Ibadan, is shortly to undertake such a study of E.M.F. in several widely separated parts of Africa.

In conclusion I need hardly emphasize the absolute necessity for team-work between the clinician, laboratory worker, and epidemiologist; in fact, the clinician must have a good grounding in epidemiology and the epidemiologist should be in substantial measure a clinician.

ADDENDUM.—At the Conference in Colombia it became apparent that an important cause of myocardial fibrosis is Chagas's disease (*Trypanosoma cruzi* infection), in which, while the infection is often contracted in childhood, cardiac failure may not supervene until middle life. Histologically, tissue reaction seems to differ from African E.M.F. in that it is much more cellular; indeed, it is essentially an inflammatory reaction. It is clear that a careful study is necessary of the distribution of cases of African E.M.F. in relation to exposure to *T. gambiense* and *T. rhodesiense* infection.

REFERENCES

Arnold, R. M. (1954). *W. Indian med. J.*, **3**, 1.

Bedford, D. E., and Konstam, G. L. S. (1946). *Brit. Heart J.*, **8**, 236.

Bras, G., Berry, D. M., and György, P. (1957). *Lancet*, **1**, 960.

Brigden, W (1957). Ibid., **2**, 1179, 1243.

Chapelle, C. E. de la, and Kossmann, C. E. (1954). *Circulation*, **10**, 747.

Davidson, J. (1935). *J. Path. Bact.*, **40**, 285.

Davies, J. N P. (1956). In *Cardiovascular Epidemiology* (edited by A. Keys and P. D. White), p. 100. Hoeber-Harper, New York

—— and Ball, J. D. (1955). *Brit. Heart J.*, **17**, 337.

Dick, G. W. A., Haddow, A. J., Best, A. M., and Smithburn, K. C. (1948). *Lancet*, **2**, 286.

Edge, J. R. (1946). Ibid., **2**, 675.

Gallavardin, L., and Gravier, L. (1929). *Arch. Mal. Cœur*, **22**, 379.

Gillanders, A. D. (1951). *Brit. Heart J.*, **13**, 177.

Gray, I. R. (1951). Ibid., **13**, 387.

Löffler, W. (1936) *Schweiz. med. Wschr.*, **17**, 817.

Selye, H. (1958). *Lancet*, **1**, 1351.

Selzer, G., Parker, R. G. F., and Sapeika, N. (1951). *Brit. J. exp. Path.*, **32**, 14.

Smith, J. J., and Furth, J. (1943). *Arch. intern. Med.*, **71**, 602.

Williams, A. W., Ball, J. D., and Davies, J. N. P. (1954). *Trans. roy. Soc. trop. Med. Hyg.*, **48**, 290.

Starting nearly 100 years ago with one rowboat, one medical officer, and one sanitary inspector, the City of London Corporation as Port Health Authority of the Port of London has gradually built up a comprehensive Port Health Service, whose main preoccupation is with imports—imports of food and imports of disease. By a day and night watch on shipping, including ships from the Far East, dangerous and infectious diseases are prevented from entering into the Port of London and beyond that to greater London and the United Kingdom itself—15,000 ships from foreign lands enter the Port of London every year. No case of smallpox has arrived in the port since 1951, and for at least the last 50 years there has been no outbreak of smallpox in the United Kingdom that could be attributed to any smallpox admitted through the port. There is also a regular spot check on foodstuffs entering the port, which is by far the greatest importing port in the United Kingdom —for instance, 85% of Britain's meat supplies enters the Port of London. Similarly the Corporation's elaborate system of rodent control in the port helps to prevent the spread of infectious diseases and the contamination and destruction of foodstuffs. These parts of the Corporation Port Health Service, like the Quarantine Service at the entrance to the port, are of benefit not only to greater London but to the nation as a whole. ("The Work of the Port of London Health Authority": Dr. J. Greenwood Wilson, at the Royal Institute of Public Health and Hygiene.)

AN EVALUATION OF CONTINUOUS ANTIBIOTIC THERAPY IN CHRONIC BRONCHITIS

BY

J. McC. MURDOCH, F.R.F.P.S., M.R.C.P.Ed.
Senior Medical Registrar, Royal Infirmary, Edinburgh

W. J. H. LECKIE, M.B., Ch.B., M.R.C.P.Ed.
Medical Registrar, Royal Infirmary, Edinburgh

JEAN DOWNIE, A.B.
Research Student, Department of Bacteriology, University of Edinburgh

R. H. A. SWAIN, M.A., M.D.
Reader, Department of Bacteriology, University of Edinburgh

AND

J. C. GOULD, M.B., Ch.B., B.Sc.
Senior Lecturer, Department of Bacteriology, University of Edinburgh

Chronic bronchitis is a major cause of industrial invalidism in the United Kingdom resulting, for example, in the loss of 22 million male working-days between June, 1955, and June, 1956, or about 25% of all male sickness incapacity. Therefore any measures which can be adopted to reduce this enormous morbidity, with its consequent drain on the national economy, must be assessed as accurately as possible.

In addition to infection, many complex factors, such as climate, atmospheric pollution, heredity, and social and occupational habits, operate in the causation of chronic bronchitis. These have been reviewed in three recent books (Stuart-Harris and Hanley, 1957; Ogilvie and Newell, 1957; Oswald, 1958) and require no further comment here. The infective element is most obvious in acute exacerbations which are responsible for much of the invalidism. The association of *Haemophilus influenzae* and the pneumococcus with these exacerbations is now well recognized (May, 1953; Stewart, 1959). Eradication of these pathogens from the bronchi might prevent further destruction of already damaged broncho-pulmonary tissue, thus reducing recurrent winter ill-health and ultimately prolonging life (L. M. Reid, 1955, 1956). For this reason much attention in recent years has been paid to the treatment of patients suffering from chronic bronchitis with various forms of long-term chemotherapy.

Continuous administration of tetracyclines during the winter months has given good results (Helm *et al.*, 1956; May and Oswald, 1956; Moyes and Kershaw, 1957; Buchanan *et al.*, 1958; Edwards and Fear, 1958; Moyes and Kalinowski, 1959). But there is still uncertainty about the selection of patients, the optimal dosage, and the justification of the expense involved (Elmes *et al.* 1957; Stuart-Harris, 1959). Further, the administration of broad-spectrum antibiotics for long periods may lead to the overgrowth of resistant micro-organisms within the host (Williams, 1954), and their dissemination among patients outside hospitals, especially in the case of *Staphylococcus aureus*, is a potential hazard.

The epidemiological significance of such a spread of staphylococci in the community is not known and

remains a matter of controversy (Rountree and Rheuben, 1956).

