

Tetracycline levels in bronchial secretions

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SYNOPSIS The excretion of tetracycline HCl in sputum was studied in 42 hospital patients suffering from various respiratory disorders who were receiving oral tetracycline therapy of 1.0 gram daily in divided doses.

The tetracycline levels were estimated biologically by a cup-plate agar diffusion method using *B. cereus*. Two separate methods of sputum homogenization are described: enzymic liquefaction with pronase, a powerful proteolytic enzyme, and ultracentrifugation at 103,000 g for three hours which sediments the viscous mucoids leaving 80-90% as a clear, non-viscous supernatant. The two methods allowed duplicate assays on sputum samples and generally showed close agreement. The ultracentrifugation technique is favoured for normal purposes because of its simplicity and the avoidance of any biochemical interference.

A close correlation was shown between the tetracycline levels in sputum samples and the average serum level for each patient. Over the serum tetracycline range 1.0-5.0 µg/ml, the mean sputum tetracycline levels after centrifugation were 23-18%, and the average level was 0.50 µg/ml.

The levels found in sputum were compared with the minimum inhibitory concentration of tetracycline for strains of *H. influenzae* and *Strep. pneumoniae*. Eighty-six percent of 29 *H. influenzae* strains tested and all *Strep. pneumoniae* strains tested were sensitive to 0.5 µg/ml tetracycline. The remaining *H. influenzae* strains had a minimum inhibitory concentration of 1.0 µg/ml or higher, a tetracycline level in sputum which appeared to be outside the mean range at the oral dosage of 1.0 gram daily.

Hence it is suggested that a small proportion of patients with lower respiratory tract infections due to *H. influenzae* would be unresponsive, or only partially responsive, to this usual therapeutic range of tetracycline.

Bacterial infection is believed to be an important factor in influencing the prognosis of patients with chronic bronchitis (Reid, 1954; Fletcher, 1959 and 1965), and although its exact role in the acute episodes which characterize the clinical picture of the disease is not clear (Davis, Grobow, Tompsett, and McClement, 1961), antibacterial agents have been widely advocated and employed in treatment.

It is generally held that *Haemophilus in-*

fluenzae and, less certainly, *Streptococcus pneumoniae*, are the most important bacterial pathogens in bronchitis (Mulder, 1938; Mulder, Goslings, van der Plas, and Lopes Cardozo, 1952; May, 1953). As these organisms are sensitive *in vitro* to drugs of the tetracycline series, these antibiotics have been widely used in the treatment of chronic bronchitis. Many clinical and bacteriological studies of therapeutic trials with tetracyclines have been reported with varied results, and, although the

higher dose schedules have been most effective, complete elimination of *H. influenzae* from sputum has never been reported. There is no evidence to suggest that the persistent organisms are more resistant to tetracyclines and their survival may be due to failure to attain therapeutically adequate levels of tetracycline in the respiratory tract. Bronchial infections are essentially inflammations of the mucosal surface with little invasion of the tissues by the organisms (Hers and Mulder, 1953). The elimination of respiratory pathogens may, therefore, rely on the level of the antibiotic drug within the bronchial secretions.

The purpose of this work was to measure the tetracycline excretion in the bronchial secretions of patients receiving tetracycline orally in normal therapeutic dosage, and to relate these levels to the minimum inhibitory concentration (MIC) of tetracycline for strains of *H. influenzae* and *Strep. pneumoniae*. Preliminary studies were directed at finding a simple but satisfactory method of homogenization of sputum, which would allow accurate measurement of biologically active compounds.

Materials and Methods

PATIENTS

Forty-two hospital patients, suffering from various acute and chronic respiratory disorders and producing significant amounts of sputum, were studied. Each received 1.0 g tetracycline HCl daily in four divided doses. In some cases a loading dose of 2.0 g was given during the first 24 hours. Other drugs were given when necessary but the only other antibacterial agents were sulphonamides, which were administered to a few patients. The bacteriostatic effects of sulphonamides were neutralized in the assay procedure.

The patients were instructed to swallow the capsules whole rather than to chew them and to expectorate sputum from the chest with as little salivary contamination as possible. The sputum was collected over 24- or 48-hour periods, depending on the volume, in 250 ml screw-capped jars, and the samples were stored at 4°C for a maximum of 10 days before the assay. Venous blood samples were obtained from each patient on two or more separate days, and approximately three hours after a preceding tetracycline capsule. The serum was separated immediately and stored at -10°C until the assay.

