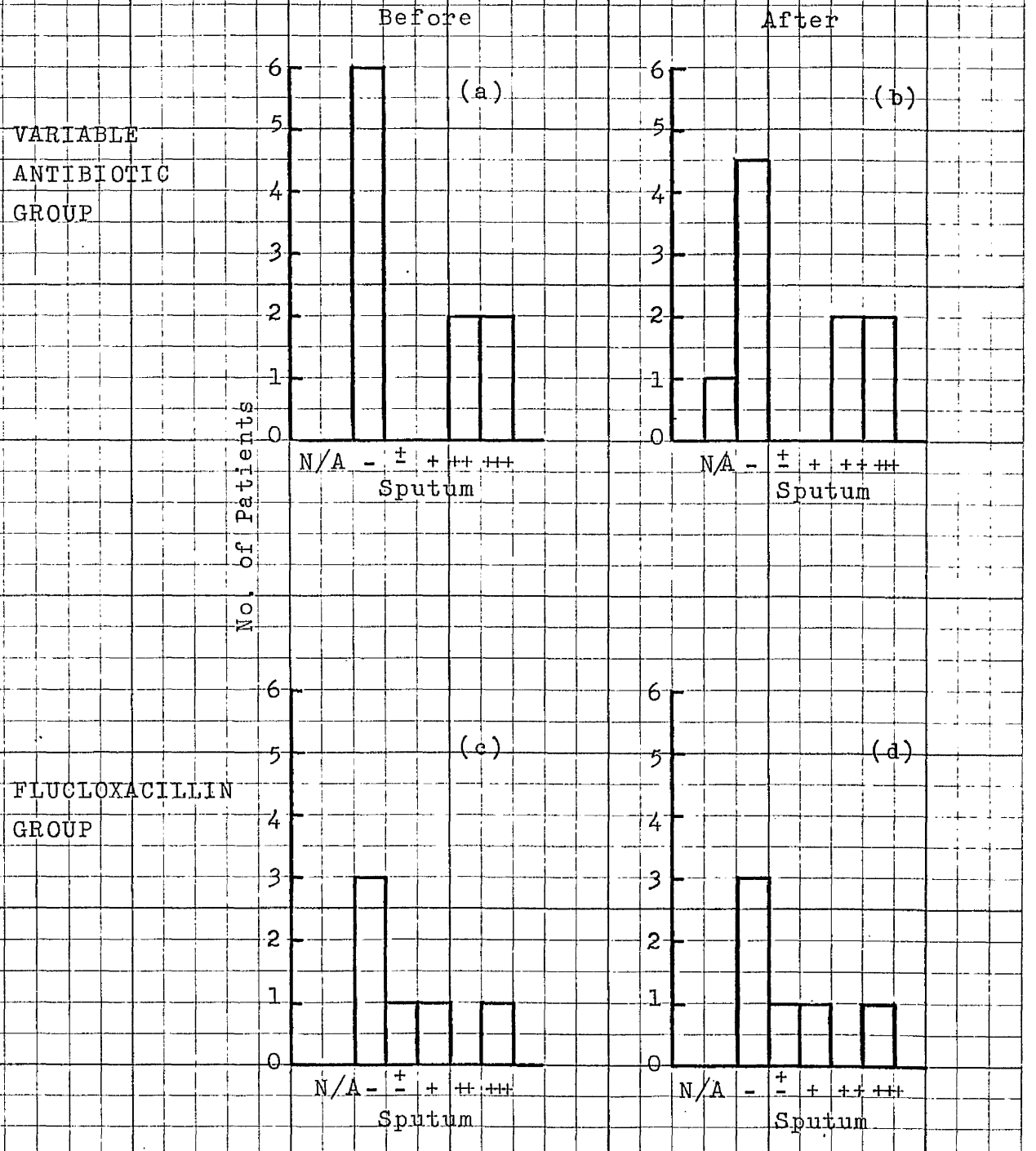


Fig.4. Amount of sputum expectorated by CF patients (a) and (c) pre-trial; (b) and (d) post-trial
N/A = Data not available; - = no sputum;
± = very small quantity of sputum;
+ = small quantity of sputum; ++ = 2ml or less/expectoration; +++ = 5ml/expectoration.



Pulmonary Changes in CF Patients During the Investigation

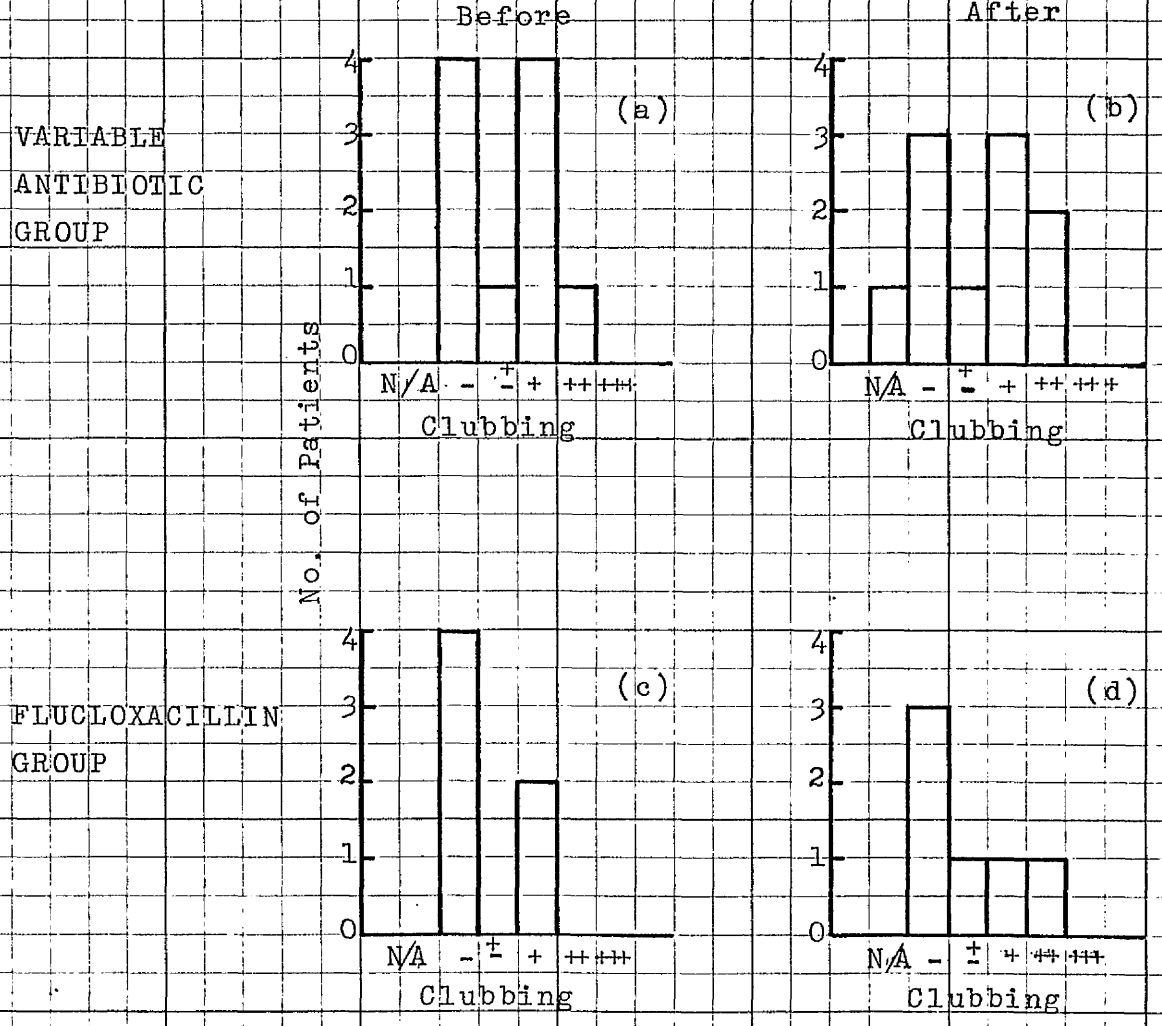
The extent of broncho-pulmonary disease in CF can be assessed by physical and radiological examination. They are complementary in determining the pulmonary changes which may occur as the disease progresses.

Pulmonary Changes Evident on Physical Examination

(1) Cough : The most prominent and constant symptom of pulmonary involvement is cough. The cough may, at first, be dry and hacking but with progression of the disease it becomes productive and often troublesome at night. Figure 3 shows the severity of cough in patients of both groups at the beginning and end of the investigation. One of the patients in the Variable Antibiotic group who had no cough initially developed a slight cough during the 18 months (HW). His cough, however, was non-productive. In the Flucloxacillin group, DA who had a non-productive cough initially, responded to treatment sufficiently that coughing ceased. LM, although having a severe cough throughout, ceased night coughing shortly after the investigation started.

(2) Sputum : Severity of sputum production was assessed by the frequency of sputum produced daily and the ease of expectoration i.e. produced during coughing or only by physiotherapy. Figure 4 illustrates the amounts of sputum expectorated by the CF patients and shows that there was no change during the investigation.

Fig. 5. Degree of clubbing in CF patients
(a) and (c) pre-trial; (b) and (d) post-trial
N/A = Data not available; - = no clubbing;
± = slight clubbing; + = mild clubbing;
++ = moderate clubbing; +++ = pronounced clubbing.



(3) Digital Clubbing : Digital clubbing is one of the physical manifestations of pulmonary changes. It arises from the enlargement of the bronchial artery and the systemic vascular supply to the lungs. Figure 5 shows the degree of clubbing for both groups. In the Variable Antibiotic group DS had mild clubbing at the start of the investigation, but this had become moderately severe after 18 months. This patient also had severe cough and copious sputum production. In the Flucloxacillin group, YH developed very mild clubbing - a first sign of pulmonary involvement. LM had progressed from mild clubbing to moderately severe clubbing during the investigation.

Pulmonary Changes Evident on Radiological Examination

The radiographic manifestations of pulmonary disease in CF are often seemingly out of proportion to the clinical status of the patient. The earliest radiological signs of disease are usually bronchial thickening and irregular areas of hyperinflation. In advanced disease, segmental or lobar atelectasis, cyst formation, extensive bronchiectasis, retained secretions, and extensive infiltrates are seen. Appendices 1 and 2 summarize the severity of radiological and physical findings in both groups of patients at the start and end of the investigation. No overall improvement in the radiological findings was observed. EB showed slight deterioration and LM marked deterioration.

Table 13

Hospital admission rate of CF patients

Group	Patient	Hospital Admissions 18 months pre-trial	18 months during trial
VARIABLE ANTIBIOTIC	TK	1	1
	LC	0	0
	AL	1	---
	HW	1	0
	IL	0	0
	JD	1	0
	ER	0	0
	EB	1	0
	DS	4	1
	JG	0	0
	MEAN RATE	0.9	0.2
FLUCLOXACILLIN	AT	4	1
	YH	1	1
	DB	0	0
	PA	0	0
	LM	2	3
	DA	0	0
	MEAN RATE	1.1	0.8

Hospitalization for Acute Exacerbation

The number of times a CF patient is admitted to hospital can, in some cases, be dependent on the physician-in-charge. However, those involved in treating CF agree with Wood et al., (1976) that in order to avoid unnecessary exposure to infection patients should have as little contact as possible with the hospital environment.

The number of hospital admissions therefore became another parameter which could be employed in assessing clinical progress (Table 13). A fall in the rate of hospital admissions would have a two-fold effect. Firstly the cost to the health service. It is less expensive to treat a patient at home than in hospital. Secondly, the benefit to the patient. A child is usually far happier in an environment it knows. There is therefore a psychological advantage in keeping the child with its parents and relying on them for administration of therapy.

In Table 13 those patients who had little or no cough and no sputum (no-sputum producers) were either admitted once or not at all during the 18 months pre-investigation (i.e. TK, LC, AL, HW, JD, JG, YH, DB, DA). Of these patients TK and YH were admitted during the investigation. TK had a rectal prolapse which required admission in the day-bed area for treatment by alcohol injection. YH had an exacerbation which required an 18 day admission.

The remaining patients in both groups are those who had both cough and sputum expectoration (sputum producers). Of these patients IL, ER and PA had no admissions either pre or

during the investigation. EB was admitted once pre-investigation but not during the investigation.

However, probably the most significant difference can be seen in the admission rate for DS and AT. In both instances this dropped from 4 admissions pre-investigation to only one during the investigation. In the case of DS this was merely for 4 days observation. AT did require intravenous aminoglycoside therapy for an exacerbation caused by Pr.mirabilis and P. aeruginosa.

LM was the only patient who had more hospital admissions during than before the investigation. The two admissions pre-investigation were during the first 5 months of the 18 month period and were both of 14 days duration. The first of the 3 admissions during the investigation occurred 19 months later. This was of 7 days duration and was followed after one month by an acute exacerbation which required hospitalization for 36 days. The third admission occurred 3 months later and was for observation after haemoptysis was detected. This was of 3 days duration. This patient died 6 weeks later.

The decrease in admission rate during the investigation was not significant ($p > 0.05$, Students' t-test) either for the Variable Antibiotic group or the Flucloxacillin group($p > 0.05$).