In an attempt to throw further light on the problems involved in the management of chronic bronchitis with continuous chemotherapy, two "double-blind" trials of continuous tetracycline and oxytetracycline ("terramycin") therapy were undertaken during the periods October 1 to March 31, 1957–8 and 1958–9, respectively. In the earlier period 23 patients were observed, and in the later period 75 patients, including 20 from the previous winter. The clinical and bacteriological results of these trials are reported here.

Materials and Methods for Both Trials

Clinical

The patients were drawn from general practices and were known to live and work in the vicinity of the Royal Infirmary and the University of Edinburgh. Each had suffered from chronic bronchitis for five years or more, characterized by a persistent cough, exertional dyspnoea, and recurrent winter invalidism due to acute exacerbations of the disease. The history, full clinical examination, and chest radiography excluded concomitant disease. Assessment of the disease in each patient was recorded on a form (see Chart), and was completed during the months of September, 1957, and September, 1958.

INITIAL ASSESSMENT CHART

Name: Age: Occupation:
Address: Weight:
Approximate duration of bronchitis:
Smoking habits:
Respiratory disability (Sinclair, 1955) I Grade 1
" 2
" 3
" 4
" 5
II Frequency of cough:
(a) a.m. only
(b) continuous
Physical disability:
Time lost from work during winter of 1956–7, 1957–8
(amplify for earlier years if possible)
Sputum: I Rough estimate of 24-hr. volume
II Usual character of sputum: (a) clear
(b) mucopurulent
(c) purulent
III Bacteriology
General health: 1. Appetite: (a) normal
(b) impaired by nausea and retching after coughing.
2. Bowel function:
3. General remarks:
Physical Examination:
Chest Radiograph:

During the periods October 1 to March 31, 1957–8 and 1958–9 the patients were seen at fortnightly intervals after their normal working hours in one room of the clinical laboratory in the Royal Infirmary, which was not part of the main hospital building and which was used almost exclusively for the investigation of patients suffering from metabolic diseases. The patients were instructed to contact the hospital if they became ill, and were then attended to in their homes by one of us as well as their family doctor. The patients were managed at home and recourse to hospital admission was not made. The family doctor had previously agreed to withhold specific therapy in these instances.

Bacteriological

Sputum.—Patients were instructed to produce an early morning specimen of sputum in a sterile 2-oz. (60-ml.) honey-pot and hand it in to the laboratory within two hours of expectoration. At least two specimens from each patient were examined in September, 1957, and September, 1958. Thereafter specimens were obtained at fortnightly intervals, or more often if clinically indicated.

Examination of Sputum. — Each specimen was examined with the naked eye and classified as mucoid, mucopurulent, or purulent. The specimen was divided into two; one part was homogenized by the addition of an equal volume of 1% pancreatin solution and incubated at 37° C. for one hour (Rawlins, 1953, 1955). Both the homogenized and untreated portions of sputum were examined as follows: (*a*) Standard loopfuls were transferred to measured areas on glass slides and smears of uniform thickness were made. These were Gram and Leishman stained. Microscopical counts of the morphological types of bacteria present and estimations of the cellular exudate, including eosinophils, were obtained. (*b*) Standard loopfuls of sputum were transferred to a selection of culture media which included blood-agar, heated blood-agar, and milk-agar (Mackie and McCartney, 1953); these were spread uniformly over the surface of the plates so that an approximate count of the numbers of each colony type could be made. (*c*) The antibiotic sensitivity of the micro-organisms present in each specimen was determined by the disk-diffusion test (Gould and Bowie, 1952). The disks were placed on one of the diagnostic blood-agar plates at the time of initial inoculation. When necessary the results of these tests were confirmed on subcultures of the individual species. (*d*) Subcultures of each species isolated were examined for fuller identification.

Nasal Swabs.—The anterior nares of each patient were swabbed on two occasions in the assessment period and thereafter at six-weekly intervals throughout each winter. Similar swabs were also taken from the medical personnel coming into contact with the patients. These swabs were cultured on milk-agar for the detection of *Staph. aureus.*

Faeces.—Samples of stool were obtained from each patient at time intervals corresponding to the nasal swabs. Additional specimens were obtained if a patient complained of diarrhoea. The stools were plated on to MacConkey medium and salt-milk-agar. Approximate counts of the faecal flora were made and colonies selected for antibiotic sensitivity testing. Colonies of *Staph. aureus*, if present, were compared by bacteriophage-typing with those isolated from the nose swabs.

Examination of Dust.—Airborne and settling dust in the room used for seeing the patients was examined for *Staph. aureus* by air-samp'ing and settling-plate techniques (Wallace and Duguid, 1952). The aerial staphylococci were compared with those obtained from the patients.

Clinical Material and Methods Peculiar to 1957-8

The initial assessments of the 23 patients made in September, 1957, are shown in Table I. This also shows how the treatment groups were allotted by the randomization method of Bradford Hill (1955). Contemporary reports in the literature (English *et al.*,

1956; Fairbrother and Southall, 1957; Welch, 1957) suggested that a combination of oleandomycin and tetracycline delayed the emergence of antibiotic resistance, particularly in respect of *Staph. aureus*. Therefore "sigmamycin" (167 mg. of tetracycline and 83 mg. of oleandomycin in each capsule) was chosen as the active agent. The inactive capsules contained lactose.

Treatment was begun on January 1, 1958, and consisted of one capsule four times daily until March 31. The capsules were identical in appearance, neither the clinician nor the patient being aware of their contents. Those patients who by chance drew treatment in group A received the inactive preparation; those in group B the active preparation. Eleven patients drew A and 12 drew B. If a "head cold" developed the patient was asked to increase the number of capsules to six a day, and if looseness of the stools occurred, to visit the clinician, so that the severity of the diarrhoea could be assessed and stools obtained for culture. Details of the time each employed patient lost from work owing to bronchitis was recorded throughout the winter. The character of the sputum as assessed by the patient was noted at each visit to the clinician. If the patient described his phlegm as "white and clear" this was recorded as mucoid, and similarly "yellow" as mucopurulent, and "green" as purulent. These comments were compared with the bacteriologists' findings after the end of the trial, or sooner if the patient was withdrawn from the trial because of clinical illness.

Bacteriological Methods.—Specimens of sputum, anterior nasal swabs, and faeces were examined as described. These specimens taken before January 1, 1958, were thus pretreatment specimens.