TETRACYCLINE

Tetracycline hydrochloride in 250 mg capsules were used for administration to the patients.

Microcrystalline tetracycline hydrochloride¹ (Achromycin Lederle) was used in the preparation of standard solutions for the bio-assays.

ENZYMES

The following enzyme preparations were used: pancreatin (BDH), trypsin (Worthington Biochemical Corp., Freehold, N.J.), and Pronase-P (Kaken Chemical Company, Tokyo).

MEDIA

Difco Penassay agar was used in the tetracycline bio-assay. A fluid medium containing co-enzyme I (DPN) and haematin (Holt, 1962) was used for determining the antibiotic sensitivity (MIC) of the *H. influenzae* strains. For estimating the MIC of *Strep. pneumoniae* strains, a fluid medium with the following final composition was used:

Casamino acids (Difco Technical)	20.0g
l-cysteine	0.2g
1% CaCl ₂ ·6H ₂ O solution	1.0ml
Pot. dihydrogen phosphate	0.5g
Yeast extract (Difco)	5.0g
Sod. chloride	2.0g
l-tryptophan	5.0g
Distilled water to 1 litre	

The pH was corrected to 7.8, and the medium autoclaved for 15 to 20 min at 10 lb/sq inch. After autoclaving, sterile 50% glucose solution was added to give a final concentration of 0.5%.

BACTERIAL STRAINS

Twenty strains of *H. influenzae*² and two strains of *H. parainfluenzae* were freshly isolated from sputum on heated blood agar. The identification of these organisms was confirmed by their requirements for haematin and coenzyme I. The organisms were subcultured and maintained in a fluid medium (Holt, 1962). Nine stock strains of *H. influenzae* (NCTC 4560, 4842, 8143, 8465, 8468, 8469, 8470, 8472, and 8473), and two stock strains of *H. parainfluenzae* (NCTC 4101 and 7857) were similarly tested and maintained in this medium.

Eleven strains of *Strep. pneumoniae* were isolated from sputum. These were identified by their typical colonial appearance, the production of greening on blood agar plates, sensitivity to Optochin, and bile solubility. They were subcultured and maintained in the synthetic broth described above.

HOMOGENIZATION OF SPUTUM

As tetracyclines are acid- and alkali-labile compounds (McCormick, Fox, Smith, Bitler,

¹Kindly donated by Cyanamid of Great Britain Ltd.

²Eight strains were obtained through the courtesy of Professor B. Lacey.

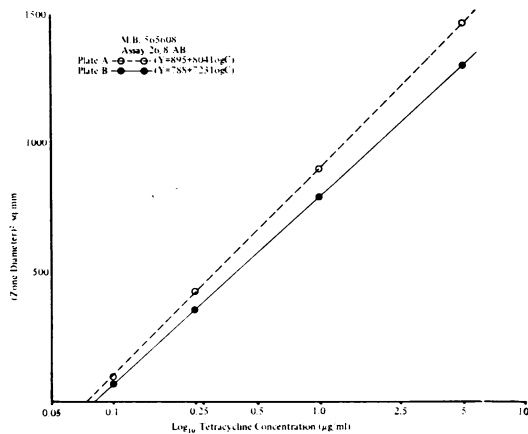


Fig. 1 Tetracycline concentration of four standard solutions.

Reichenthal, Origoni, Muller, Winterbottom, and Doerschuk, 1957), care must be taken during homogenization of sputum to preserve antibiotic activity. Proteolysis with several enzyme preparations, including 2% pancreatin (Rawlins, 1953), trypsin, and chymotrypsin, all gave unsatisfactory or variable results but consistently good homogenization was obtained with Pronase-P, an enzyme isolated from *Streptomyces griseus*. This enzyme has maximum proteolytic activity at pH 8.0 and an optimum temperature range of 40 to 60°C; calcium ions are essential for optimum activity (Nomoto and Narahashi, 1959).

Pronase was freshly prepared as a 10 mg/ml solution in tris-(hydroxy-methyl)-aminomethane (Tris)/HCl buffer, pH 8.1, at 37°C (Gomori, 1946), containing in addition 0.005M calcium chloride. Approximately 10 g aliquots of sputum were weighed into capped test tubes and 10% w/v Tris/HCl buffer was added. Pronase solution was then added to give a final concentration of 0.1 mg pronase/g sputum. The tubes were incubated at 42°C in a water bath for up to one and a half hours, until liquefaction was complete. These digests were then used directly in the bio-assay.