SECTION II : BACTERIOLOGICAL OBSERVATIONS

Records of Isolations of Pathogens

Results of Nose and Throat Swabs and Sputum Specimens

The nose and throat swabs and sputum specimens were obtained

Table 14

Frequency of isolations of pathogens from specimens in the Variable Antibiotic and Flucloxacillin groups

Organism	No. of Isolates / No. of Specimens (%)	
	VARIABLE ANTIBIOTIC GROUP	FLUCLOXACILLIN GROUP
S. aureus	61/490 (12)	59/175 (34)
H. influenzae	33/490 (7)	19/175 (11)
P. aeruginosa	105/490 (21)	9/175 (5) **
H. parahaemolyticus	26/490 (5)	2/175 (1) *
H. parainfluenzae	7/490 (1)	3/175 (2)
St. faecalis	23/490 (5)	1/175 (1) *
Pr. mirabilis	5/490 (1)	19/175 (11) **
E. coli	13/490 (3)	6/175 (3)

* = denotes significant difference ($p < 0.05$)

** = denotes highly significant difference ($p < 0.001$)

Table 15

Bacteriological analysis of the sets of specimens from the CF patients.

Nature of Specimen	Number (%) of Specimens Yielding Pathogenic Bacteria			
	VARIABLE ANTIBIOTIC GROUP		FLUCLOXACILLIN GROUP	
	SP	NSP	SP	NSP
NOSE SWAB	3/84 (4)	32/122 (26)	4/27 (15)	14/43 (33)
THROAT SWAB	32/81 (40)	36/122 (30)	10/25 (40)	12/43 (28)
SPUTUM	74/81 (91)**	----	37/37 (100)**	----

SP = Sputum producers; NSP = No-sputum producers

---- = No specimen obtained

** = denotes highly significant difference ($p < 0.001$) Sputum specimens compared with the other specimens of SP and NSP within the same group.

as described in the Materials and Methods.

During the 18 months of the investigation a total of 490 specimens were taken from the Variable Antibiotic group and 175 specimens from the Flucloxacillin group. Table 14 shows the frequency of the isolations of each organism from the 2 groups.

The data showed that P. aeruginosa, H. parahaemolyticus and St.faecalis were isolated at a higher frequency from the Variable Antibiotic group than the Flucloxacillin group. This difference was significant for H. parahaemolyticus and St. faecalis ($p < 0.05$) and highly significant for P. aeruginosa ($p < 0.001$). Pr. mirabilis was the only organism which was isolated at a greater frequency from the Flucloxacillin group than the Variable Antibiotic group ($p < 0.001$).

There was no significant difference between the groups for the isolation of the other organisms ($p > 0.05$).

Sputum is, of course, the specimen of choice from CF patients. However, in some patients, especially those under the age of 5 years, it is very difficult to obtain sputum without using suction. It was therefore thought that in these patients, nose and throat swabs would be an acceptable substitute for sputum. Nose and throat swabs were also taken from patients who could produce sputum.

Thus, at every visit, 2 specimens were obtained from patients unable to produce sputum and 3 from those who could produce sputum. A comparison of the number of these specimens which yielded pathogens was made for each group (Table 15).-

Table 16

Isolation of pathogenic bacteria from CF patients at the beginning and end of the trial

Organism	Number (%) of Patients with Organism				
	VARIABLE ANTIBIOTIC GROUP		FLUCLOXACILLIN GROUP		Non-CF Patients
	Initial	Final	Initial	Final	
S. aureus	5/10 (50)**	7/10(70)**	4/6(67)•	3/6(50)	32/200(16)•**
H. influenzae	3/10 (30)	4/10(40)*	4/6(67)•	1/6(17)	15/200(8)•*
P. aeruginosa	2/10 (20)**	4/10(40)**	1/6(17)	2/6(33)••	1/200(0.5)•**
Miscellaneous	3/10 (30)	7/10(70)	3/6(50)	1/6(17)	76/200(38)
Commensals Only	4/10 (40)	2/10(20)	0/6(0)	1/6(17)	94/200(47)

* = denotes significant difference ($p < 0.05$) Variable Antibiotic Group vs non-CF patients.

** = denotes highly significant difference ($p < 0.001$) "

• = denotes significant difference ($p < 0.05$) Flucloxacillin Group vs non-CF patients.

•• = denotes highly significant difference ($p < 0.001$) "

Here each of the 2 groups was sub-divided into sputum producers and no-sputum producers. Consequently, the sputum specimens were taken as the base for comparison.

Analysis for the Variable Antibiotic group showed that the number of sputum specimens which yielded pathogens was significantly greater than the number of nose and throat swabs which yielded pathogens ($p < 0.001$). This result was irrespective of whether the swabs had been taken from sputum producers or no-sputum producers.

The same highly significant difference was obtained for the Flucloxacillin group.

In order to determine the effect of the 2 methods of therapy on the bacterial flora of the groups, the pathogenic bacteria isolated at the beginning and end of the investigation from CF patients were compared (Table 16). There was no significant difference in the number of patients in each of the groups whose specimens yielded any of the pathogens at the beginning of the investigation from those at the end of the investigation ($p > 0.1$).

However, when these results were compared with analogous specimens taken from non-CF patients, the differences became significant.

There was a highly significant difference in the isolation of S. aureus and P. aeruginosa from the CF patients in the Variable Antibiotic group compared to those of the non-CF patients ($p < 0.001$). This difference applied to both initial and final isolations of pathogens. S. aureus was isolated

from 50 per cent of CF patients initially and 70 per cent finally. For non-CF patients the overall figure was 16 per cent. The corresponding results for P. aeruginosa was 20 per cent and 40 per cent from CF patients and 0.5 per cent from non-CF patients. The P. aeruginosa isolated from the non-CF patients was grown from a sputum specimen from a patient recovering from cardiac surgery.

There was no significant difference initially for H. influenzae ($p > 0.1$) but at the end of the investigation this organism was isolated from 40 per cent of the CF patients in this group compared to only 8 per cent of non-CF patients. Here the difference was highly significant ($p < 0.01$).

In the Flucloxacillin group the number of patients with S. aureus and H. influenzae fell during the period of the investigation from 67 per cent to 50 per cent, and 67 per cent to 17 per cent respectively. These differences when compared to those of non-CF patients, were significant ($p < 0.01$) at the beginning of the investigation. However, at the end of the investigation the differences were not significant ($p > 0.1$).

This result suggests that the treatment for the Flucloxacillin group was effective for both S. aureus and H. influenzae.

The isolation of P. aeruginosa showed a different trend. At the beginning of the investigation only one patient in this group yielded this organism. When compared to the non-CF patients the difference was not significant ($p > 0.05$). The difference between non-CF patients and those of the Flucloxacillin group was significant however at the end of the investigation ($p < 0.001$).

Table 17

Isolation rates of pathogenic bacteria during the investigation.

ORGANISM	Number (%) of patients with organism	
	Sputum Producers	No-Sputum Producers
S. aureus	6/7 (86)	6/9 (67)
H. influenzae	5/7 (71)	6/9 (67)
P. aeruginosa	6/7 (86)	0/9 (0) *
Miscellaneous	7/7 (100)	8/9 (89)
Commensals Only	0/7 (0)	3/9 (33)

* = denotes highly significant difference ($p < 0.01$)

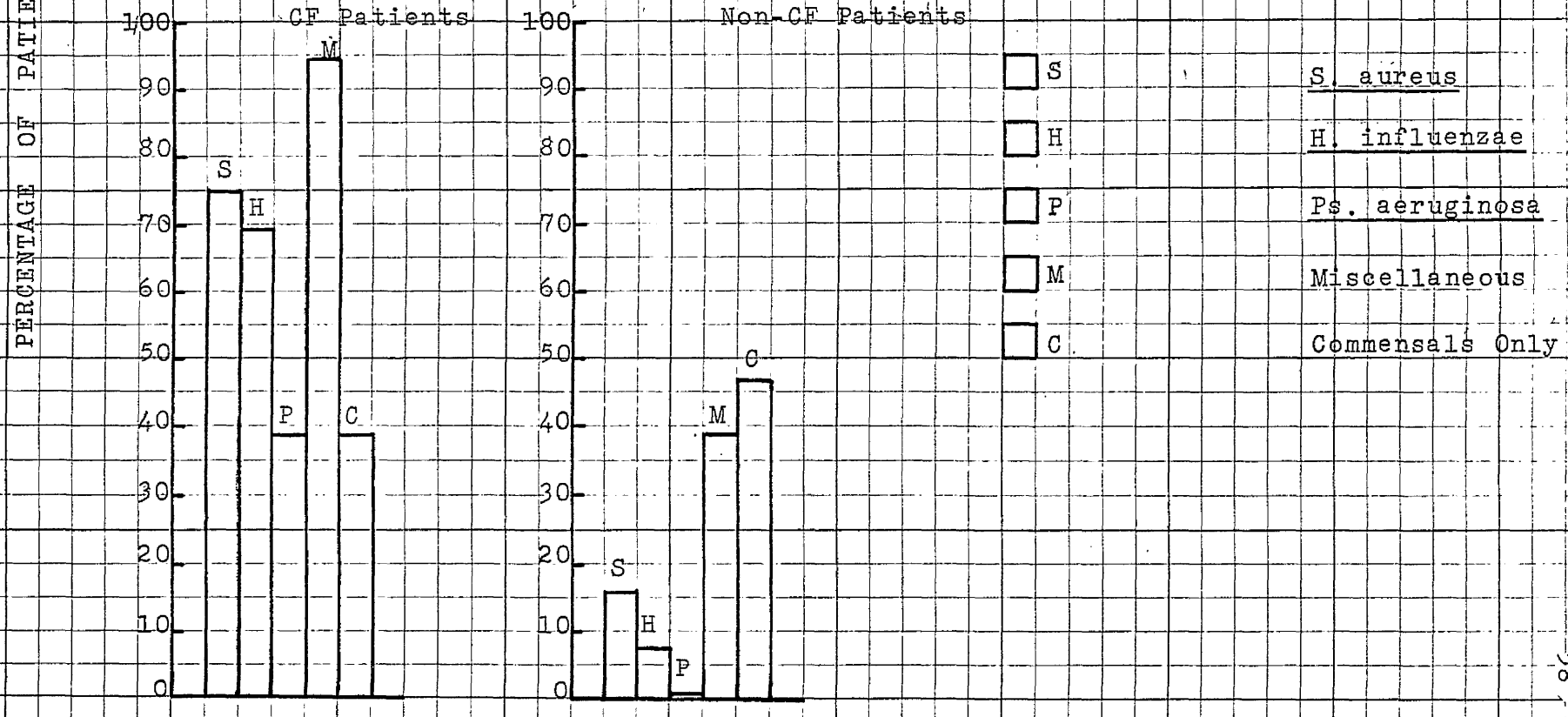
Table 18

Isolation rates of pathogenic bacteria during the investigation

Organism	Number (%) of Patients with organism	
	VARIABLE ANTIBIOTIC GROUP	FLUCLOXACILLIN GROUP
S. aureus	8/10 (80)	4/6 (67)
H. influenzae	6/10 (60)	5/6 (83)
P. aeruginosa	4/10 (40)	2/6 (33)
Miscellaneous	10/10 (100)	5/6 (83)*
Commensals Only	2/10 (20)	1/6 (17)

* = denotes significant difference ($p < 0.05$)

Fig. 6. Isolation rates of pathogenic bacteria from CF and non-CF patients.



Epidemiology of CF Patients

Figure 6 shows the isolation rates of pathogenic bacteria from all CF patients and non-CF patients. The figure depicts the percentage and number of patients who, during the 18 month period of the investigation harboured the bacteria in question in the respiratory tract at one or more examinations.

The results show that of the three major pathogens associated with CF S. aureus was the predominating organism (75 per cent) followed by H. influenzae (69 per cent) and P. aeruginosa (38 per cent). The pattern is the same for non-CF patients but reflect a much lower isolation rate i.e. S. aureus (16 per cent), H. influenzae (8 per cent), and P. aeruginosa (0.5 per cent). The differences in the isolation rates between the 2 categories of patients are highly significant ($p < 0.001$).

In order to determine whether there were any significant differences in the isolation rates of the major pathogens within the CF patients, the data were arranged such that a comparison could be made between sputum producers and no-sputum producers (Table 17) and patients in the Variable Antibiotic and Flucloxacillin groups (Table 18).

The ranking of predominant organisms was the same for the Variable Antibiotic group as that given in Figure 6 i.e. S. aureus (80 per cent), H. influenzae (60 per cent), and P. aeruginosa (40 per cent). This order changed somewhat for the Flucloxacillin group. Here H. influenzae was the predominating organism (83 per cent) followed by S. aureus

Table 19

Number of isolations of each pathogen from the respiratory tract of CF patients.

Organism	RESPIRATORY TRACT SITE				
	Lower Respiratory Tract Only	Throat Only	Nose Only	Combination	Total
S. aureus	39	15	26	17	97
H. influenzae	21	3	16	3	43
P. aeruginosa	60	4	0	25	89
Miscellaneous	47	50	12	10	119
Total	167	72	54	55	348

(67 per cent) and P. aeruginosa (33 per cent). The differences were not significant between the 2 groups for the major pathogens ($p > 0.05$). However, for the miscellaneous organisms, the differences were significant ($p < 0.05$).

The isolation rates in Table 17 show that S. aureus and P. aeruginosa were isolated with equal frequency (86 per cent) followed by H. influenzae (71 per cent) in sputum producers. S. aureus and H. influenzae were isolated with equal frequency (67 per cent) from no-sputum producers. P. aeruginosa was not isolated at all from these patients. This suggests that P. aeruginosa tends to be localized in those CF patients with advanced pulmonary infection. Indeed the only significant difference was seen in the isolation rates of P. aeruginosa in sputum producers and no-sputum producers. Here the difference was highly significant ($p < 0.01$).