Clinical Material and Methods Peculiar to 1958–9

The same initial assessments were made for 75 patients in September, 1959. These are shown in Table II, which also shows the allotment to treatment groups. During the previous year there were reports in the literature of cross-resistance between oleandomycin and erythromycin (Garrod, 1957; Jones and Finland, 1957a, 1957b). It was therefore decided to employ oxytetracycline alone as the active agent, and this was drawn by those in group A, group B receiving identical capsules containing lactose. Treatment consisted of one 250-mg. capsule four times daily from October 1, 1958, to March 31, 1959. Instructions to the patients were similar to those of the previous year.

Bacteriological Methods.—Similar investigations were carried out as in 1957–8 with the addition of serological examination of specimens of sera taken in October, 1958, during the treatment period after any exacerbation, and again after the completion of treatment. The sera were titrated for agglutinating, and complement-fixing activities against strains of *H. influenzae* isolated from the corresponding patients. An attempt was also made to ascertain the role of the influenza viruses A, B, and C, and the adenoviruses in the production of acute exacerbations of chronic bronchitis by measuring the presence of antibody in the sera of these patients. Specimens of venous blood were taken at the end of November, 1958, and in March, 1959. Additional specimens were taken during convalescence from any patient with an influenza-like illness.

Results from Both Trials

Clinical Aspects

During both winters the assessment of each patient was based on the subjective and objective findings entered at each fortnightly interview on a tabulated facsimile of the case record (see Chart). The patients were recorded as "improved," "unchanged," or "worse" at each assessment, and a similar overall assessment was made at the end of each winter.

The smoking habits of all the patients were unchanged throughout both winters. Subjectively the patients who received the active preparations were improved as regards appetite, frequency of cough, and exertional dyspnoea much more often than those receiving the inactive preparation. Acute exacerbations of bronchitis which caused a marked increase in cough, with the production of copious mucopurulent or purulent sputum, increase in dyspnoea, and constitutional upset sufficient to force the patient to bed were considered to be a failure of treatment. Similarly, the development of side-effects severe enough to cause the patient distress was also considered a failure of treatment. Withdrawals from the trials were determined on the basis of the following factors: (*a*) the development of other disease; (*b*) the family doctor's decision to change treatment; and (*c*) default by the patient.

Bacteriological Aspects

The bacteriological results were obtained in complete ignorance of the clinical findings except where it was imperative that the clinician have the information for the treatment of the patient. The nature and relative numbers of the species of bacteria in each specimen of sputum were used to assess the progress of the patients. If the flora remained the same in a series of specimens the patient was regarded as unchanged. A recording of worse was made if one or more of these organisms

TABLE I.—*Initial Assessment of Patients*, 1957–8

Treatment Group	No. of Patients	Age Group							Sex		Social Grade					Years of Bronchitis				Dyspnoea Grade				
		10–	20–	30–	40–	50–	60–	70–	M	F	I	II	III	IV	V	5–	10–	15–	20+	1	2	3	4	5
A (inactive)	11	—	—	2	3	2	4	—	8	3	1	1	2	6	1	2	0	3	6	1	4	1	4	1
B (active)	12	1	3	—	—	5	2	1	8	4	0	3	4	4	1	5	3	0	4	2	3	4	3	—

TABLE II.—*Initial Assessment of Patients*, 1958–9

Treatment Group	No. of Patients	Age Group							Sex		Social Grade					Years of Bronchitis				Dyspnoea Grade				
		10–	20–	30–	40–	50–	60–	70–	M	F	I	II	III	IV	V	5–	10–	15–	20+	1	2	3	4	5
A (active)	35	3	2	1	3	10	12	4	24	11	1	7	12	8	7	13	3	4	15	3	6	12	11	3
B (inactive)	40	0	1	3	6	15	15	0	26	14	1	5	10	14	10	10	9	6	15	1	8	19	11	1

appeared in large numbers, and improved if they disappeared. Particular attention was paid to the incidence of *H. influenzae*, the pneumococcus, *Staph. aureus*, streptococci groups A, C, and D, and coliform strains, and their association with purulence in successive samples of sputum.

H. influenzae was isolated more frequently and in larger numbers from homogenized than from untreated specimens of sputum. The numbers of pneumococci, streptococci, and coliforms were often smaller in the homogenized specimens. The fullest information was obtained by comparing cultures prepared by both methods. The results given below are thus combined observations.

H. influenzae was isolated from more than half of all purulent specimens of sputum, less than one-third of mucopurulent specimens, and one-tenth of mucoid specimens. Similarly, the pneumococcus was isolated much more often from purulent sputa, but *Staph. aureus*, coliform species, and streptococci were isolated almost as frequently from mucoid sputa (Table III). The patients' estimates of the character of their sputa agreed with that of the bacteriologist in only one-third of all specimens. Microscopical examination confirmed the nature of the exudate, and in only one instance were eosinophil polymorphonuclear cells present in large numbers.

TABLE III.—*Frequency of Isolation of Micro-organisms from Specimens of Sputa from Patients Who Did Not Receive Antibiotic*

Micro-organism	Percentage of Specimens from which Micro-organism Isolated					
	Purulent Sputa		Mucopurulent Sputa		Mucoid Sputa	
	1957–8	1958–9	1957–8	1958–9	1957–8	1958–9
H. influenzae ..	40	58	29	30	17	15
Pneumococcus ..	55	37	29	23	26	15
Staph. aureus ..	21	4	36	10	4	11
Str. pyogenes ..	0	2	0	3	2	4
Coliforms and proteus	7	6	2	4	1	8
Total No. of specimens	42	106	93	284	75	191

Statistical Aspects

The allotment of the patients to two treatment groups by randomization has approximately balanced out any variable features over both trial periods. Accordingly it has been possible to compare results obtained from the treated and untreated groups in each winter by statistical analysis.

Results, 1957–8

Clinical

Eleven of the 12 patients who received the active preparation during the period January to March, 1958, were clinically improved and one remained unchanged. Ten did not lose any days from work, and the remaining two lost only five days. But during the observation period (October–December, 1957) 6 of the 12 patients lost 90 days. In contrast only one of the 11 patients who received the inactive preparation was clinically improved, while the remainder got worse. Nine of these latter lost 153 days from work during the treatment period and 5 lost 97 days during the observation period. The figures for the days off work because of bronchitis in these patients as supplied by the Ministry of Labour and National Insurance were unreliable, showing a gross discrepancy when compared with the actual times that the patients were known to be off work. Because of this, comparison of the invalidism in the winters before the trials with the trial periods themselves was obviously impossible.

If a particular person is away from work one day because of bronchitis it is likely that he will be away on the following day. It is therefore more informative to compare the number of people who lost days off work with the number who lost none, rather than the total days lost by the groups. Table IV shows that there is no statistical difference between the two groups in the number of patients losing days from work during the observation period, while Table V shows a significant difference in favour of active treatment.