SEPARATION OF PHASES OF SPUTUM GEL

In chemical and structural studies on bovine cervical mucus, Gibbons (1959) found that centrifugation at 103,000 *g* for up to five hours separated the macromolecular glycoproteins forming the discontinuous phase of the gel from the continuous phase which forms a non-viscous supernatant solution. This method was found to be applicable to sputum.

Sputum samples were centrifuged at 103,000 *g* for three hours under continuous refrigeration. Non-viscous supernatants comprising 80-90% w/w of the original sputum specimens were

separated, and these were stored at -10°C until they were assayed. In some experiments the discontinuous phase functions were homogenized with pronase, using an enzyme concentration of 2 mg/g sputum and an incubation period of three hours.

ASSAY OF TETRACYCLINE

The tetracycline concentration in both serum and sputum samples was measured biologically by an agar diffusion method using the punch hole technique and with *Bacillus cereus* (NCIB 8012; ATCC 9634) as test organism (modified from Grove and Randall, 1955). Large assay plates (30 × 30 cm) were prepared with a base layer of 75 ml phosphate-buffered Difco Penassay agar (pH 5.6), and a top seeded layer, comprising 50 ml of the same agar, in which had been incorporated 2.9×10^4 /ml *B. cereus* spores. Seven-millimetre diameter wells were cut to an assay design template. The standard tetracycline solutions were prepared from micro-crystalline tetracycline HCl with 0.01N HCl to 1 mg/ml concentration, and then diluted with sterile distilled water to 10 µg/ml concentration. Aqueous dilutions of 5.0, 1.0, 0.25, and 0.1 µg/ml TC concentration were used as standard solutions in the assay. In some assays 10 mg/100 ml potassium para-aminobenzoate was added to the agar to neutralize the bacteriostatic effect of sulphonamides.

An 8 × 8 quasi-latin square design was used incorporating 12 separate test samples, and standard tetracycline solutions at four levels of concentration. This allowed four estimations at each level. Three standard drops (0.06ml) of each test sample were added to the appropriate well and the plate was allowed to stand at room temperature for one hour before incubation at 30°C for 16 to 20 hours.

The diameters of the inhibition zones around the wells were measured in two directions at 90° to each other and an average was recorded. Standard curves were constructed for each plate on semi-log paper. The concentration of each of the four standard solutions was plotted on the log₁₀ scale against the square of the mean diameter of the inhibition zones (Fig. 1). The concentrations in the unknown samples were then read off from these curves. Thus tetracycline levels were measured directly in the test solutions without attempting to allow for the variable protein concentration. A 10% correction factor was allowed for the dilution of the pronase-digested samples by the Tris/HCl buffer. Sterilization of sputum samples before bioassay was found to be unnecessary.

The lowest concentration of tetracycline consistently giving an observable inhibition zone around the wells was 0.09 µg/ml. Pronase digests of antibiotic-free sputum samples showed

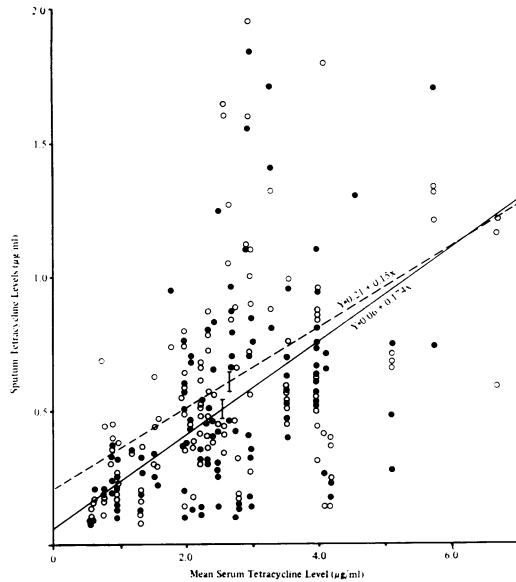


Fig. 2 Tetracycline levels in sputum compared with mean levels in serum from 42 patients.