Location of Pathogenic Bacteria in the Respiratory Tract

Table 19 shows the number of isolations of each pathogen from the different sites of the respiratory tract. The upper respiratory tract encompasses the nose and throat and the lower respiratory tract consists of the trachea, bronchi, bronchioles, and the alveoli. Sputum is obtained from the bronchi. All three of the major pathogens were isolated mainly from the lower respiratory tract. The miscellaneous organisms seemed fairly evenly distributed between the lower respiratory tract and throat only, being rarely isolated from the nose.

The most commonly isolated organism from the nose only was

S. aureus. This was not unexpected since the nares and skin are the commonest source of this organism in humans.

Analysis showed that the isolates for S. aureus were significantly greater from the lower respiratory tract than the throat ($p < 0.001$). There was no significant difference when the number of isolations was compared for the lower respiratory tract and nose only ($p > 0.05$).

Similarly H. influenzae was also isolated in larger numbers from the lower respiratory tract closely followed by nose only. The differences between the lower respiratory tract and throat and nose and throat are significant ($p < 0.01$).

The distribution of P. aeruginosa was completely different. This organism was almost exclusively isolated from the lower respiratory tract. In the cases where this organism was isolated from the throat only, the patient, PA, did produce sputum but not on every occasion. Therefore, this was a contamination rather than actual colonization. The combined isolations referred to lower respiratory tract and throat only for this organism. On no occasion was P. aeruginosa isolated from the nose. The differences here are highly significant ($p < 0.001$).

Analysis of the total isolations of all organisms at each site show that the differences between lower respiratory tract isolations and those of other respiratory tract sites were significant (lower respiratory tract vs. throat, $p < 0.05$; lower respiratory tract vs. nose, $p < 0.01$), whereas comparing

Table 20

Isolation of pathogenic bacteria from nose swabs of Sputum producers and No-sputum producers.

Organism	Number (%) of Nose Cultures which yielded bacteria	
	Sputum Producers	No-sputum Producers
S. aureus	5/108 (5)	34/165 (21) **
H. influenzae	1/108 (1)	17/165 (10) *
P. aeruginosa	0/108 (0)	0/165 (0)
Miscellaneous	1/108 (1)	12/165 (7) *
Commensals Only	101/108 (94)	129/165 (78) **

* = denotes significant difference ($p < 0.05$)

** = denotes highly significant difference ($p < 0.001$)

nose and throat i.e. upper respiratory tract isolations were not significant ($p > 0.05$).

Thus as would be expected in CF patients, the lungs are the main source of potential pathogens in this condition.

During the period of the trial, as each patient record was compiled, a striking difference emerged between sputum producers and no-sputum producers. This was irrespective of which of the 2 groups they belonged to. The number of nose swabs which were positive for pathogens in no-sputum producers was greater than those of sputum producers. These data were therefore included in a separate table to determine if this difference was significant (Table 20).

Again by far the commonest organism in the nose cultures of no-sputum producers was S. aureus (21 per cent of all cultures). With the exception of P. aeruginosa (which, as previously stated, was not isolated from the nose) the percentage of nose cultures from no-sputum producers which yielded bacteria was significantly greater than cultures from sputum producers ($p < 0.05$). For S. aureus this difference was highly significant ($p < 0.001$).

Since the nose cultures of no-sputum producers yielded a significantly greater number of pathogenic bacteria than those of sputum producers, one might expect the nose cultures of sputum producers to have a higher percentage of cultures which yielded commensals. This was indeed the case, for 94 per cent of nose cultures from sputum producers had commensals, compared to 78 per cent of no-sputum producers. This difference was highly significant ($p < 0.001$).

Table 21

Antibiotic sensitivities of the main pathogens in CF compared with those of the same bacterial species isolated from non-CF patients.

Antibiotic	Number (%) of Organisms Sensitive		Value of p_2 in X^2-test
	CF Patients	Non-CF Patients	
Staphylococcus aureus			
Benzylpenicillin	8/120 (7)	0/32 (0)	> 0.1
Ampicillin	8/120 (7)	0/32 (0)	> 0.1
+ 14 other antibiotics showed no significant difference (see Appendix 3)			
Haemophilus influenzae			
Ampicillin	51/52 (98)	9/15 (60)	< 0.001
+ 15 other antibiotics showed no significant difference (see Appendix 3)			
Pseudomonas aeruginosa			
Carbenicillin	4/66 (6)	2/11 (18)	> 0.1
Tetracycline	31/66 (47)	1/11 (9)	< 0.05
Gentamicin	66/66 (100)	8/11 (73)	< 0.001
+ 10 other antibiotics showed no significant difference (see Appendix 3)			
Pseudomonas aeruginosa (Rough vs Mucoid strain)*			
Cotrimoxazole	0/66 (0)	9/48 (19)	< 0.001
Sulphonamide	4/66 (6)	11/48 (23)	< 0.01
Ampicillin	0/66 (0)	6/48 (13)	< 0.01
+ 10 other antibiotics showed no significant difference (see Appendix 3)			

* = The number (%) of organisms sensitive refer to rough and mucoid strains both from CF patients.

Table 22

Antibiotic sensitivities of pathogens isolated from CF patients compared with those of the same bacterial species from non-CF patients.

Antibiotic	Number (%) of Organisms Sensitive		Value of p in X^2-test
	CF Patients	Non-CF Patients	
Haemophilus parainfluenzae			
Benzylpenicillin	8/10 (80)	3/8 (38)	< 0.05
Cephradine	3/10 (30)	7/8 (88)	< 0.05
+ 14 other antibiotics showed no significant difference (see Appendix 4)			
Escherichia coli			
Ampicillin	16/19 (84)	78/133 (59)	< 0.05
Carbenicillin	17/19 (90)	80/133 (60)	< 0.05
Tetracycline	19/19 (100)	109/133 (82)	< 0.05
Cephradine	16/19 (84)	130/133 (98)	< 0.01
+ 9 other antibiotics showed no significant difference (see Appendix 5)			
Proteus mirabilis			
Cotrimoxazole	16/24 (67)	30/31 (97)	< 0.01
Sulphonamide	10/24 (42)	30/31 (97)	< 0.001
Ampicillin	24/24 (100)	26/31 (84)	< 0.05
Carbenicillin	24/24 (100)	26/31 (84)	< 0.05
Tetracycline	18/24 (75)	1/31 (3)	< 0.001
Cephradine	16/24 (67)	30/31 (97)	< 0.01
+ 7 other antibiotics showed no significant difference (see Appendix 5)			
Streptococcus faecalis			
Cotrimoxazole	21/24 (88)	0/52 (0)	< 0.001
Sulphonamide	22/24 (92)	0/52 (0)	< 0.001
Carbenicillin	9/24 (38)	51/52 (98)	< 0.001
Tetracycline	24/24 (100)	30/52 (58)	< 0.001
Cephradine	24/24 (100)	25/52 (48)	< 0.001
+ 8 other antibiotics showed no significant difference (see Appendix 5).			

Antibiotic Sensitivities

The antibiotic sensitivities of the pathogens isolated from the CF patients were determined using the disk diffusion technique as outlined in the Materials and Methods. The antibiotic sensitivities were compared to those of non-CF patients (Appendices 3, 4, 5).

Tables 21 and 22 summarise the data in the appendices. These tables show the antibiotic sensitivities of the pathogens, which were significantly different, from CF and non-CF patients as detailed below.

The differences in the numbers of isolates of S. aureus from CF and non-CF patients sensitive to the antibiotics tested were not significant ($p > 0.05$). H. influenzae isolated from CF patients was significantly more sensitive to ampicillin than the same organism isolated from non-CF patients ($p < 0.001$).

A two-way comparison was made for P. aeruginosa viz. CF (rough strain) vs. non-CF patients and rough strain vs. mucoid strain (both from CF patients) (Table 21).

The only antibiotics which showed a significant difference between CF and non-CF patients were tetracycline and gentamicin. Of the isolates from CF patients 47 per cent were sensitive to tetracycline compared to 9 per cent from non-CF patients ($p < 0.05$). The corresponding figures for gentamicin were 100 per cent and 73 per cent respectively ($p < 0.001$). However, when the antibiotic sensitivities of the rough and mucoid strains of P. aeruginosa from CF patients were compared the results were noteworthy.

No significant differences emerged for the antibiotics which were largely specific for gram-negative organisms i.e. carbenicillin, gentamicin, tobramycin, neomycin and colistin. The most striking differences were seen in the sensitivity of the mucoid strains to cotrimoxazole, sulphonamide, and ampicillin. None of the rough strains were sensitive to cotrimoxazole compared to 19 per cent of the mucoid strains. Only 6 per cent of the rough strains were sensitive to sulphonamide whereas 23 per cent of the mucoid strains were sensitive. Again none of the rough strains were sensitive to ampicillin compared to 13 per cent of mucoid strains. These differences were highly significant ($p < 0.01$). *P. aeruginosa* can, in some instances, be sensitive to cotrimoxazole and sulphonamide. It is usually resistant to penicillins with the exception of carbenicillin which is a synthetic penicillin specific for gram-negative organisms. Thus, it would appear that the mucoid strain of *P. aeruginosa* is, at least *in vitro*, more sensitive to antibiotics than the rough strain.

No significant differences were apparent between CF and non-CF patients for *H. parahaemolyticus*. Of the isolates of *H. parainfluenzae* 80 per cent from CF patients were sensitive to benzylpenicillin compared to only 38 per cent of non-CF patients. This difference was significant ($p < 0.05$). However, the isolates of *H. parainfluenzae* from CF patients were less sensitive to cephradine (30 per cent) than those from non-CF patients (88 per cent). This difference was also significant ($p < 0.05$).

E. coli from CF patients were significantly more sensitive to ampicillin, carbenicillin, and tetracycline than those from non-CF patients ($p < 0.05$). However, this organism was

Table 23

Distribution of Antibiograms and percentage of patients harbouring Staphylococcus aureus

ANTIBIOGRAM	Percentage of Strains		Percentage of Patients harbouring strains	
	VARIABLE ANTIBIOTIC GROUP (61 strains)	FLUCLOXA-CILLIN GROUP (59 strains)	VARIABLE ANTIBIOTIC GROUP (10 Patients)	FLUCLOXA-CILLIN GROUP (6 Patients)
P PN	77	88	80	67
Sensitive	10	2	20	17
P Pn E	7	7	10	17
P Pn E N	2	0	10	0
P Pn N K	2	0	10	0
P Pn E N K	2	0	10	0
P Pn Te E	0	2	0	17

For explanation of abbreviations see Table 2 (page 19).

Table 24

Distribution of Antibiograms and percentage of patients harbouring Haemophilus influenzae

ANTIBIOGRAMS	VARIABLE ANTIBIOTIC GROUP (31 strains)	FLUCLOXA-CILLIN GROUP (21 strains)	VARIABLE ANTIBIOTIC GROUP (10 Patients)	FLUCLOXA-CILLIN GROUP (6 Patients)
P Ob Ce My	23	0	30	0
Ob Ce My	19	29	50	33
Ob Ce My Fd	16	24	30	17
P Ob Ce My Fd	13	0	20	0
Ob My Fd	10	19	30	17
P Ob My Fd	0	10	0	17
Ob My	0	10	0	17

For explanation of abbreviations see Table 2 (page19).

Table 25

Distribution of Antibiograms and percentage of patients harbouring Pseudomonas aeruginosa (Rough strain)

ANTIBIOGRAM	Percentage of Strains		Percentage of Patients harbouring strains	
	VARIABLE ANTIBIOTIC GROUP (58strains)	FLUCLOXA-CILLIN GROUP (8strains)	VARIABLE ANTIBIOTIC GROUP (10 Patients)	FLUCLOXA-CILLIN GROUP (6 Patients)
Sxt Sf Pn Py Te Kz Cr Ce K	36	38	30	17
Sxt Sf Pn Py Kz Cr Ce K	26	50	40	17
Sxt Sf Pn Py Te Kz Cr Ce N K	12	0	20	0
Sxt Sf Pn Py Kz Cr Ce Ct K	7	0	20	0
Sxt Pn Py Kz Cr Ce K	7	0	10	0
Sxt Sf Pn Kz Cr Ce K	5	0	30	0
Sxt Sf Pn Py Te Kz Cr Ce	3	13	20	17

For explanation of abbreviations see Table 2 (page 19).

Table 26

Distribution of Antibiograms and percentage of patients harbouring Pseudomonas aeruginosa (Mucoid strain)

ANTIBIOGRAM	Percentage of Strains		Percentage of Patients harbouring strains	
	VARIABLE ANTIBIOTIC GROUP (47strains)	FLUCLOXA-CILLIN GROUP (1strain)	VARIABLE ANTIBIOTIC GROUP (10Patients)	FLUCLOXA-CILLIN GROUP (6Patients)
Sxt Sf Pn Py Te Kz Cr Ce K	45	0	20	0
Sxt Sf Pn Py Kz Cr Ce K	17	0	20	0
Sxt Sf Pn Py Te Kz Cr Ce	6	100	10	17
Sxt Sf Pn Py Te Kz Cr Ce N K	6	0	10	0
Kz Cr Ce	6	0	10	0
Pn Py Kz Cr Ce K	6	0	10	0
Kz Ce	4	0	10	0

For explanation of abbreviations see Table 2 (page 19).