TABLE IV.—*Patients Off Work Because of Bronchitis During Observation Period October–December, 1957*

Time Lost	No. of Patients Allotted to		Total
	Group A	Group B	
Some days ..	5	6	11
No ,, ..	6	6	12
Total	11	12	23

These figures are the nearest whole numbers to the "expected" values, so that there is no significant difference between the two groups.

TABLE V.—*Patients Off Work Because of Bronchitis During Treatment Period January–March, 1958*

Time Lost	No. of Patients Receiving		Total
	Inactive Capsules	Active Capsules	
Some days ..	9	2	11
No ,, ..	2	10	12
Total ..	11	12	23

Comparison of the two treatment groups by the 'exact" test gives P= 0·00278, so that the difference in favour of active capsules is highly significant.

Bacteriological

Of the 12 patients receiving active treatment 11 were recorded as improved and 1 as worse. All of the 11 patients on inactive treatment were recorded as worse. The frequency with which *H. influenzae*, pneumococcus, *Staph. aureus*, and coliform species were isolated from these patients' sputa is detailed in Table VI. It is clear that both *H. influenzae* and the pneumococcus were isolated much more frequently from those receiving inactive treatment (see also Fig. 1). *Staph. aureus* was

TABLE VI.—*Frequency of Isolation of Micro-organisms from Patients During the Period January 1 to March 31, 1958*

Isolation of		No. Receiving Inactive Capsules	No. Receiving Active Capsules	P Value
H. influenzae	Frequent	9	2	0·00091
	Infrequent or absent	2	10	
Pneumococcus	Frequent	11	4	0·00101
	Infrequent or absent	0	8	
H. influenzae + pneumococcus	Frequent	9	1	0·00059
	Infrequent or absent	2	11	
Staph. aureus	Frequent	7	1	0·00841
	Infrequent or absent	4	11	
Coliforms	Frequent	4	3	0·444
	Infrequent or absent	7	9	

P is calculated by "exact" test. Difference significantly in favour of active treatment for *H. influenzae*, pneumococcus, *H. influenzae* plus pneumococcus, and *Staph. aureus*.

TABLE VII.—*Numbers of Patients who had Sigmamycin-resistant Micro-organisms in Their Sputum During Winter* 1957–8

Micro-organism	No. of Patients with Sputa Containing Sigmamycin-resistant Strains		
	Before Treatment	During Treatment With	
		Active Capsules	Inactive Capsules
H. influenzae	0	2	0
Pneumococcus	0	0	0
Staph. aureus	0	2	0
Str. pyogenes	0	0	0
Coliforms	0	2	0
Total No. of patients ..	23	11	12

isolated with a significantly higher frequency from the sputa of those receiving active treatment but coliforms were not.

Antibiotic Sensitivity. — Details of sigmamycin-resistant strains isolated from the patients, both before and during treatment, are shown in Table VII. No pre-treatment strains were resistant to sigmamycin, but several strains of staphylococci and coliforms resistant to this antibiotic combination were isolated from the patients receiving it. The strains of *Staph. aureus* recovered from the sputa of patients known to be nasal carriers were identical to those from their nares. Antibiotic-resistant strains when isolated from carriers did not correspond to their carrier strains, and in most cases corresponded in phage type to those isolated from the hospital environment.

Twenty patients who were treated during both winters were bacteriologically investigated in the summer of 1958. Eleven of them had received active treatment during the first winter. Of these, 4 had sigmamycin-resistant strains of bacteria predominant in their sputa in April, 1958. When re-examined in September, 1958, none of these strains had persisted and the flora in each patient was similar to that found in September, 1957. From the bacteriological point of view, therefore, these patients were regarded as untreated at the beginning of the second winter. There was no change in the flora of the nine patients receiving the inactive preparation.

Results, 1958–9

Clinical

Of the 35 patients receiving active treatment, 26 completed the course: 23 were improved, 2 were worse, and 1 failed treatment because of persistent nausea. The remaining 9 were withdrawn for the following reasons: 3 defaulted, 2 left the area, 2 developed other diseases—namely, acute pancreatitis and cerebral thrombosis—and 2 had their treatment changed by their family doctor. Twenty-two did not lose any days from work, while four lost 210 days. In contrast, only 19 of the 40 patients receiving inactive treatment completed the course and were clinically unchanged, though 10 felt better in themselves. Twenty of the remainder had acute exacerbations and required antibiotic treatment; 1 defaulted. Thirteen of the 19 patients completing the course lost no days from work, while the 26 remaining patients lost 358 days. Table VIII shows again a significant difference in favour of active treatment by comparing the number of people who lost some days with those who lost none.

TABLE VIII.—*Patients Off Work Because of Bronchitis During Treatment Period October–March,* 1958–9

Time Lost	No. of Patients Receiving		Total
	Active Capsules	Inactive Capsules	
Some days ..	13	27	40
No ,, ..	22	13	35
Total	35	40	75

Comparison of the two treatment groups: $\chi^2 = 6{\cdot}91$, giving $0{\cdot}01 > P > 0{\cdot}005$, a significant difference in favour of the active capsules.

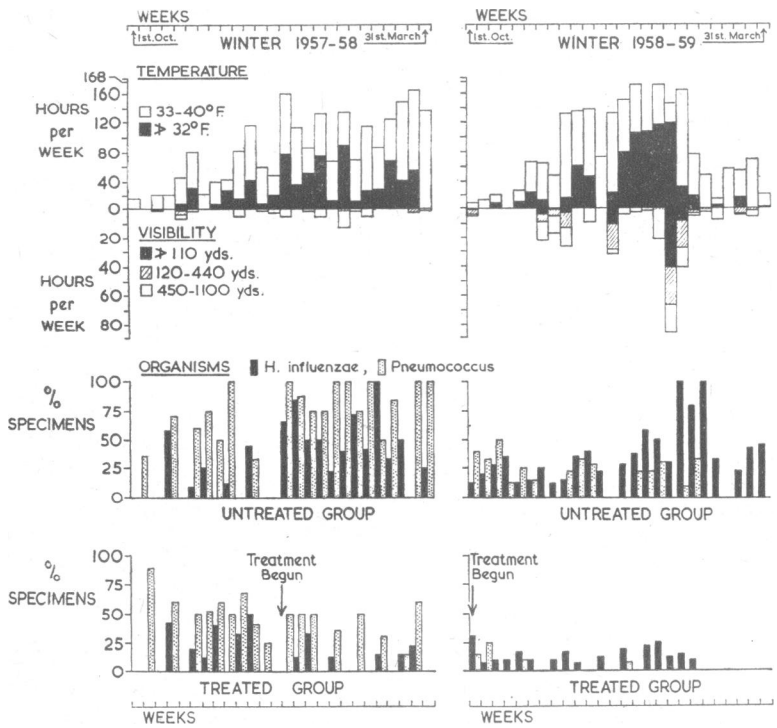

FIG. 1.—Charts showing number of hours a week when visibility and temperature were within certain ranges, and the percentage of specimens each week from which organisms were isolated.