Serum TC ($\mu\text{g/ml}$)	Pre-assay Treatment Methods			
	Ultracentrifugation ($y = 0.06 + 0.17x$)		Pronase Digestion ($y = 0.21 + 0.15x$)	
	Sputum TC ($\mu\text{g/ml}$)	% Serum Level	Sputum TC ($\mu\text{g/ml}$)	% Serum Level
1.0	0.23 (0.13-0.33) ¹	23 (13-33) ¹	0.36 (0.26-0.46)	36 (26-46)
2.5	0.50 (0.44-0.56)	20 (18-22)	0.59 (0.53-0.65)	23 (21-26)
5.0	0.93 (0.79-1.07)	19 (16-21)	0.96 (0.82-1.1)	19 (16-22)
7.5	1.36 (1.10-1.62)	18 (15-22)	1.34 (1.08-1.60)	18 (14-21)

Table Calculated values of tetracycline (TC) concentrations in sputum at particular TC levels in serum

¹95% limits in brackets.

an inhibition zone equivalent to a tetracycline level of not greater than 0.13 $\mu\text{g/ml}$. This activity was shown not to be a property of the pronase of Tris/HCl buffer but appeared to be a property of the proteolytic digest of sputum. The biological activity of tetracycline in Tris/HCl buffer between pH 5.0 and 8.75 was found to be substantially unchanged even after three days. The addition of pronase to an aqueous tetracycline solution in concentrations up to 100 mg% at 42°C for two hours also had no effect on the biological activity.

ESTIMATIONS OF MINIMUM INHIBITORY CONCENTRATION OF TETRACYCLINE

Haemophilus influenzae

The minimum inhibitory concentration (MIC) of tetracycline for strains of *H. influenzae* was measured in a fluid medium incorporating two-fold dilutions of standard tetracycline, beginning at 8 $\mu\text{g/ml}$ concentration. Of an overnight culture 0.02 ml was added and the tubes were incubated aerobically for 16 to 20 hours at 37°C. The turbidity due to growth was subsequently recorded. The end point was further defined by counting the viable organisms in the tubes about the end point. A hundredfold reduction in the count, compared with controls, was taken as evidence of inhibition of growth by tetracycline.

Streptococcus pneumoniae

Double-strength media (vide supra) was added (1:1) to two-fold serial dilutions of tetracycline solutions in a series of screw-capped bijou bottles, beginning with a concentration of 8 $\mu\text{g/ml}$. Of an overnight culture, 0.02 ml was then added and the tubes were incubated at 37°C for 24 hours. Turbidity due to growth was subsequently recorded. A sharp end point was obtained.

Results

OVERALL VARIATION IN TETRACYCLINE LEVELS IN SPUTUM

The tetracycline levels in the sputum and serum samples of all 42 patients studied are shown graphically in Figure 2. The daily sputum levels have been plotted against the average serum tetracycline level for each patient. The assay values in sputum samples treated by pronase digestion and ultracentrifugation are plotted separately and regression curves constructed for each method. Tetracycline was detected in all sputum samples obtained during oral therapy but the levels were low during the initial 24-hour collection period and often only reached a peak after 48 hours, so the results from samples taken in the first 24-hour period have been excluded from the statistical analysis.

The results show a fairly wide scatter about a broad mean, but the regression lines show a very close agreement between the tetracycline levels estimated after enzymic homogenization and those after ultracentrifugation. The average serum tetracycline level in 76 estimations was 2.68 $\mu\text{g/ml}$, with the majority of values falling in the range of 1.0-4.0 $\mu\text{g/ml}$. The average sputum tetracycline levels were 0.50 $\mu\text{g/ml}$ after ultracentrifugation and 0.61 $\mu\text{g/ml}$ after pronase digestion. The calculated mean levels

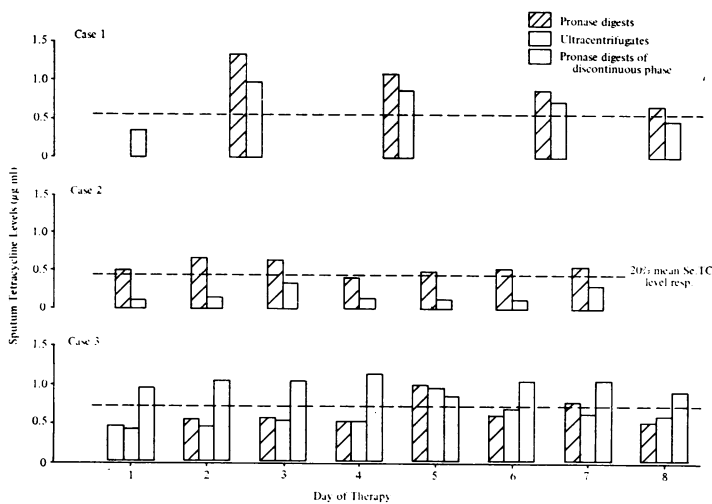


Fig. 3 Variation in sputum tetracycline levels.