Table 27

Distribution of Antibiograms and percentage of patients harbouring Haemophilus parainfluenzae

ANTIBIOGRAM	Percentage of Strains		Percentage of Patients harbouring strains	
	VARIABLE ANTIBIOTIC GROUP (7 strains)	FLUCLOXACILLIN GROUP (3 strains)	VARIABLE ANTIBIOTIC GROUP (10 Patients)	FLUCLOXACILLIN GROUP (6 Patients)
Ob Ce My Fd	29	0	20	0
P Ob Ce My Fd	29	0	20	0
Sf Ob Ce My	14	0	10	0
Ob Ce My	14	33	10	17
Ob My Fd	14	67	10	33

For explanation of abbreviations see Table 2 (page 19).

Table 28

Distribution of Antibiograms and percentage of patients harbouring Streptococcus faecalis

ANTIBIOGRAM	Percentage of Strains		Percentage of Patients harbouring strains	
	VARIABLE ANTIBIOTIC GROUP (23strains)	FLUCLOXACILLIN GROUP (1strain)	VARIABLE ANTIBIOTIC GROUP (10Patients)	FLUCLOXACILLIN GROUP (6Patients)
Py Ct	43	100	50	17
Ct	30	0	50	0
Sxt Py Ct	9	0	10	0
Sxt Ct	4	0	10	0

For explanation of abbreviations see Table 2 (page 19).

Table 29

Distribution of Antibiograms and percentage of patients harbouring Proteus mirabilis

ANTIBIOGRAM	Percentage of Strains		Percentage of Patients harbouring strains	
	VARIABLE ANTIBIOTIC GROUP (5 strains)	FLUCLOXACILLIN GROUP (19 strains)	VARIABLE ANTIBIOTIC GROUP (10 Patients)	FLUCLOXACILLIN GROUP (6 Patients)
Sf Ct	40	26	10	33
Sxt Ct	20	5	10	17
Ct	0	26	0	17
Sxt Sf Ce Ct	0	11	0	17
Sxt Sf Te Ce Ct	0	16	0	17

For explanation of abbreviations see Table 2 (page 19).

Table 30

Distribution of Antibiograms and percentage of patients harbouring Escherichia coli

ANTIBIOGRAM	Percentage of Strains		Percentage of Patients harbouring strains	
	VARIABLE ANTIBIOTIC GROUP (13 strains)	FLUCLOXACILLIN GROUP (6 strains)	VARIABLE ANTIBIOTIC GROUP (10 Patients)	FLUCLOXACILLIN GROUP (6 Patients)
Sensitive	46	100	20	17
Ce	15	0	10	0
Sf Pn Py	8	0	10	0
Sf Pn	8	0	10	0
Pn Py K	8	0	10	0

For explanation of abbreviations see Table 2 (page 19).

significantly more sensitive to cephradine when isolated from non-CF patients (98 per cent) than CF patients (84 per cent) ($p < 0.01$).

The isolates of Pr. mirabilis from CF patients were more sensitive to ampicillin, carbenicillin, and tetracycline ($p < 0.05$). Those from non-CF patients were more sensitive to cotrimoxazole, sulphonamide, and cephradine ($p < 0.01$). The comparative resistance of CF isolates to cotrimoxazole, sulphonamide, and cephradine were due to one patient (AT). This patient had chronic carriage of Pr. mirabilis. His particular strain of Pr. mirabilis rapidly became resistant to these antibiotics. The resistance was not lost during the period of the trial.

St. faecalis from CF patients were significantly more sensitive to cotrimoxazole, sulphonamide, and cephradine ($p < 0.001$) whereas those from non-CF patients were significantly more sensitive to carbenicillin ($p < 0.001$).

Antibiograms of Pathogenic Bacteria

Antibiograms of every pathogen were compiled for each group of CF patients. This made the detection of specific resistances easier, especially if they were frequently found in one group of patients or one particular patient within that group.

The most frequently found antibiograms for the pathogens are listed in Tables 23, 24, 25, 26, 27, 28, 29 and 30. Statistical analysis showed only Table 27 with a significant difference. The number of strains of the Flucloxacillin group with the "sensitive" antibiogram was significantly greater than those in the Variable Antibiotic group ($p < 0.05$).

Table 31

Number of Antibiograms for each pathogen in the two groups of CF patients.

Organism	Number of Antibiograms in each group per total number of Antibiograms	
	VARIABLE ANTIBIOTIC GROUP	FLUCLOXACILLIN GROUP
S. aureus	7/9	5/9
H. influenzae	11/15	8/15
P. aeruginosa	9/15	3/15 *
H. parahaemolyticus	3/3	1/3
H. parainfluenzae	5/5	2/5
St. faecalis	7/7	1/7 *
P. mirabilis	4/10	8/10
E. coli	7/7	1/7

* = denotes significant difference ($p < 0.05$)

Fig. 7. Phage-type of S. aureus isolated from CF patients for which phage-typing results were available (60 per cent of S. aureus).

NT = Not typable; w = weak reaction;

+ = some additional reactions also present;

N = nose swab; T = throat swab; S = sputum;

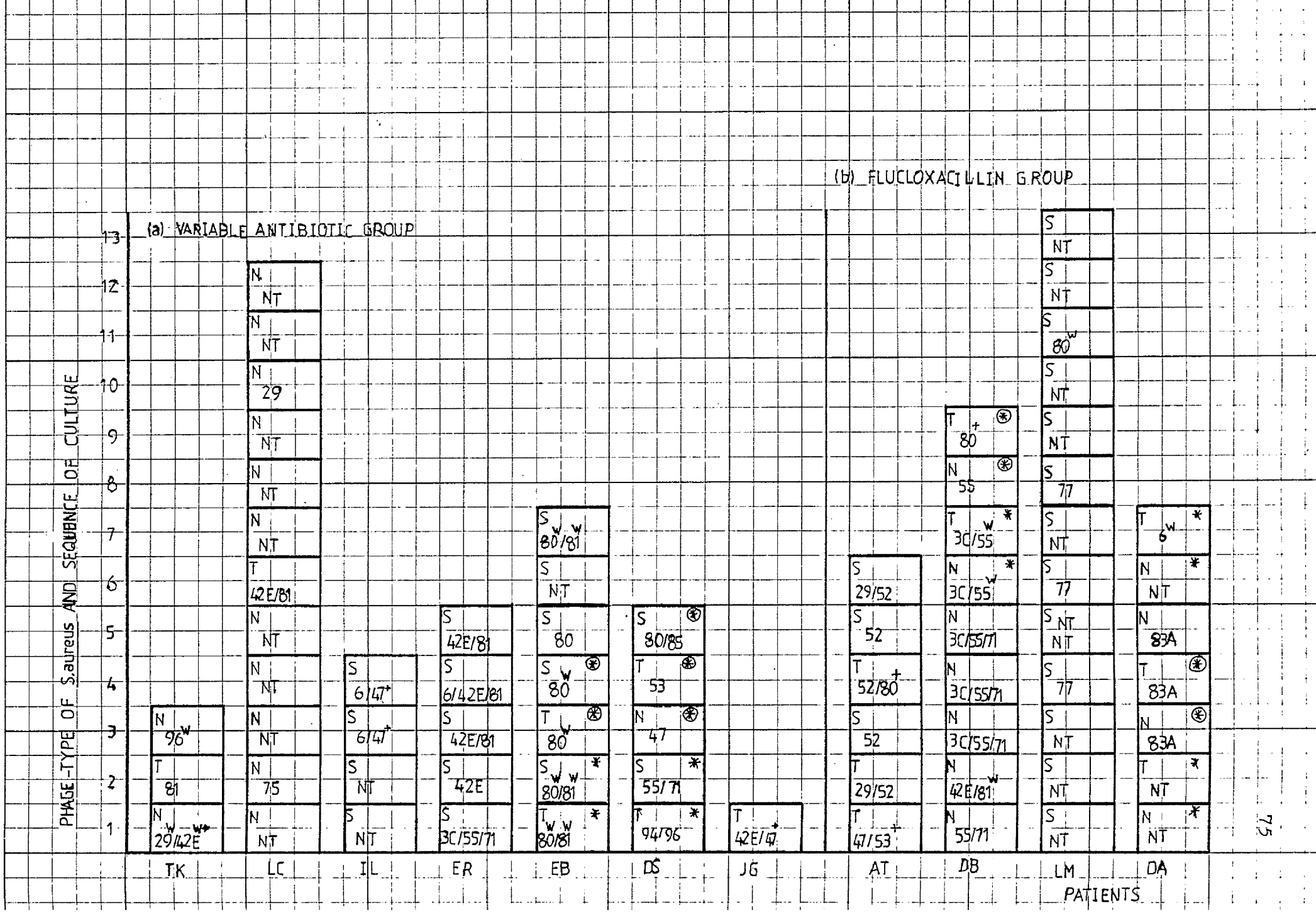
* or ⊛ specimens taken on same day

Phage-types in full :- JG1 = 42E/47/54/75/81;

IL3 and IL4 = $6/47/54/75/88/81^{+}$;

AT1 = 47/53/54/75/77/81;

TK1 = $29^{W}/42E^{W}/75^{W}/81^{W}$.


(a) VARIABLE ANTIBIOTIC GROUP
(b) FLUCLOXACILLIN GROUP
PHAGE-TYPE OF S.aureus AND SEQUENCE OF CULTURE
13
12
11
10
9
8
7
6
5
4
3
2
1
TK
LC
IL
ER
EB
DS
JG
AT
DB
LM
DA
PATIENTS

The total number of antibiograms in each group were compared for significant differences (Table 31). The Variable Antibiotic group had significantly more antibiograms for P. aeruginosa St. faecalis, and E. coli than the Flucloxacillin group ($p < 0.05$).

Although St. faecalis may appear to have greater antibiotic variation in patients in the Variable Antibiotic group it should be noted that this organism was almost uniquely isolated from this group. Only specimens from YH of the Flucloxacillin group yielded St. faecalis and on only one occasion.

The same can be said of P. aeruginosa. This organism was only isolated on nine occasions from the Flucloxacillin group and from 2 patients (PA, AT), whereas the 105 isolates of P. aeruginosa from the Variable Antibiotic group were from 4 patients (DS, ER, EB, IL).

Phage Typing of Staphylococci

Phage Types of S. aureus from CF and non-CF Patients

In order to determine whether a patient was colonized with the same strain of S. aureus at all sites of the respiratory tract the S. aureus were phage-typed as well as being tested for antibiotic sensitivity. Approximately 60 per cent of all S. aureus isolated were phage-typed.

Figure 7 shows both the phage-type and sequence of isolation of S. aureus from the 2 groups of patients under investigation during the 18 months of the trial. The organism, itself, was largely isolated from sputum and nose swabs.

The phage-type of S. aureus, isolated from more than one

site of the respiratory tract from specimens taken on the same day, from the same individual were usually identical. Of the specimens whose S. aureus were phage-typed only 44 per cent yielded S. aureus of different phage-types. DS was the only patient whose specimens taken on the same day yielded S. aureus from all three sites i.e. nose, throat, and lower respiratory tract. The antibiotic sensitivity pattern for all three organisms was identical but the phage-types were different, although all belonged to phage-group III.

There was no evidence to suggest that there was a link between antibiotic sensitivity and phage-type. The only exception to this was patient IL. On 2 occasions S. aureus grown from his sputum specimens gave phage reactions in group III (6/47/54/75/77/88/81^{+}). This particular phage group has been shown to be common in S. aureus which carry the plasmid for resistance to kanamycin and neomycin (Lacey, 1971a). This is a linked resistance. The S. aureus from IL were resistant to these antibiotics.

It can also be seen from figure 7 that most of the patients were colonized by S. aureus belonging to one group e.g. LC and LM:- both with non-typable phage; EB:- phage of the 80/81 complex; ER:- group III and miscellaneous; AT:-group I; DA:- 83A and non-typable; DB:- group II especially the 55/71 complex.

This pattern of phage-typing was compared to that of non-CF patients who were also chronic carriers of S. aureus. The best example of this from the hospital records were patients in Ward 1C (Renal Unit). These patients were the only ones who,

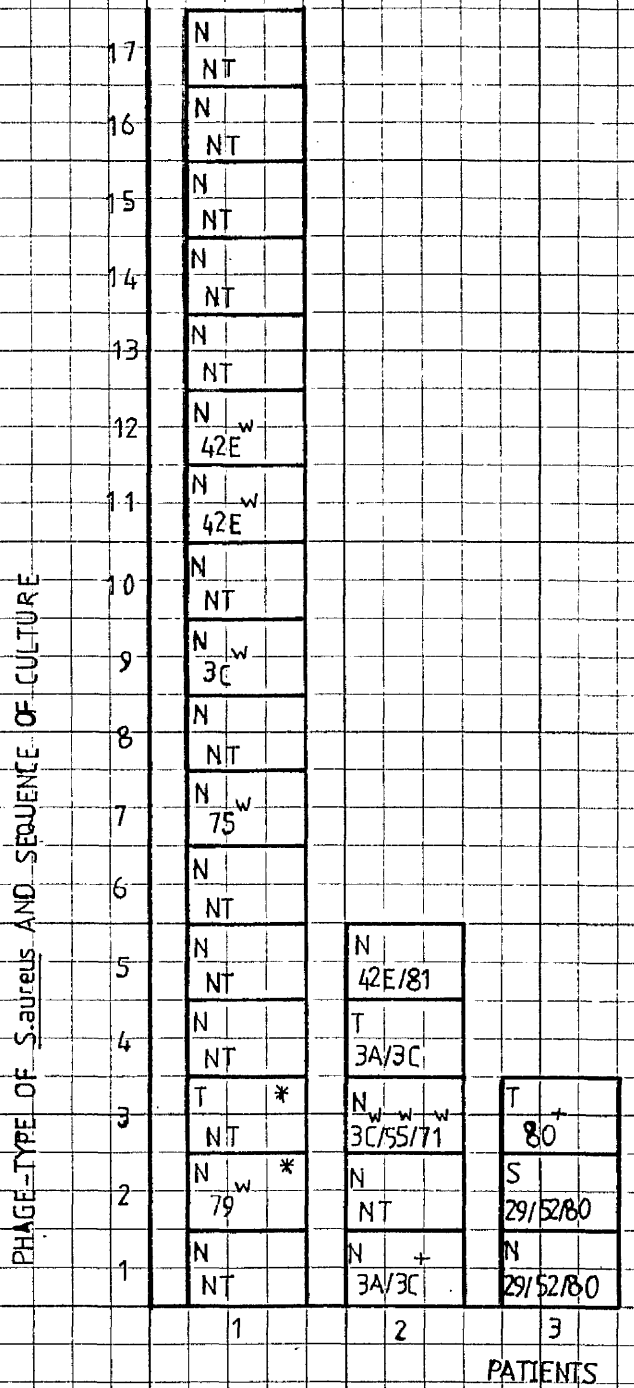
Table 32

Phage groups of *S. aureus* in nose and throat swabs and sputum cultures from CF and non-CF Patients.