Patients in dyspnoea grades 3, 4, and 5 were more liable to acute exacerbations of their disease. Sixteen of 23 patients who had acute exacerbations in both groups did so during the months of January and February, 1959, when the incidence of cold and fog was much higher than in the other winter months of 1958–9 (see Fig. 1).

Bacteriological

Of the 26 patients completing the course of active treatment, 20 were recorded as improved, 2 as unchanged, and 4 as worse, including the 2 who were clinically worse. Of the remaining 9 patients up to the time of their withdrawal, 7 were improved and 2 were worse. In contrast, of the 19 patients completing the course and receiving "dummy" capsules, only 1 was improved, 3 were unchanged, and 15 were worse. The remaining 20 patients in this group were all worse, and were considered as failures of treatment. The frequency with which *H. influenzae*, pneumococcus, *Staph. aureus*, and coliform strains were isolated from the patients' sputa is

detailed in Table IX. The results clearly show that *H. influenzae* and the pneumococcus had the same significance as in the previous winter. *H. influenzae*, however, was isolated relatively more often and the pneumococcus less often than in the previous winter (Table III). The former organism was most often isolated during the cold and foggy period (see Fig. 1). Neither *Staph. aureus* nor coliform strains were isolated with significantly higher frequency from either treatment group.

The number of patients with sputa containing oxytetracycline-resistant strains is shown in Table X. Strains of several species resistant to oxytetracycline were isolated, the greatest number appearing in patients receiving this antibiotic. It is particularly noteworthy, however, that strains of *Staph. aureus* resistant to oxytetracycline were isolated as often from patients receiving the inactive preparations as those treated with oxytetracycline. The majority of the resistant strains were of a single phage pattern—namely, 42B/52/80—

TABLE IX.—*Frequency of Isolation of Micro-organisms from Patients During the Period October* 1, 1958, *to March* 31, 1959

Isolation of		No. Receiving Inactive Capsules	No. Receiving Active Capsules	χ^2	P Value
H. influenzae	Frequent	27	4	21·96	<0·001
	Infrequent or absent	13	31		
Pneumococcus	Frequent	18	3	10·55	<0·005
	Infrequent or absent	22	32		>0·001
H. influenzae + pneumococcus	Frequent	17	3	9·31	<0·005
	Infrequent or absent	23	32		>0·001
Staph. aureus	Frequent	10	9	Nearest whole numbers to "expected" values	
	Infrequent or absent	30	26		
Coliforms	Frequent	11	9	,,	,,
	Infrequent or absent	29	26		

Differences significantly in favour of active treatment for *H. influenzae*, pneumococcus, and *H. influenzae* plus pneumococcus.

TABLE X.—*Number of Patients Who Had Oxytetracycline-resistant Micro-organisms in Their Sputum During Winter* 1958–9

Micro-organism	No. of Patients with Sputa Containing Oxytetracycline-resistant Strains			
	Before Treatment	During Treatment With		
		Active Capsules	Inactive Capsules	Inactive + Active
H. influenzae ..	0	4	0	3
Pneumococcus ..	0	0	0	0
Staph. aureus ..	2	6	6	3
Str. pyogenes ..	0	0	0	0
Coliforms ..	5	3	5	4
Total No. of patients	75	35 (9)	19 (1)	20

Numbers in parentheses refer to withdrawals from the trial who were not given antibiotic thereafter (see text).

TABLE XI.—*Occurrence of Staph. aureus in Nares and Faeces of* 45 *Patients Completing Course of Treatment During Winter* 1958–9

	No. of Persons with *Staph. aureus*					
	Before Treatment		After Treatment with			
			Active Capsules		Inactive Capsules	
	OS	OR	OS	OR	OS	OR
In nares	9	2	4	2	4	1
,, faeces ..	5	0	3	2	4	0

OS = Oxytetracycline-sensitive. OR = Oxytetracycline-resistant.

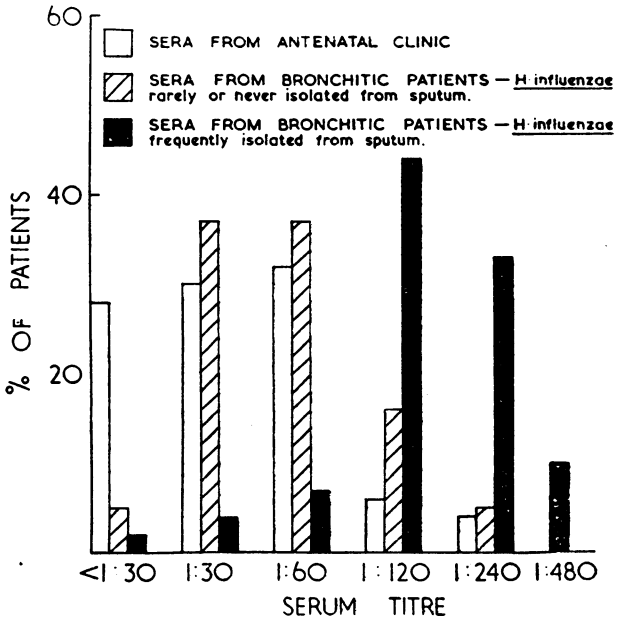

FIG. 2.—*H. influenzae* antibody titres of sera.

and resistant to multiple antibiotics. This strain was isolated from the dust of the room in which these patients were interviewed but not from the medical personnel using the room. The number of staphylococcal nasal carriers did not increase during the winter, nor did the proportion of those harbouring oxytetracycline-resistant strains. Only two specimens of stools from the patients receiving oxytetracycline yielded *Staph. aureus* resistant to this antibiotic, and none from the patients on the inactive preparation (Table XI).

The serum antibody titres for *H. influenzae* were measured in the patients during the second winter and compared with those found in healthy adults. The results of these are shown in Fig. 2. There is a close similarity in the titres of sera from patients whose sputa did not contain *H. influenzae* and those of healthy adults. Most of the sera from patients with this organism in their sputa had much higher antibody titres.