at serum tetracycline values are shown in the Table. The levels were higher in the pronase treatment samples at the lower serum levels, but almost identical with those obtained after ultracentrifugation in the higher serum range. Over the range 1.0-5.0 $\mu\text{g/ml}$ in serum, the mean sputum levels were 0.23 to 0.93 $\mu\text{g/ml}$ after ultracentrifugation, and 0.36-0.96 $\mu\text{g/ml}$ after pronase digestion. These sputum tetracycline concentration ranges are 23-18% and 36-19% of the serum levels respectively.

VARIATION IN SPUTUM TETRACYCLINE LEVELS IN INDIVIDUAL PATIENTS

Although good agreement was obtained overall the variation in the sputum levels of tetracycline was found to be considerable even amongst samples from a single patient. This variation is illustrated in the following clinical examples. The results are shown in Figure 3.

Case 1

W.B., a man aged 50 years, was admitted to hospital following a road traffic accident with a fractured shaft of the right femur. He had a past history of a longstanding chronic productive cough and of recurrent winter bronchitis. The studies were performed during an acute episode of bronchitis. There was no evidence of any other disease. Blood samples were obtained on days 5 and 8 of treatment with tetracycline and the serum tetracycline levels were estimated as 2.75 and 2.6 $\mu\text{g/ml}$ respectively.

Case 2

The patient, M.B., was a woman aged 30 years, who had suffered from severe bronchiectasis for

several years. During this study she produced 15-45 ml of very tenacious purulent sputum daily. Cultural studies on the first sputum specimen demonstrated a pure growth of *H. influenzae*. This organism was not isolated at the end of the study but the culture plates were overgrown with a *Proteus* species. The serum tetracycline levels on days 3 and 6 were 1.9 $\mu\text{g/ml}$ and 2.6 $\mu\text{g/ml}$ respectively (average of 2.25 $\mu\text{g/ml}$).

Case 3

A 70-year-old man, F.M., an inpatient with a fractured shaft of the femur, had an acute exacerbation of chronic bronchitis while in hospital and was placed on oral tetracycline 250 mg four times daily. He produced moderate volumes of mucoid sputum daily. Serum tetracycline levels on days 4 and 7 were 3.8 and 3.3 $\mu\text{g/ml}$ respectively, an average of 3.55 $\mu\text{g/ml}$.

The maximum tetracycline levels in sputum were obtained in these examples by the second day, and thereafter remained fairly constant throughout the course of the study. The levels in samples after pronase digestion were usually higher than after ultracentrifugation, and markedly so in case 2. The tetracycline levels found in each patient correspond more closely when compared as percentage values of the respective average serum tetracycline levels.

In case 2 the tetracycline levels after pronase digestion, at 18 to 29% of the average serum level, were of the usual order found in sputum but the values in supernatant samples after ultracentrifugation, at 5 to 14%, were unusually low. These low levels presumably indicate that a high proportion of the tetracycline was sedimented with the discontinuous phase and possibly was protein-bound. The sputum from this patient was unusually viscid and the supernatant portion was only approximately 50% compared with the usual 80-90%.

The tetracycline concentration was estimated in the discontinuous phase of sputum samples in case 3. Assay after pronase digestion of the gel residue from ultracentrifugation gave a range of 0.86 to 1.14 $\mu\text{g/g}$, or 24 to 32% of the average serum level. These levels are consistently higher than the whole sputum measurements and suggest differential binding by the sedimented protein fraction. On the assumption that pronase releases the protein-bound tetracycline it was calculated for day 3 that 21% of the total tetracycline was sedimented in that sample. The removal of such a proportion of the tetracycline is seen to alter the level only slightly as assessed by assay after the use of the two different treatment methods.