S. aureus Phage Group	Special Features of Phage Group	Number (%) of Strains CF Patients	Non-CF Patients (Chronic Carriage)	Non-CF Patients (Randomly Selected)
I	Some strains cause widespread outbreaks of severe sepsis in hospitals	11/72 (16)	4/25 (16)	24/100 (24)
II	Some strains cause vesicular and exfoliative skin lesions	9/72 (13)	4/25 (16)	4/100 (4)
III	Cause enterotoxic food-poisoning	11/72 (16)	3/25 (12)	17/100 (17)
IV	Rarely of human origin	0/72 (0)	0/25 (0)	0/100 (0)
I + III		1/72 (1)	0/25 (0)	4/100 (4)
II + III		0/72 (0)	0/25 (0)	1/100 (1)
I + III + M		1/72 (1)	0/25 (0)	1/100 (1)
I + M		3/72 (4)	0/25 (0)	5/100 (5)
III + M		9/72 (13)	1/25 (4)	6/100 (6)
M		3/72 (6)	0/25 (0)	18/100 (18)
N.T.	Not uncommon in patients receiving Prophylaxis	25/72 (35)	13/25 (52)	20/100 (20)

Group I - 29, 52, 52A, 79, 80; Group II - 3A, 3C, 55, 71; Group III - 6, 42E, 47, 53, 54, 75, 77, 83A, 85; M (Miscellaneous) - 81, 88, 94, 96; N.T. - Not typable.

Fig. 8. Phage-type of S. aureus from non-CF patients with chronic carriage (renal patients)
Legend as for Fig. 7.
Phage-type in full :- 2(1) = 3A/3C/55/71



like CF patients, had regular specimens taken from the upper respiratory tract. Only 3 of these patients had chronic carriage of S. aureus (Figure 8). These 3 patients each appear to be colonized with S. aureus of predominantly one group. Patient 1:- non-typable; patient 2:- group II; and patient 3:- group I. This is similar to CF patients.

The phage-types for both the CF patients and non-CF patients (chronic carriage) were arranged into their phage groups and compared with those of 100 randomly selected non-CF patients who did not have a chronic respiratory infection. (Table 32).

Non-typable strains of S. aureus were the most frequently isolated from CF patients (33 per cent of the specimens). This is a not uncommon finding in patients receiving prophylaxis. Non-typable strains were also the most frequently isolated from the non-CF patients (chronic carriage). These patients would also be receiving prophylaxis.

When all three categories of patients were compared the following emerges :

There was no significant difference in the number of S. aureus belonging to each phage-group between CF and non-CF patients (chronic carriage) ($p > 0.05$ in all cases). However, when the CF patients and non-CF patients (chronic carriage) were compared to non-CF patients (randomly selected) significant differences were found. These occurred in the same 3 phage groups. Thus for CF vs. non-CF patients (randomly selected) the numbers of S. aureus belonging to phage groups II and non-typable phage were significantly greater in CF patients than non-CF ($p < 0.05$). The comparison of non-CF patients (chronic

carriage) and non-CF patients (randomly selected) also showed significant differences for the same phage groups ($p < 0.05$). No individual phage group was found to predominate.

DISCUSSION

Clinical Assessment

The clinical progress of the Variable Antibiotic group (who received only 1 antibiotic, changed as required) and the Flucloxacillin group (who received anti-staphylococcal prophylaxis plus an additional antibiotic for 10 days, if required) was assessed.

The clinical data revealed no overall significant difference between the two groups. All of the patients gained weight and height during the 18 months of the investigation, with the exception of JG (Variable Antibiotic group) and LM (Flucloxacillin group), both of whom experienced weight loss, the more serious being that of LM.

Despite the increases in weight and height, however, only 2 patients, AT and YH of the Flucloxacillin group showed a marked improvement. The weight of AT was increased from lying between the 3rd and 10th centiles to between the 10th and 25th centiles. The height of YH increased from lying between the 10th and 25th centiles to between the 25th and 50th centiles.

Patient LM whose weight decreased by 2Kg during the course of the investigation remained in the severely underweight category. According to Kraemer et al., (1978) patients in this category have the poorest prognosis for survival, and indeed this patient died during the investigation.

Although no difference was apparent for weight and height between the 2 groups of patients, differences were observed between the sexes. The greatest difference was seen in the weight charts of the male and female patients. The weight of the females was retarded more than that of the males after 10

years of age. Usually, at this age the pre-pubertal growth spurt would be expected to commence in females.

The striking fall in weight after the age of 10 years was also observed by Gurivitz et al., (1979). Of their female patients 67 per cent fell below the 50th centile for weight compared to 50 per cent of their male patients. They commented that this drop in weight corresponded to a deterioration in pulmonary function. They also suggested that this may explain why the survival rate for males in CF is greater than that of females. Thus the data in this investigation appear to support the hypothesis of these two teams of workers that exacerbation of pulmonary disease is associated with weight loss which in turn results in poor survival.

Pulmonary Changes

The broncho-pulmonary aspects of the disease did not alter radically in either group during the investigation. This applied to changes evident both in physical and radiological examinations.

Hospital Admissions

The mean rate of hospital admissions decreased in both groups during the investigation, thus indicating an improved status. This was seen even in patients with advanced pulmonary involvement viz. DS and AT. Both of these patients had only one admission during the investigation compared to four before the investigation.

Thus both regimens of therapy appear to have a significant

beneficial effect on the rate of hospital admissions. Clearly this is useful both in terms of reduced cost to the health service and for the general well-being of the child.

Support for the success of these regimens is evident from data received after the work of this investigation had ceased. Patient DS was admitted three times in the 5 months after this investigation whereas patient AT had no further admissions in the same period. This was probably due, in part, to the fact that patient DS returned to the previous state of specimens for culture being taken only at 6 monthly intervals. The parents of patient AT, with the aid of their General Practitioner, continued the monthly cultures.

Bacteriological Assessment

S. aureus was long believed to be the etiological agent in bronchopneumonia in CF patients (di Sant 'Agnese and Andersen, 1946). This organism was also regarded as being the principal cause of lung damage in CF (Lawson, 1970). Recently, however, the pathogenic role of *P. aeruginosa* and *H. influenzae* have also been considered in CF (May *et al.*, 1972; Mearns *et al.*, 1972; Høiby, 1974a; McCrae and Raeburn, 1974).

Assessing both groups of patients collectively in this investigation, the results showed that *S. aureus* was the most frequently isolated organism with 75 per cent of patients harbouring it. It was followed by *H. influenzae* (69 per cent) and *P. aeruginosa* (38 per cent). *S. aureus* and *P. aeruginosa* were isolated with equal frequency (86 per cent) from patients with advanced pulmonary infection (sputum producers). *S. aureus*

and H. influenzae were isolated with equal frequency (67 per cent) from no-sputum producers. Thus for this cohort of patients, although S. aureus is the most frequently isolated organism overall, in patients with advanced pulmonary disease this position is shared with P. aeruginosa. The order of ranking the main pathogens associated with CF in this investigation was the same as that found by May et al., (1972). Høiby (1974a) also found S. aureus was the predominating organism. 90 per cent of his patients harboured S. aureus compared to 64 per cent with P. aeruginosa and 37 per cent with H. influenzae.

However, Mearns et al., (1972) found that the prevalence of S. aureus in their patients decreased with a concomitant rise in P. aeruginosa. This pattern occurred in all age groups and irrespective of the severity of lung disease.

McCrae and Raeburn (1974) did not isolate S. aureus from any of their CF patients. Their investigation assessed 35 CF patients attending the Royal Hospital for Sick Children, Edinburgh Sputum was obtained from 13 patients. The age range of the patients was 1-13 years (median age 6.2 years). They found H. influenzae was the predominant pathogen which was replaced in older patients by P. aeruginosa.

Perhaps if this investigation had continued for a further 18 months, P. aeruginosa may also have emerged here as the predominant pathogen, especially in the sputum producers.

Despite the predominance of S. aureus in CF, Høiby (1974a) and McCrae and Raeburn (1974) believe that P. aeruginosa is the principal threat to patients with CF since satisfactory control of infection by this organism is not possible.

The absence of P. aeruginosa from the no-sputum producers appears to confirm the predilection of P. aeruginosa for the CF lung. P. aeruginosa was not isolated from the nose-swabs of any of the CF patients, even those who harboured the organism in both throat and sputum. This agrees with the findings of Laraya-Cussay et al., (1976).

The ability of other organisms to establish themselves in the CF lung was also noted e.g. Pr. mirabilis and E. coli. This seems to lend support to the theory that CF children are susceptible from birth to infection of the lower respiratory tract by any potential pathogen to which they may be exposed (May et al., 1972). Thus chemoprophylaxis directed towards the prevention of staphylococcal infection may, in fact, be contributing to the increase in other infections in CF viz. P. aeruginosa.

The Effect of Therapy on Pulmonary Infection.

The results indicated that the problem of obtaining alternative specimens to sputum, which is the specimen of choice in CF, could be circumvented by culturing a throat swab from patients who were either too young to cough-up sputum or could not produce sputum. Iacocca et al., (1963) also concluded that where sputum was unobtainable from CF patients throat swabs were an acceptable substitute.

In this investigation also, S. aureus and P. aeruginosa were frequently cultured simultaneously from the throat and sputum. This was probably due to the throat being contaminated by sputum during the coughing which occurs in the CF patient.

Nose swabs in sputum producers were of little value since they reflected neither the flora of the throat nor sputum. However, in no-sputum producers the same organism was frequently isolated from both nose and throat. This was especially true of S. aureus.

The results of the bacterial flora present in both groups at the beginning and end of the investigation indicated that the therapeutic regimen of the Flucloxacillin group seemed to be more successful in the treatment of S. aureus and H. influenzae However, this was only by comparison with non-CF patients and not to the other CF patients in the Variable Antibiotic group.

Thus the treatment of the Flucloxacillin group succeeded in that the specimens taken from this group at the end of the investigation yielded the main bacterial species with a similar frequency to that of non-CF patients.

The failure of antibiotic therapy to eradicate P. aeruginosa is well documented in the literature (Hoff et al., 1974; Shwachman et al., 1975). However, both regimens appeared to keep P. aeruginosa in check since only 1 patient (AT) of the 6 who harboured the organism was admitted to hospital for therapy. Pr. mirabilis was also implicated in the pulmonary infection of this patient.

One fact which did emerge was that antibiotic therapy must be maintained for at least 10 days. The original five patients (TK, AL, IL, DS, YH) were visited weekly and consequently frequently had their antibiotic therapy changed weekly. Recurring infection by the organism being treated was frequent.

When the number of patients being treated rose to the final

total of 16, visits were made monthly. Therapy was therefore maintained for 4 weeks in the Variable Antibiotic group and 10 days in the Flucloxacillin group. Recurring infection rarely occurred. Maintenance of therapy for 2-3 weeks has been advocated by Wood *et al.*, (1976).

The Role of the Mucoid Strain of *P. aeruginosa* in CF

P. aeruginosa is frequently isolated from CF patients in both a non-mucoid and mucoid form - the latter form is peculiar to CF. The ability of *P. aeruginosa* to establish itself in the CF lung is unique since it is rarely a pathogen in other chronic pulmonary diseases.

It has been impossible to comment in this investigation on the proposed order of infection in CF patients (Burns and May, 1968; Kilbourn, 1978), since none of the patients were newly-diagnosed cases. However, the author was fortunate in observing initial colonization by *P. aeruginosa* in 4 of the patients (IL, EB, DS, AT). All of these had established pulmonary infection with other bacteria and were sputum producers.