The antibody titres to influenza virus A, B, and C, and the adenovirus group in the sera of 45 patients who completed the course of treatment, are shown in Table XII. In five patients an attack of virus influenza was suspected on clinical grounds; in two of these there was a significant rise of antibody titre to virus A and in one to C, but in the remaining two no rise was demonstrable. In the remaining 40 patients in whom there was no reason to suspect clinical influenza, 29 showed negative serological findings. However, 11 of this group appear to have contracted subclinical virus infection, because two showed antibody rise to virus A, five to B, three to C, and one to the group antigen of the adenovirus group.

TABLE XII.—*Antibody Titres in Sera of* 45 *Patients Observed During Entire Period* 1958–9

No Rise in Antibody	No. with Fourfold or More Rise in Antibody to			
	Virus A	Virus B	Virus C	Adenovirus
31	4	5	4	1

Correlation of Clinical and Bacteriological Results

There was good correlation at any given time between the clinical state of each patient and the degree of purulence of the sputum, the presence or absence of *H. influenzae* and/or the pneumococcus (Table XIII). Acute exacerbations of bronchitis were usually associated with the appearance of large numbers of these species in the sputum.

TABLE XIII.—*Correlation of Clinical and Bacteriological Findings Over Both Winters in 98 Patients*

Clinical Correlation with			
Purulence		Bacteriological Findings	
Agreed	Disagreed	Agreed	Disagreed
68%	32%	79%	21%

TABLE XIV.—*Side-effects of Treatment Observed in Both Winters*

Side-effect	1957–8		1958–9	
	Active Capsules	Inactive Capsules	Active Capsules	Inactive Capsules
Nausea	1	0	1	2
Diarrhoea* persistent	3	0	3	0
,, transient	4	0	5	0
Abdominal colic	0	0	1	0
"Black tongue"	0	0	1	0
Pruritus ani	0	0	1	0
Skin rash	0	0	1	1
Vomiting	0	0	0	1

* The term "persistent" describes the passage of loose ill-formed stools (four daily approximately) throughout treatment: "transient" describes the same symptoms disappearing within six weeks of starting treatment.

Side-effects

The side-effects (Table XIV) were almost entirely confined to those receiving active treatment during both winters. Diarrhoea was the most frequent, but was never severe enough to warrant stopping treatment; its incidence was no higher with oxytetracycline than with tetracycline. Bacteriological examination of these stools failed to reveal any gross disturbance of the normal intestinal flora, and antibiotic-resistant strains of *Staph. aureus* and yeasts were not detected. Persistent nausea arose in one patient receiving oxytetracycline, and was severe enough to justify discontinuation of treatment.

Discussion

The results of these trials show clearly that advanced chronic bronchitic subjects benefit greatly from continuous daily treatment with tetracyclines during the winter months. This is especially the case in the elderly patients with advanced disease. Out-patient supervision and domiciliary treatment during exacerbations have proved practicable. The absence of recurrent winter invalidism and consequent loss of time from work has been an advantage of continuous therapy, as shown by the comparison of days lost by treated as against untreated patients. Further, it seems probable that treatment should be begun in the autumn, as the 23 patients in 1957 who did not receive such treatment during this period lost considerably more time from work than those receiving oxytetracycline in the corresponding months of 1958. The occurrence of an acute exacerbation of bronchitis in those patients receiving inactive capsules led to withdrawal from the trial. The patients continued to be seen, however, and were treated with oxytetracycline in the same manner as those on active treatment. These patients responded slowly, both clinically and bacteriologically to this therapy, but lost more time from work than those treated with oxytetracycline from the outset.

The treatment of exacerbations in a large general hospital such as the Royal Infirmary of Edinburgh carries with it the dangers of superinfection with hospital pathogens resistant to the tetracyclines, and is also very expensive, the average weekly cost per patient being £22. Continuous treatment with oxytetracycline in a dose of 1 g. daily for six months costs £63 14s. in general practice. This means expenditure of less than £2 10s. a week per patient. When sickness benefit and industrial financial loss are added to hospital expenditure it is our view that the expense involved in continuous prophylaxis with oxytetracycline is relatively small. While these trials have included a large number of bacteriological investigations we do not consider these necessary in the diagnosis and management of chronic bronchitis in this way.

The degree of purulence of the sputum is a useful guide to the efficiency of antibiotic treatment, since there is a good correlation between it, the clinical state of the patient, and the presence of *H. influenzae* and pneumococcus. However, the patient's opinion of the purulent or non-purulent nature of his sputum is quite unreliable, less than a third of our patients giving an accurate description. It is therefore important for the doctor treating bronchitic patients to examine the sputum himself from time to time. The dose of oxytetracycline should be adjusted so that the sputum remains mucoid; this appears to be about 1 g. daily in four divided doses.

Ten (25%) of the patients receiving the inactive preparation during the second winter showed a striking "placebo effect" (Wolf and Pinsky, 1954) despite the fact that there was neither clinical nor bacteriological improvement in their condition throughout the winter. This illustrates the value to the bronchitic patient of seeing his doctor at frequent intervals.

Looseness of stools was troublesome in 15 out of 47 patients receiving active treatment, but was not severe enough to warrant withdrawal of the drugs, and in general was a transient symptom. In no case was it associated with a significant change in bowel flora. The occurrence of other side-effects to the antibiotics used was negligible. In the second winter, in the affected patients acute exacerbations of bronchitis were particularly associated with very cold and foggy weather; the production of purulent sputum containing large numbers of *H. influenzae* was observed more often (see Fig. 1). This agrees with previous reports in the literature (Hewitt, 1956; D. D. Reid, 1956; Stocks, 1959).

Conditions for the isolation of pathogenic micro-organisms from the large number of sputum specimens were highly favourable. Early-morning specimens were obtained frequently from each patient and examined within two hours. Despite this, *H. influenzae* and pneumococcus were rarely recovered from all specimens from the same individual, but the frequency with which these organisms were associated with acute exacerbations supports the view that they play an important part in the aetiology of infective episodes of chronic bronchitis (Mulder *et al.*, 1952; Hers and Mulder, 1953; *Lancet*, 1955; Mulder, 1956; Oswald, 1958). So also does their repeated isolation from purulent sputa and their virtual absence from mucoid specimens.

Additional confirmation of the role of *H. influenzae* has been the demonstration of high antibody titres in the sera of the patients who regularly yielded this micro-organism from their sputa, which suggests that *H. influenzae* has the invasive properties which lead to the inflammatory destruction of broncho-pulmonary tissue demonstrable in chronic bronchitis (L. M. Reid, 1954, 1955, 1956). Bronchoscopic aspirates yield *H. influenzae* in most patients with purulent sputum (Brumfitt *et al.*, 1957). This method is impracticable for routine use, and so examination of sputum remains necessary if information about the bacterial flora is desired; but we must emphasize that the examination of single isolated specimens of sputum may often give inadequate information.

The strains of *Staph. aureus* isolated before treatment were usually sensitive to the tetracyclines and invariably to sigmamycin. Some strains isolated during and after treatment were resistant to these antibiotics. The nasal carriage rate was unaffected, as was the presence of *Staph. aureus* in the stools. In the second winter this organism appeared in 12 patients, 6 of whom were receiving oxytetracycline and 6 the dummy capsules. Ten of these strains were of phage type 42B/52/80, a common hospital type, and all were resistant to the tetracyclines. In none of the patients was there any clinical change attributable to staphylococcal infection. These patients had no direct contact with the usual out-patient or ward environments, but it was assumed that they had acquired this strain of staphylococcus during their periodic visits to the room used for interviewing them. Examination of the dust in this room revealed this organism, and it seems likely that these patients inspired it in dust. Reports of similar trials (May and Oswald, 1956) have referred to the danger of patients acquiring antibiotic-resistant strains of *Staph. aureus* following continuous chemotherapy. Apart from the above-mentioned episodes, this has not been our experience, perhaps because of the policy of keeping our patients away from the general hospital environment. We therefore believe that where it is possible to treat chronic bronchitic patients in their own homes this is highly preferable to admission to a general hospital.

In this series there was no evidence that overgrowth by yeasts resistant to tetracyclines was encouraged in the treated patients. Qualitative change of the intestinal flora did not occur even in patients with diarrhoea, and this symptom may have been due to a direct chemical effect on the intestinal motility by the tetracyclines. These findings suggest that opinions expressed (*Lancet*, 1954; Stuart-Harris, 1959) may overstress the danger of seriously altering the natural surface flora of the body by continuous small doses of therapy with broad-spectrum antibiotics.

It is of interest that three out of five patients with influenza-like illnesses during the trial showed proof of a virus aetiology of these episodes by a significant rise in serum antibody in their sera. However, as 11 other patients also had a rise in antibody titre to influenza virus without associated clinical illness some patients with chronic bronchitis appear to be able to overcome the experience of influenzal infection without the serious consequence of an acute exacerbation. These rises in titre occurred between December and March, 1959. Until the end of December, in Edinburgh there was no laboratory evidence of acute infections of the respiratory tract due to the influenza or adenoviruses. Early in 1959 there was ample laboratory evidence of a brisk outbreak of virus respiratory disease due to influenza virus A (Asian strain) and the B virus, but not to virus C. Despite this, a rise of antibody to virus C was found in three cases when the sera from our patients were examined.

Summary

Two double-blind trials of continuous daily tetracycline-oleandomycin and oxytetracycline treatment in patients with chronic bronchitis were carried out during the winters 1957–8 and 1958–9 respectively. Twenty-three patients were observed in the first trial and 75, including 20 from the former, were seen in the second.

The antibiotic-treated groups were greatly benefited as compared with the groups receiving "dummy" capsules. Improvement was judged on the basis of reduction in the number and severity of acute exacerbations, by eradication of *H. influenzae* and pneumococcus from the sputum, and by statistical comparison of sickness in the treated as against the untreated groups.

Serial sputum examinations were of value in the assessment of each patient's progress, but single specimens did not necessarily reflect the condition, since *H. influenzae* and the pneumococcus were not isolated from every specimen.

A significant rise in antibody titre to the influenza viruses was demonstrated in 14 patients during the second trial, but only 3 of them had clinical influenza. Subclinical infection in the remaining 11 did not obviously affect their bronchitis.

Side-effects were infrequent and not serious. Diarrhoea was mildly troublesome in 15 out of 47 patients receiving antibiotics.

Gross disturbance of normal surface flora of the respiratory and intestinal tracts was not evident. Antibiotic-resistant strains of coliform species and *Staph. aureus* occurred in some patients, mainly in those receiving antibiotics, but they were not significantly associated with clinical deterioration.

It was found practicable to manage the patients on an out-patient or domiciliary basis. This had the advantages of a lower overall cost than hospital treatment, which, in any case, carries with it the attendant dangers of superinfection with antibiotic-resistant and often highly virulent micro-organisms. It is therefore preferable, should exacerbations of bronchitis arise, to treat them in the home rather than to seek immediate admission to hospital. It is concluded that continuous daily oxytetracycline therapy in advanced cases of chronic bronchitis, though apparently expensive, is less costly than the treatment of exacerbation of the disease in hospital. It is also safe and can be recommended as a reliable method of preventing such exacerbations.

We thank Pfizer Ltd., Folkestone and Sandwich, for supplies of sigmamycin and terramycin, and for a generous grant to defray the expenses of the trials. We have to acknowledge assistance in the statistical analysis of Mr. I. D. Hill. The Chief Meteorological Officer, Edinburgh, and the Manager of the Ministry of Pensions and National Insurance, Edinburgh, kindly supplied information regarding climatic conditions and individual invalidism during the periods of both trials. We are grateful to Dr. E. Adler, Drs. A. and D. Cruikshank, Dr. M. Goldberg, Dr. G. W. Gordon, Dr. D. W. Maclean, and Dr. R. A. K. Ross for

referring patients to the infirmary. We are greatly indebted to Professors Robert Cruickshank and D. M. Dunlop for valuable criticism and help in the preparation of this paper.