The non-mucoid or rough strain of *P. aeruginosa* appeared first in every patient. This is in accordance with the findings of Doggett *et al.*, (1966) who observed that a non-mucoid strain of *P. aeruginosa* always preceeded the mucoid form. Two of the patients (IL and DS) became colonized with the mucoid form between 1 and 6 weeks after receiving aminoglycoside therapy (gentamicin). This had raised the possibility that the slime envelope may have been produced *in vivo* in response to aminoglycoside therapy. However, the mucoid strain was isolated

from AT who had not been given aminoglycoside therapy. Only flucloxacillin had been given to this patient. EB did not receive aminoglycoside therapy since the sputum culture yielded only a light growth of P. aeruginosa and it was decided not to treat this particular infection in order to observe if the mucoid strain emerged without an aminoglycoside being administered. This patient, therefore, was only prescribed cloxacillin and septrin. After harbouring P. aeruginosa for 3 months no mucoid strains were cultured from his sputum.

One of the reasons proposed for the frequency of mucoid strains in the respiratory tract of CF patients was that they were selected by means of antibacterial chemotherapy (Iacocca et al., 1963; Govan, 1976).

This was thought unlikely by Doggett et al., (1966) since they reported that mucoid strains were also seen in patients who had not received any antibiotic therapy.

Recently, however, Govan and Fyfe (1978) have shown that mucoid strains can be isolated in vitro by selection with antibiotics. The antibiotics which these workers used were tobramycin (an aminoglycoside), carbenicillin and flucloxacillin. In light of both these findings and those of this investigation, it seems very probable that antibiotic therapy plays a part in the selection of the mucoid strain of P. aeruginosa in pulmonary infection in CF.

Despite the fact that antimicrobial therapy only kept P. aeruginosa in check, and did not eradicate the organism, it was observed that the quantity of slime envelope surrounding each colony in vitro was considerably reduced following therapy with

gentamicin or tobramycin. Therefore treating *P. aeruginosa* specifically is not entirely to no avail. Reducing the amount of slime produced by the organism is probably beneficial to the patient in that the slime plus the viscous mucus produced by the CF lung must contribute to the blocking of the bronchioles.

Antibiotic Sensitivity of pathogens in CF

The comparison of the antibiotic sensitivities of the bacterial pathogens between CF and non-CF patients produced unexpected results. Many of the pathogens isolated from CF patients were more susceptible to some antibiotics than those isolated from non-CF patients. This increased sensitivity has not been previously reported with the exception of *P. aeruginosa*. The previous comparison for *P. aeruginosa* was between the rough and mucoid strains of this organism which were both isolated from CF patients. No comparison has been made between CF and non-CF patients. In this investigation increased sensitivity was seen in the following organisms:- *H. influenzae*, *P. aeruginosa*, *H. parainfluenzae*, *E. coli*, *Pr. mirabilis* and *St. faecalis*.

Two recent reports have commented on the antibiotic susceptibility of the mucoid and rough strains of *P. aeruginosa* isolated from CF patients (Govan and Fyfe, 1978; Thomassen *et al*. (1979). Govan and Fyfe found the mucoid strains were more resistant to carbenicillin, flucloxacillin, and tobramycin than the rough strains but more sensitive to tetracycline. Thomassen *et al*., obtained slightly different results. They reported that both strains were resistant to tetracycline and kanamycin. There was a higher percentage of mucoid strains sensitive to

gentamicin, carbenicillin, and tobramycin than rough strains.

In this investigation no difference was found in the susceptibility of either strain to tetracycline, gentamicin, tobramycin, and carbenicillin. Both strains were sensitive, although the zone of inhibition surrounding the antibiotic disk was usually larger for the mucoid strains than the rough strains. The mucoid strains were also more sensitive to cotrimoxazole and sulphonamide than the rough strains. Neither strain was sensitive to kanamycin. These results appear to be more in agreement with those of Thomassen and co-workers. This may be due to the technique employed. This investigation and that of Thomassen used the disk diffusion technique whereas Govan and Fyfe used the more sensitive single cell technique.

The variation in reports on the sensitivity of rough and mucoid strains of P. aeruginosa suggest that at all times antibiotic sensitivity tests should be performed before choosing the antibiotic for treatment.

The sensitivity of P. aeruginosa to cotrimoxazole and sulphonamide by some mucoid strains (notably those from DS) was exploited in order to determine if septrin was an acceptable alternative to gentamicin or tobramycin. This would have provided an oral antibiotic for this organism rather than a course of intramuscular injections. However, although the organism was sensitive in vitro, septrin was ineffective in vivo. The failure of septrin to act in vivo is probably due to a permeability barrier in the lungs. One of the components of septrin, sulphamethoxazole, does not penetrate well into the bronchial secretions (Reeves, 1971).

Permeability may also explain the differences between CF and non-CF patients in antibiotic susceptibility of the other pathogenic bacteria. The pathogens from CF patients may be so protected by the mucus in the CF lung and the great difficulty of achieving the minimal inhibitory concentration of the antibiotic in the lung that, *in vitro*, deprived of this armour, the organisms are more sensitive to antibiotics. Thus they are not subject to selection pressure *in vivo*.

H. influenzae from CF patients only differed in their sensitivity to ampicillin from that of non-CF patients. Here it is the isolates of the non-CF patients which are deviating from the expected. These findings for *H. influenzae* are similar to those of Cargill and Saleh (1974). They reported that none of the strains of *H. influenzae* isolated from purulent sputum specimens were resistant to ampicillin.

S. aureus showed no variability between CF and non-CF patients in sensitivity to the antibiotics tested. All strains of *S. aureus* were sensitive to cloxacillin. Since this antibiotic has been given to the CF patients in this investigatio almost from the time of diagnosis, and with little respite, it is perhaps surprising that resistance did not develop.

Cloxacillin is a member of the penicillinase-resistant penicillins, the first of which was methicillin. Methicillin resistance is not as common as would be expected. Perhaps the lack of cloxacillin-resistant strains of *S. aureus* is due to similar reasons as that for methicillin. Lacey (1972) reported that although the genes responsible for methicillin resistance were plasmid-borne, they were transduced only at low frequency

and to a narrow range of recipients. Transfer did not occur in mixed cultures. He therefore concluded that methicillin-resistant staphylococci probably arose from a single clone. It has also been reported (Cruickshank et al., 1973) that methicillin-resistant strains do not destroy methicillin or cloxacillin (as penicillin-resistant strains destroy penicillin) and are capable of growth in the presence of low therapeutic doses of methicillin and cloxacillin. Thus the absence of cloxacillin-resistance in CF patients is still to be explained.

Antibiotic Resistance Patterns in CF

Most of the reports on resistance patterns (and sensitivity patterns) found in CF pathogens have been confined to those of S. aureus (Huang et al., 1961; Huang and Sheng, 1963; Hoff and Høiby, 1975).

Antibiograms which were compiled for all the pathogens isolated from the CF patients showed that the number of resistance patterns for each pathogen did not depend solely on the actual number of times the pathogen was cultured from each group.

P. aeruginosa was isolated on 9 occasions from the Flucloxacillin group and from 2 patients, whereas the 105 isolates in the Variable Antibiotic group were from 4 patients. However, there were 13 times more isolates of H. parahaemolyticus from the Variable Antibiotic group than the Flucloxacillin group, yet there were only 3 different antibiograms. Also, for S. aureus, there was a total of 120 isolates with only 9 antibiograms.

Clearly the number of organisms isolated does not determine the number of antibiograms. The possibility of genetic transfer of resistance and the effect of long term antibiotic therapy should be considered.

The antibiograms found in the isolates from CF patients were compared with those of non-CF patients in order to determine if resistance to certain antibiotics was linked and perhaps commonly found in the local population. However, there was little evidence to support this idea, especially amongst the gram-negative pathogens. The antibiograms for these organisms tended to be unique for the patient who harboured the organism. The gram-negative pathogens (P. aeruginosa, Pr. mirabilis, E. coli) showed evidence of cross-resistance i.e. bacteria resistant to one member of a group of antibiotics are usually resistant to the others, especially if the chemical composition is very similar.

Pr. mirabilis isolated from patient AT exhibited very well how resistance arises from the use of antibiotics. This organism was originally only resistant to colistin. Following therapy with septrin it rapidly developed resistance to sulphonamide and trimethoprim : (the components of septrin). Cephradine was then substituted for septrin. Resistance to cephradine developed following 3 months continuous therapy with this antibiotic. It seems highly probable that either R-factors or transduction were responsible for the acquisition of resistance to these drugs.

S. aureus showed evidence of linked resistances. Of the S. aureus isolated 92 per cent were resistant to penicillin. As the CF patients were regularly in contact with the hospital environment, resistance to penicillin was expected. Resistance is due to the production of β-lactamase. Since ampicillin is also susceptible to this enzyme, the organism was assumed to be resistant to this antibiotic if it was also resistant to penicillin since it too is susceptible to the action of β-lactamase. This was irrespective of the zone of inhibition surrounding the ampicillin disk.

Resistance to penicillin, and in many instances, antibiotic resistance in staphylococci is due to the acquisition or presence of plasmids (Novick and Bouanchaud, 1971). Plasmids are also responsible for resistance to tetracycline, erythromycin, neomycin, and fusidic acid (Lacey, 1971a; Lacey, 1971b; Lacey and Grinsted, 1972; Lacey and Rosdahl, 1974).

Linked resistances for some antibiotics have been reported. These are penicillin and erythromycin (Mitsuhashi et al., 1965); and neomycin, kanamycin, and erythromycin (Annear and Grubb,1972).

In this investigation linked-resistance to neomycin, kanamycin, and erythromycin in the same antibiogram occurred in 3 per cent of all strains isolated, as was resistance to neomycin and kanamycin, and erythromycin and neomycin. All of these strains were also resistant to penicillin and ampicillin. Linked resistance to penicillin and erythromycin was encountered in 7 per cent of isolates.

No information could be found in the literature of antibiotic resistance patterns for the haemophilus species, including

H. influenzae. Resistance in these organisms may be due in part to natural resistance e.g. cloxacillin. All haemophili are resistant to this antibiotic.

If the data from the sensitivity and resistance patterns are compared with the original pre-designed sequence of antibiotics (Table 3) for treating S. aureus, H. influenzae and P. aeruginosa, it can be seen that the order of choice was correct. The pre-designed sequence could therefore be used with reasonable confidence in choosing an appropriate antibiotic for treatment before the full results of the antibiotic sensitivity tests are available.

Phage-types of S. aureus from CF patients

Previous authors had stated that the S. aureus of the 80/81 complex were more frequently isolated from CF patients than non-CF patients with chronic respiratory infections (Huang and Sheng, 1963). There was no evidence of greater frequency of this phage-type in this investigation. Only patient EB was colonized with S. aureus of the 80/81 complex.

This is in agreement with other authors who found no predominant phage pattern for patients with CF (Iacocca et al., 1963; Hoff and Høiby, 1975). However, Hoff and Høiby reported that S. aureus of the "epidemic complexes" 80 and 83A were isolated in significant numbers. But this was not found in the present investigation.

Although predominance of S. aureus of one particular phage-type was not found, persistance of S. aureus of the same phage-group was observed in CF patients. This was also found by

Iacocca et al., (1963). Most of the patients were colonized by S. aureus belonging to one phage-group or of a particular phage-type within that group. This pattern was also seen in the non-CF patients (chronic carriage). The CF patients who were colonized by S. aureus belonging to one phage-group were persistant carriers of the organism (LC, EB, AT, DB, LM, DA).

This persistance of one phage-group may be due to the phenomenon of bacterial interference between strains of S. aureus (Shinefield et al., 1974). These workers found that super-infection by other strains of S. aureus was not established in patients who were carriers of S. aureus. Shinefield and co-workers also found that interference between strains of S. aureus was site-specific i.e. throat or nose.

It was postulated at the start of this investigation that as the nares are the most common source of S. aureus in humans the lungs of CF patients may become infected with their own strain of organism i.e. could the S. aureus progress from the nares → throat → sputum ? Only patient DS yielded S. aureus from all three sites in specimens taken on the same day. Although the S. aureus belonged to the same phage-group (III), the phage-types were all different. Thus there was no evidence to support the migration hypothesis.

Patients who could produce sputum also rarely yielded S. aureus in their nose or throat swabs. If this organism was isolated it was from the sputum only. Thus the upper respiratory tract does not appear to be the source of S. aureus in pulmonary infection in the CF patient. The frequency of isolation of non-typable strains (33 per cent) was that expected in patients

receiving antibiotic therapy (Anderson and Williams, 1956).

Although no predominant phage-types were found, a significar difference was found in the number of strains belonging to phage-group II in both CF patients and non-CF patients (chronic carriage) compared to those of non-CF patients (randomly selected). This phage-group contains S. aureus which cause vesicular and exfoliative skin lesions.

No correlation between phage-type and antibiotic sensitivity pattern was found.

Conclusions

Thus it can be concluded that monthly bacterial cultures of nose, throat and sputum can contribute to maintaining the clinical status of the CF patient; even if an overall clinical improvement was not apparent in the small series of patients studied here.

Both methods of therapy, when applied consistently, are of value. The regimen of the Flucloxacillin group was slightly more effective than the Variable Antibiotic group, especially in controlling S. aureus and H. influenzae. However, the final frequency with which these organisms were isolated was not significantly different from that of the Variable Antibiotic group. This therefore casts doubt on the value of anti-staphylococcal prophylaxis. The Flucloxacillin group, it will be remembered, received continuous therapy with flucloxacillin.

The results also indicated that bacteriological examination of the patient at 6-month intervals is quite inadequate. It is not the antibiotic regimen which is important, but rather the frequency with which infection is monitored, so that appropriate antibiotics can be administered when required.

In retrospect it would probably have been more efficient to have had lung function tests performed at the beginning and end of the investigation. However, these were not made available.

From the literature it is apparent that the practice of the past 20 years of controlling infection with the varied use of antibiotics has had only a limited success. Thus it is true that CF patients are now living longer and have a better quality of life, but complete eradication of infection has never been achieved. The doubts cast concerning the value of anti-staphylococcal prophylaxis suggests that any future investigation should consider including a control group which does not receive prophylaxis and only an antibiotic in response to a specific infection.

REFERENCES

ANDERSEN, D.H. (1938). Cystic fibrosis of the pancreas and its relation to coeliac disease: a clinical and pathologic study. American Journal of Diseases of Children 56: 344-399.

ANDERSON, E.S. and WILLIAMS, R.E.O. (1956). Bacteriophage typing of enteric pathogens and staphylococci and its uses in epidemiology. Journal of Clinical Pathology 9: 94-127.

ANNEAR, D.I. and GRUBB, W.B. (1972). Linked and unstable resistance to kanamycin and penicillin, and diffusible pigment production, in an isolate of Staphylococcus aureus. Journal of Medical Microbiology 5: 109-111.

BIGGAR, W.D., HOLMES, B. and GOOD, R.A. (1971). Opsonic defect in patients with cystic fibrosis. Proceedings of the National Academy of Sciences 68: 1716-1719.

BLACKFAN, K.D. and MAY, C.D. (1938). Inspissation of secretion, dilatation of the ducts and acini, atrophy and fibrosis of the pancreas in infants. A clinical note. Journal of Pediatrics 13: 627-634.

BOXERBAUM, B., KAGUMBA, M. and MATTHEWS, L.W. (1973). Selective inhibition of phagocytic activity of rabbit alveolar macrophage by cystic fibrosis serum. American Review of Respiratory Disease 108: 777-783.

BURNS, M.W. and MAY, J.R. (1968). Bacterial precipitins in serum of patients with cystic fibrosis. Lancet(i): 270-272.

CARGILL, S.J. and SALEH, F.V. (1974). Sensitivity patterns of respiratory type Haemophilus influenzae from purulent sputum specimens. Scottish Medical Journal 19: 253-256.

CARLSON, D.M. and MATTHEWS, L.W. (1966). Polyuronic acids produced by Pseudomonas aeruginosa. Biochemistry 5: 2817-2822.

COWAN, S.T. and STEEL, K.J. (1974). Manual for the Identificatior of Medical Bacteria. Cambridge University Press.

CRUIKSHANK, R., DUGUID, J.P., MARMION, B.P. and SWAIN, R.H.A. (1973). Medical Microbiology Vol. I: Microbial Infections p. 240-241. Churchill Livingstone. Edinburgh and London.

DI SANT'AGNESE, P.A. and ANDERSEN, D.H. (1946). Chemotherapy in infection of the respiratory tract association with cystic fibrosis of the pancreas. American Journal of Diseases of Children 72: 17-61.

DI SANT'AGNESE, P.A. and TALAMO, R.C. (1967). Pathogenesis and pathophysiology of cystic fibrosis of the pancreas. New England Journal of Medicine 277: 1287-1294, 1344-1352, and 1399-1408.

DOERSHUK, C.F., MATTHEWS, L.W., TUCKER, A.S., NUDELMAN, H., EDDY, G., WISE, M. and SPECTOR, S. (1964). A five year clinical evaluation of a therapeutic program for patients with cystic fibrosis. Journal of Pediatrics 65: 677-693.

DOGGETT, R.G., HARRISON, G.M. and WALLIS, E.S. (1964). Comparison of some properties of Pseudomonas aeruginosa isolated from infections in persons with and without cystic fibrosis. Journal of Bacteriology 87: 427-431.

DOGGETT, R.G., HARRISON, G.M., STILLWELL, R.N. and WALLIS, E.S. (1965). Enzymatic action on the capsular material produced by Pseudomonas aeruginosa of cystic fibrosis origin. Journal of Bacteriology 89: 476-480.

DOGGETT, R.G., HARRISON, G.M., STILLWELL, R.N. and WALLIS, E.S. (1966). An atypical Pseudomonas aeruginosa associated with cystic fibrosis of the pancreas. Journal of Pediatrics 68: 215-221.

EICHENWALD, H.F. (1960). Staphylococcal infection in CF. In Research on Cystic Fibrosis, pp. 89-120. Edited by R.McIntosh. Baltimore: The French-Bray Printing Company.

EVANS, L.R. and LINKER, A. (1973). Production and characterization of the slime polysaccharide of Pseudomonas aeruginosa. Journal of Bacteriology 116: 915-924.

FANCONI, G., UEHLINGER, E. and KNAUER, C. (1936). Das Coeliakiesyndrom bei angeborener zystischer Pankreasfibromatose und Bronchiektasien. Wiener Medizinische Wochenschrift 86: 753-756.

FARBER, S. (1945). Some organic digestive disturbances in early life. Journal of the Michigan Medical Society 44: 587-594.

FINNEY, D.J., LATSCHA, R., BENNETT, B.M. and HSU, P. (1963). Tables for Testing Significance in a 2 x 2 Contingency Table. Cambridge University Press.

GIBBONS, A., ALLAN, J.D., HOLZEL, A. and McFARLANE, H. (1976). Cell-mediated immunity in patients with cystic fibrosis. British Medical Journal 1: 120-122.

GIBSON, L.E. and COOKE, R.E. (1959). A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine iontophoresis. Pediatrics 23: 545-549.

GOVAN, J.R.W. (1975). Mucoid strains of Pseudomonas aeruginosa: The influence of culture medium on the stability of mucus production. Journal of Medical Microbiology 8: 513-522.

GOVAN, J.R.W. (1976). Antibiotic therapy and cystic fibrosis: increased resistance of mucoid Pseudomonas aeruginosa to carbenicillin. Journal of Antimicrobial Chemotherapy 2: 215-217.

GOVAN, J.R.W. and FYFE, J.A.M. (1978). Mucoid Pseudomonas aeruginosa and cystic fibrosis: resistance of the mucoid form to carbenicillin, flucloxacillin and tobramycin and the isolation of mucoid variants in vitro. Journal of Antimicrobial Chemotherapy 4: 233-240.

GURIVITZ, D., COREY, M., FRANCIS, P.W.J., CROZIER, D. and LEVISON, H. (1979). Survival. In Perspectives in Cystic Fibrosis. Pediatric Clinics of North America 26: 603-615.

HALBERT, S.P., DI SANT'AGNESE, P.A. and KATEK, F.R. (1960). Staphylococcal antibodies in cystic fibrosis of the pancreas. Pediatrics 26: 792-799.

HALBERT, S.P. (1967). Immunological aspects of cystic fibrosis. Modern Problems in Pediatrics 10: 144-157.

HOFF, G.E. SCHIØTZ, P.O. and PAULSEN, J. (1974). Tobramycin treatment of Pseudomonas aeruginosa infections in cystic fibrosis. Scandanavian Journal of Infectious Diseases 6: 333-337.

HOFF, G.E. AND HØIBY, N. (1975). Staphylococcus aureus in Cystic Fibrosis: Antibiotic sensitivity and phage types during the latest decade. Acta Pathologica et Microbiologica Scandinavica B 83: 219-225.

HØIBY, N. and AXELSEN, N.H. (1973). Identification and quantitation of precipitins against Pseudomonas aeruginosa in patients with cystic fibrosis by means of crossed immunoelectrophoresis with intermediate gel. Acta Pathologica et Microbiologica Scandinavica B 81: 298-308.

HØIBY, N. (1974a). Epidemiological investigations of the respiratory tract bacteriology in patients with cystic fibrosis. Acta Pathologica et Microbiologica Scandinavica B 82: 541-550.

HØIBY, N. (1974b). Pseudomonas aeruginosa infection in cystic fibrosis. Relationship between mucoid strains of Ps. aeruginosa and the humoral immune response. Acta Pathologica et Microbiologica Scandinavica B 82: 551-558.

HUANG, N.N., VAN LOON, E.L. and SHENG, K.T. (1961). The flora of the respiratory tract of patients with cystic fibrosis of the pancreas. Journal of Pediatrics 59: 512-521.

HUANG, N.N. and SHENG, K.T. (1963). Staphylococcal carrier rates of patients with cystic fibrosis and of members of their families. Journal of Pediatrics 62: 36-43.

IACOCCA, V.F., SIBINGA, M.S. and BARBEVO, G.J. (1963). Respiratory tract bacteriology in cystic fibrosis. American Journal of Diseases of Children 106: 315-324.

KILBOURN, J.P. (1978). Bacterial content and ionic composition of sputum in cystic fibrosis. Lancet(i): 334.

KRAEMER, R., RUDEBERG, A., HADORN, B. and ROSSI, E. (1978). Relative underweight in cystic fibrosis and its prognostic value. Acta Paediatrica Scandinavica 67: 33-37.

LACEY, R.W. (1971a). High-frequency transfer of neomycin resistance between naturally occurring strains of Staphylococcu aureus. Journal of Medical Microbiology 4: 73-84.

LACEY, R.W. (1971b). Transfer of tetracycline-resistance between strains of Staphylococcus aureus in mixed cultures. Journal of Medical Microbiology 69: 229-237.

LACEY, R.W. (1972). Genetic control in methicillin-resistant strains of Staphylococcus aureus. Journal of Medical Microbiology 5: 497-508.

LACEY, R.W. and GRINSTED, J. (1972). Linkage of fusidic acid resistance to the penicillinase plasmid in Staphylococcus aureus. Journal of General Microbiology 73: 501-508.

LACEY, R.W. and ROSDAHL, V.T. (1974). An unusual "penicillinase plasmid" in Staphylococcus aureus; evidence for its transfer under natural conditions. Journal of Medical Microbiology 7: 1-9.

LARAYA-CUASAY, L.R., CUNDY, K.R. and HUANG, N.N. (1976). Pseudomonas carrier rates of patients with cystic fibrosis and members of their families. Journal of Pediatrics 89: 23-26.

LAWSON, D. (1970). Bacteriology of the respiratory tract in cystic fibrosis - a hypothesis. In The Control of Chemotherapy, pp. 69-77. Edited by P.J. Watt, Livingstone: Edinburgh.

LINKER, A. and JONES, R.S. (1964). A polysaccharide resembling alginic acid from a Pseudomonas micro-organism. Nature 204: 187-188.

McCRAE, W.M. and RAEBURN, J.A. (1974). The patterns of infection in cystic fibrosis. Scottish Medical Journal 19: 187-190.

McFARLANE, H., HOLZEL, A., BRENCHLEY, P., ALLAN, J.D., WALLWORK, J[illegible] SINGER, B.E. and WORSLEY, B. (1975). Immune complexes in cystic fibrosis. British Medical Journal 1: 423-428.

MARTIN, D.R. (1973). Mucoid variation in Pseudomonas aruginosa induced by the action of phage. Journal of Medical Microbiology 6: 111-118.

MARTINEZ-TELLO, F.J., BRAUN, D. and BLANC, W.A. (1968). Immunoglobulin production in bronchial mucosa and bronchial lymph nodes, particularly in cystic fibrosis of the pancreas. Journal of Immunology 101: 989-1003.

MAY, R.J., HERRICK, N.C. and THOMPSON, D. (1972). Bacterial infection in cystic fibrosis. Archives of Diseases of Childhood 47: 908-912.

MEARNS, M.B., LONGBOTTOM, J.L. and BATTEN, J.C. (1967). Precipitating antibodies to Aspergillus fumigatus in cystic fibrosis. Lancet(i): 538-539.

MEARNS, M.B., HUNT, G.H. and RUSHWORTH, R. (1972). Bacterial flora of the respiratory tract in patients with cystic fibrosis 1950-1971. Archives of Diseases of Childhood 47: 902-907.

MITCHELL-HEGGS, P., MEARNS, M.B. and BATTEN, J.C. (1976). Cystic fibrosis in adolescents and adults. Quarterly Journal of Medicine 15: 479-504.

MITSUHASHI, S., HASHIMOTO, H., KONO, M. and MORIMURA, M. (1965). Drug resistance of staphylococci. II Joint elimination and joint transduction of the determinants of penicillinase production and resistance to macrolide antibiotics. Journal of Bacteriology 89: 988-992.

NOVICK, R.P. and BOUANCHAUD, D. (1971). Extrachromosomal nature of drug resistance in Staphylococcal aureus. Annals of the New York Academy of Sciences 182: 279-294.

POLYMENIDIS, Z., LUDWIG, H. and GOTZ, M. (1973). Cystic fibrosis and HLA antigens. Lancet(i): 1452.

REEVES, D.J. (1971). Sulphamethoxazole/trimethoprim: the first two years. Journal of Clinical Pathology 24: 430-437.

SCHAAP, T. and COHEN, M.M. (1976). A proposed model for the inheritance of cystic fibrosis. In Cystic Fibrosis: Projections Into the Future, pp. 291-302. Edited by J.A. Mango and R.C. Talamo. Stratton: New York.

SCHWARTZ, R.H., JOHNSTONE, D.E., HOLSCLAW, D.S. and DOOLEY, R.R. (1970). Serum precipitins to Aspergillus fumigatus in cystic fibrosis. American Journal of Diseases of Childhood 120: 432-433.

SCHWARZMANN, S. and BORING, J.R. (1971). Antiphagocytic effect of slime from a mucoid strain of Pseudomonas aeruginosa. Infection and Immunity 3: 762-767.

SHINEFIELD, H.R., RIBBLE, J.C., BORIS, M., EICHENWALD, H.F., ALY,R. and MAIBACH, H. (1974). Bacterial interference between strains of S. aureus. Annals of the New York Academy of Sciences 236: 444-455.