REFERENCES

Brumfitt, W., Willoughby, M. L. N., and Bromley, L. I. (1957). *Lancet*, **2**, 1306.
Buchanan, J., Buchanan, W. W., Melrose, A. G., McGuinness, J. B., and Price, A. U. (1958). Ibid., **2**, 719.
Edwards, G., and Fear, E. C. (1958). *Brit. med. J.*, **2**, 1010.
Elmes, P. C., Fletcher, C. M., and Dutton, A. A. C. (1957). Ibid., **2**, 1272
English, A. R., McBride, T. J., van Halsema, G., and Carlozzi, M. (1956). *Antibiot. and Chemother.*, **6**, 511.
Fairbrother, R. W., and Southall, J. E. (1957). *Lancet*, **2**, 974.
Garrod, L. P. (1957). *Brit. med. J.*, **2**, 57.
Gould, J. C., and Bowie, J. H. (1952). *Edinb. med. J.*, **59**, 178.
Helm, W. H., May, J. R., and Livingstone, J. L. (1956). *Lancet*, **1**, 775.
Hers, J. F. P., and Mulder, J. (1953). *J. Path. Bact.*, **66**, 103.
Hewitt, D. (1956). *Brit. J. prev. soc. Med.*, **10**, 45.
Hill, A. Bradford (1955). *Principles of Medical Statistics*, 6th ed. Lancet Ltd., London.
Jones, W. F., jun., and Finland, M. (1957a). *New Engl. J. Med.*, **257**, 481.
—— —— (1957b). Ibid., **257**, 536.
Lancet, 1954, **1**, 450.
—— 1955, **2**, 601.
Mackie, T. J., and McCartney, J. E. (1953). *Handbook of Practical Bacteriology*, 9th ed. Livingstone, Edinburgh.
May, J. R. (1953). *Lancet*, **2**, 534
—— and Oswald, N. C. (1956). Ibid., **2**, 814.
Moyes, E. N., and Kershaw, R. A. (1957). Ibid., **2**, 1187.
—— and Kalinowski, S. Z. (1959). *Tubercle (Lond.)*, **40**, 112.
Mulder, J. (1956). *Proc. roy. Soc. Med.*, **49**, 773.
—— Goslings, W. R. O., van der Plas, M. C., and Cardozo, P. L. (1952). *Acta med. scand.*, **143**, 32.
Ogilvie, A. G., and Newell, D. J. (1957). *Chronic Bronchitis in Newcastle-upon-Tyne*. Livingstone, Edinburgh.
Oswald, N. C. (1958). *Recent Trends in Chronic Bronchitis*. Lloyd-Luke Ltd., London.
Rawlins, G. A. (1953). *Lancet*, **2**, 538.
—— (1955). *J. med. Lab. Technol.*, **13**, 133.
Reid, D. D. (1956). *Proc. roy. Soc. Med.*, **49**, 767.
Reid, L. M. (1954). *Lancet*, **1**, 275.
—— (1955). *Thorax*, **10**, 199.
—— (1956). *Proc. roy. Soc. Med.*, **49**, 771.
Rountree, P. M., and Rheuben, J. (1956). *Med. J. Aust.*, **1**, 399.
Sinclair, J. D. (1955). *Brit. J. Tuberc.*, **49**, 157.
Stewart, F. S. (1959). *Bigger's Handbook of Bacteriology*, 7th ed. Baillière, Tindall and Cox, London.
Stocks, P. (1959). *Brit. med. J.*, **1**, 74.
Stuart-Harris, C. H. (1959). Ibid., **1**, 1606.
—— and Hanley, T. (1957). *Chronic Bronchitis, Emphysema, and Cor Pulmonale*. Wright, Bristol.
Wallace, A. T., and Duguid, J. P. (1952). *Edinb. med. J.*, **59**, 200.
Welch, H. (1957). *Antibiot. Med.*, **4**, 20.
Williams, E. (1954). *Lancet*, **2**, 999.
Wolf, S., and Pinsky, R. H. (1954). *J. Amer. med. Ass.*, **155**, 339.

A grant to Tanganyika of £2,220 from Colonial Development and Welfare Funds for laboratory research into the sterilization of tsetse flies by irradiation has been approved by the Secretary of State for the Colonies. Experiments elsewhere with irradiation at the pupal stage of another species of fly demonstrated that activated cobalt so affected the reproductive capacity of both males and females that the latter could not reproduce, and, furthermore, normal females when mated with sterilized males produced only sterile eggs and did not usually mate again. Accordingly, if a sufficient number of pupae could be released over a sufficient period of time, the fly population would die out. These results suggest that the same method might be used against these flies, but it is first necessary to discover the stage of the insect's development at which it is most susceptible to sterilization. This entails working in a laboratory with insect pupae of known age, as near as possible to a good supply of tsetse flies, and the Colonial Pesticides Research Unit at Arusha will undertake this research.

THE CHROMOSOMES IN A CASE OF PURE GONADAL DYSGENESIS

BY

D. G. HARNDEN,* Ph.D.

M.R.C. Radiobiological Research Unit, Harwell, Didcot, Berks

AND

J. S. S. STEWART, M.B., Ch.B.

Lecturer in Surgery, University of St. Andrews, Queen's College, Dundee

The normal chromosome number in man is now firmly established to be 46 (see, for example, Tjio and Levan, 1956). There are 44 autosomes, and 2 sex chromosomes which are designated XX in the female and XY in the male. In human intersexes the chromosome constitution may be abnormal, as in chromatin-positive Klinefelter's syndrome, where there are 44 autosomes and XXY sex chromosomes (Jacobs and Strong, 1959), and in chromatin-negative Turner's syndrome, where there are 44 autosomes and only one X chromosome (Ford *et al.*, 1959a). It may, however, be indistinguishable from normal. In hereditary male pseudohermaphroditism (testicular feminization) there is an apparently normal male complement of chromosomes (Jacobs *et al.*, 1959). We report here the chromosomal constitution of a patient with the syndrome which is usually described as pure gonadal dysgenesis (see discussion on nomenclature below).

Clinical Appearance and Cytological Examinations

The patient is a tall eunuchoid female who presented, aged 19, with primary amenorrhoea. Before treatment, scanty axillary and pubic hair was present, breast development was absent, and daily urinary excretion of gonadotrophins was raised. The external genitalia were of the normal female type, the clitoris being normal in size. There was a normal-sized uterus, but no gonads could be palpated on vaginal examination. Oestrogen-replacement therapy resulted in breast development, and vaginal bleeding followed oestrogen withdrawal. An oral mucosal smear was chromatin-negative, and the leucocytes showed no drumsticks. Full clinical details are given by Stewart (1959).

A skin biopsy specimen was taken from the patient's thigh, using a local analgesic, and divided into two parts. One portion was fixed immediately for nuclear sexing and the other placed in sterile Glaxo medium 199 and transferred to Harwell, where a tissue culture was established. After two weeks cells of the actively dividing culture were treated with colchicine, and orcein-stained chromosome preparations were made. The technique is described in detail by Harnden (1959).

Tissue	Chromosome Counts (No. of Cells)						Sex Chromosomes
	<45	45	46	47	48	>48	
Skin biopsy 1 ..	1	—	52	1	—	—	XY
,, ,, 2 ..	1	1	42	1	—	—	XY

The cells of the first portion were found to be chromatin-negative, no sex chromatin being present in 100 suitable nuclei. Cultured cells of the second portion contained 46 chromosomes (see Table). Twelve cells were analysed

*Now at Medical Research Council Group, Department of Radiotherapy, Western General Hospital, Edinburgh, 4.