SHWACHMAN, H., KHAW, K-T. and KOWALSKI, S.M. (1975). The management of cystic fibrosis. Observations on gentamycin when used with other antibiotics in pulmonary infections associated with cystic fibrosis. Clinical Pediatrics 14: 1115-1118.

SORENSEN, R.V., STERN, R.C. and POLMAR, S.H. (1977). Cellular immunity to bacteria: Impairment of in vitro lymphocyte responses to Pseudomonas aeruginosa in cystic fibrosis patients. Infection and Immunity 18: 735-740.

SPROUL, A. and HUANG, N.N. (1964). Growth patterns in children with cystic fibrosis. Journal of Pediatrics 65: 664-676.

STEINBERG, A.G. and MORTON, N.E. (1956). Sequential test for linkage between cystic fibrosis of the pancreas and the MNS locus. American Journal of Human Genetics 8: 177-189.

TANNER, J.M., WHITEHOUSE, H.R. and TAKAISHI, M. (1966). Standards from birth to maturity for height, weight, height velocity and weight velocity: British Children 1965. Part I Archives of Diseases of Childhood 41: 454-471. Part II Archives of Diseases of Childhood 41: 613-635.

THOMASSEN, M.J., DEMKO, C.A., BOXERBAUM, B., STERN, R.C. and KUCHENBROD, P.J. (1979). Multiple isolates of Pseudomonas aeruginosa with differing antimicrobial susceptibility patterns from patients with cystic fibrosis. Journal of Infectious Diseases 140: 873-880.

TURNBULL, L.S., TURNBULL, L.W., CROFTON, J.W. and KAY, A.B. (1977) Immunoglobulins and complement in sputum from chronic bronchitis and other pulmonary diseases. Lancet(ii): 526-529.

VIRTANEN, S. (1966). Salivary secretion of ABH blood group substances in cystic fibrosis of the pancreas. Journal of Pediatrics 68: 139-141.

WALLWORK, J.C., BRENCHLEY, P., McCARTHY, J., ALLAN, J.D., MOSS, D. WARD, A.M., HOLZEL, A., WILLIAMS, R.F. and McFARLANE, H. (1974) Some aspects of immunity in patients with cystic fibrosis. Clinical and Experimental Immunology 18: 303-320.

WARWICK, W.J. and POGUE, R.E. (1977). Cystic Fibrosis: An expanding challenge for internal medicine. Journal of the American Medical Association 238: 2159-2162.

WILLIAMS, R.J. and GOVAN, J.R.W. (1973). Pyocine typing of mucoid strains of Pseudomonas aeruginosa isolated from children with cystic fibrosis. Journal of Medical Microbiology 6: 409-412.

WOOD, R.E., BOAT, T.F. and DOERSHUK, C.F. (1976). Cystic Fibrosis. American Review of Respiratory Disease 113: 833-878.

APPENDICES

Appendix 1

Physical and radiological examination of the chest of CF Patients

			Physical findings									Radiological findings				
Group	Patient	Assessment	Cough	Sputum	Digital Clubbing	Rhonchi	Crepitations	Cyanosis	Dyspnoea	Tachypnoea	Haemoptysis	Hyperinflation	Atelectasis	Bronchial Wall Thickening	Bronchiectasis	Emphysema
VARIABLE ANTIBIOTIC	TK	Initial	-	-	-	-	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A
		Final	-	-	-	-	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A
	LC	Initial	-	-	-	-	-	-	-	-	-	+	-	-	-	-
		Final	-	-	-	-	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A
	AL	Initial	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Final	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	HW	Initial	-	-	±	-	-	-	-	-	-	+	-	-	-	-
		Final	±	-	±	-	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A
	IL	Initial	+	++	+	-	+	-	+	-	-	N/A	N/A	N/A	N/A	N/A
		Final	+	++	+	-	+	-	+	-	-	+	-	++	-	-
	JD	Initial	+	-	++	-	-	-	-	-	-	++	-	-	-	-
		Final	+	-	++	-	-	-	-	-	-	+	-	-	-	-
	ER	Initial	++	+++	+	-	-	±	-	-	-	++	-	-	-	+
		Final	+	+++	+	-	-	-	-	-	-	++	-	-	-	+
	EB	Initial	+	++	+	-	-	-	-	-	-	+	-	+	-	-
		Final	+	++	+	-	-	-	-	-	-	++	±	++	-	+
	DS	Initial	+++	+++	+	+	+	-	+	-	-	-	-	+	-	-
		Final	+++	+++	++	+	-	-	+	-	-	-	-	+	-	-
	JG	Initial	+	-	-	-	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A
		Final	+	-	-	-	-	-	-	-	-	+	-	+	+	-

N/A = Data not available; - = negative result; ± = slight; + = mild; ++ = moderate; +++ = severe

Appendix 2

Physical and radiological examination of the chest of CF patients

			Physical Findings									Radiological Findings				
Group	Patient	Assessment	Cough	Sputum	Digital Clubbing	Rhonchi	Crepitations	Cyanosis	Dyspnoea	Tachypnoea	Haemoptysis	Hyperinflation	Atelectasis	Bronchial Wall Thickening	Bronchiectasis	Emphysema
FLUCLOXACILLIN	AT	Initial	+	+	+	-	+	±	-	-	-	+	-	-	-	-
		Final	+	+	+	-	++	-	±	-	-	+	-	-	-	-
	YH	Initial	±	-	-	-	+	-	±	-	-	-	-	+	-	-
		Final	±	-	±	-	-	-	±	+	-	-	-	-	-	-
	DB	Initial	-	-	-	-	-	-	-	-	-	+	-	-	-	-
		Final	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	PA	Initial	±	±	-	-	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A
		Final	±	±	-	-	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A
	LM	Initial	+++	+++	+	-	+	-	+	+	-	+	-	-	++	++
		Final	+++	+++	++	+++	++	±	++	+	+	++	-	+	++	++
	DA	Initial	+	-	-	-	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A
		Final	-	-	-	-	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A

N/A = Data not available; - = negative result; ± slight; + = mild; ++ = moderate; +++ = severe

Appendix 3

The antibiotic sensitivities of the main pathogens isolated from the CF Patients in the investigation and non-CF Patients

Category of Antibiotic	Antibiotic	Number (%) of Isolates Sensitive to Antibiotic						
		SA		HI		PSA(Rough Strain)		PSA(Mucoid Strain)
		CF Patients	Non-CF Patients	CF Patients	Non-CF Patients	CF Patients	Non-CF Patients	CF Patients Only
Synthetic Anti-Bacterial Drugs	Cotrimoxazole	120/120(100)	32/32(100)	51/52(98)	15/15(100)	0/66(0)	1/11(9)	9/48(19)
	Sulphonamide	120/120(100)	32/32(100)	51/52(98)	14/15(93)	4/66(6)	1/11(9)	11/48(23)
Penicillins	Benzylpenicillin	8/120(7)	0/32(0)	36/52(69)	9/15(60)	N.T.	N.T.	N.T.
	Ampicillin	8/120(7)	0/32(0)	51/52(98)	9/15(60)	0/66(0)	0/11(0)	6/48(13)
	Cloxacillin	120/120(100)	32/32(100)	0/52(0)	0/15(0)	N.T.	N.T.	N.T.
	Carbenicillin	N.T.	N.T.	N.T.	N.T.	4/66(6)	2/11(18)	7/48(15)
Tetracyclines	Tetracycline	119/120(99)	32/32(100)	52/52(100)	15/15(100)	31/66(47)	1/11(9)	19/48(40)
Macrolides	Erythromycin	109/120(91)	32/32(100)	52/52(100)	14/15(93)	N.T.	N.T.	N.T.
Cephalosporins	Cephazolin	120/120(100)	32/32(100)	50/52(96)	15/15(100)	0/66(0)	0/11(0)	0/48(0)
	Cephaloridine	120/120(100)	32/32(100)	50/52(96)	15/15(100)	0/66(0)	0/11(0)	2/48(4)
	Cephradine	119/120(99)	32/32(100)	13/52(25)	6/15(40)	0/66(0)	0/11(0)	0/48(0)
Peptides	Colistin	N.T.	N.T.	N.T.	N.T.	62/66(94)	11/11(100)	48/48(100)
Aminoglycosides	Gentamicin	120/120(100)	32/32(100)	52/52(100)	15/15(100)	66/66(100)	8/11(73)	48/48(100)
	Tobramycin	120/120(100)	32/32(100)	52/52(100)	15/15(100)	66/66(100)	10/11(91)	48/48(100)
	Neomycin	116/120(97)	32/32(100)	50/52(96)	15/15(100)	59/66(89)	---	48/48(92)
	Kanamycin	118/120(98)	32/32(100)	52/52(100)	15/15(100)	4/66(6)	0/11(0)	5/48(10)
Miscellaneous	Lincomycin	120/120(100)	32/32(100)	2/52(4)	0/15(0)	N.T.	N.T.	N.T.
	Fusidic Acid	119/120(99)	32/32(100)	21/52(40)	8/15(53)	N.T.	N.T.	N.T.

N.T. = Isolate not tested against this antibiotic; --- = data not available.

Appendix 4

The antibiotic sensitivities of the pathogens from CF Patients in the investigation and non-CF Patients

Category of Antibiotic	Antibiotic	Number (%) of Isolates Sensitive to Antibiotic			
		HPI		HPH	
		CF Patients	Non-CF Patients	CF Patients	Non-CF Patients
Synthetic Anti-Bacterial Drugs	Co-trimoxazole	10/10(100)	8/8(100)	28/28(100)	9/9(100)
	Sulphonamide	9/10(90)	8/8(100)	28/28(100)	9/9(100)
Penicillins	Benzylpenicillin	8/10(80)	3/8(38)	26/28(93)	8/9(89)
	Ampicillin	10/10(100)	8/8(100)	28/28(100)	9/9(100)
	Cloxacillin	0/10(0)	0/8(0)	0/28(0)	0/9(0)
	Carbenicillin	N.T.	N.T.	N.T.	N.T.
Tetracyclines	Tetracycline	10/10(100)	8/8(100)	28/28(100)	9/9(100)
Macrolides	Erythromycin	10/10(100)	6/8(75)	28/28(100)	9/9(100)
Cephalosporins	Cephazolin	10/10(100)	8/8(100)	28/28(100)	9/9(100)
	Cephaloridine	10/10(100)	8/8(100)	28/28(100)	9/9(100)
	Cephradine	3/10(30)	7/8(88)	28/28(100)	9/9(100)
Peptides	Colistin	N.T.	N.T.	N.T.	N.T.
Aminoglycosides	Gentamicin	10/10(100)	8/8(100)	28/28(100)	9/9(100)
	Tobramycin	10/10(100)	8/8(100)	28/28(100)	9/9(100)
	Neomycin	10/10(100)	8/8(100)	28/28(100)	9/9(100)
	Kanamycin	10/10(100)	8/8(100)	28/28(100)	9/9(100)
Miscellaneous	Lincomycin	0/10(0)	0/8(0)	0/28(0)	0/9(0)
	Fusidic Acid	3/10(30)	1/8(13)	1/28(4)	1/9(17)

N.T. = Isolate not tested against this antibiotic

Appendix 5

The antibiotic sensitivities of the pathogens isolated from CF Patients in the investigation and non-CF Patients

Category of Antibiotic	Antibiotic	Number (%) of Isolates Sensitive to Antibiotic					
		EC		PM		StF	
		CF Patients	Non-CF Patients	CF Patients	Non-CF Patients	CF Patients	Non-CF Patients
Synthetic Anti-Bacterial Drugs	Cotrimoxazole	19/19(100)	120/133(90)	16/24(67)	30/31(97)	21/24(88)	0/52(0)
	Sulphonamide	15/19(79)	89/133(67)	10/24(42)	30/31(97)	22/24(92)	0/52(0)
Penicillins	Benzylpenicillin	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
	Ampicillin	16/19(84)	78/133(59)	24/24(100)	26/31(84)	24/24(100)	52/52(100)
	Cloxacillin	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
	Carbenicillin	17/19(90)	80/133(60)	24/24(100)	26/31(84)	9/24(38)	51/52(98)
Tetracyclines	Tetracycline	19/19(100)	109/133(82)	18/24(75)	1/31(3)	24/24(100)	30/52(58)
Macrolides	Erythromycin	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
Cephalosporins	Cephazolin	19/19(100)	129/133(97)	24/24(100)	30/31(97)	24/24(100)	45/52(87)
	Cephaloridine	18/19(95)	123/133(93)	24/24(100)	27/31(87)	23/24(96)	52/52(100)
	Cephradine	16/19(84)	130/133(98)	16/24(67)	30/31(97)	24/24(100)	25/52(48)
Peptides	Colistin	19/19(100)	133/133(100)	0/24(0)	0/31(0)	1/24(4)	0/52(0)
Aminoglycosides	Gentamicin	19/19(100)	133/133(100)	24/24(100)	31/31(100)	0/24(0)	0/52(0)
	Tobramycin	19/19(100)	132/133(99)	24/24(100)	30/31(97)	0/24(0)	0/52(0)
	Neomycin	19/19(100)	---	24/24(100)	---	0/24(0)	---
	Kanamycin	18/19(95)	125/133(94)	24/24(100)	29/31(94)	0/24(0)	0/52(0)
Miscellaneous	Lincomycin	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
	Fusidic Acid	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.

N.T. = Isolate not tested against this antibiotic; --- = data not available.