MINIMUM INHIBITORY CONCENTRATION OF TETRACYCLINE

The MIC of tetracycline for 20 freshly isolated strains and 9 NCTC strains of *H. influenzae*

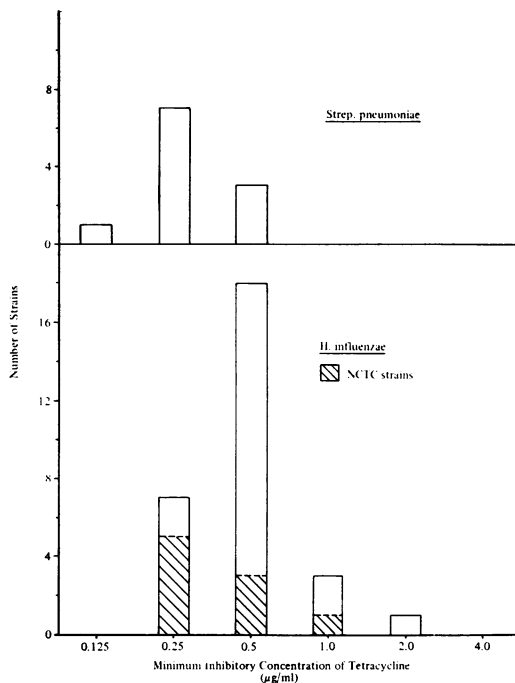


Fig. 4 Minimum inhibitory concentration of tetracycline from 11 strains of *Strep. pneumoniae*.

are shown in Figure 4. Seven strains were sensitive to 0.25 µg/ml or less and 18 to between 0.25 and 0.5 µg/ml. Thus 86% of all the strains were sensitive to 0.5 µg/ml. The MIC for three strains was 1.0 µg/ml and for one strain 2.0 µg/ml.

The results from 11 freshly isolated strains of *Strep. pneumoniae* are also shown in Figure 4. One strain was sensitive to 0.125 µg/ml or less, seven to 0.25, and three to 0.5 µg/ml tetracycline or less.

Discussion

Sputum consists of bronchial secretions variably contaminated with saliva and breakdown products of bacteria and leucocytes, including nucleoprotein. Bronchial secretions are believed to consist of a plasma exudate together with specific mucoproteins and mucopolysaccharides secreted by the bronchial glands. Albumin and other serum protein fractions have been identified in sputum but the proportions have varied in relation to the underlying disease (Brogan, 1960; Biserte, Havez, Voisin, Delahousse, Cuvelier, and Gernez-Rieux, 1961; Atassi, Barker, and Stacey, 1962; Gernez-Rieux, Biserte, Voisin, Havez, and Cuvelier, 1963).

It has been shown in normal human volunteers that with an oral dose of 250 mg four times daily, tetracycline HCl is non-cumulative, and produces a steady serum level at between 2.0 and 3.0 µg/ml (Lichter and Sobel, 1962; Putnam, Hendricks, and Welch, 1953 and 1954). Several estimates of the degree of protein-binding of tetracycline in the blood have been made but the results have varied widely. However, Wozniak (1960), in careful dialysis studies using radio-labelled H₃ tetracycline, estimated the protein-bound fraction to be 32 ± 4% in human plasma. This binding is believed to be readily reversible and not specifically with any particular protein fraction (Schach von Wittenau and Yearly, 1963). However, albumin probably contributes very largely to the protein-bound fraction, because of the large preponderance of this molecule. In studies using radio-labelled tetracyclines, Eisner and Wulf (1963) reported little difference between the tetracycline levels in serum as measured biologically and radiometrically. This suggests that the protein-bound tetracycline remains biologically active, possibly because of ready dissociation.

Kraus, Casey, and Johnson (1951) measured the salivary and plasma levels concurrently of both chlortetracycline and oxytetracycline and concluded that the plasma levels were four to 10 times those in the unstimulated saliva. Maynard, Andriola, and Prigot (1953-54) showed that in two patients receiving 1 g tetracycline daily the salivary levels were 0.22 and 0.84 µg/ml respectively. Since tetracycline is excreted in saliva and because there are no means yet available of estimating the degree of salivary contamination in sputum, it would have been preferable in this study to estimate tetracycline in uncontaminated bronchial secretions. However, these are rarely obtainable in any quantity and necessitate minor operative procedures.

The excretion of penicillin (May, 1955; Hafez, Stewart, and Burnet, 1965) and ampicillin (May and Delves, 1964 and 1965) in sputum has been extensively studied but little work has been reported on tetracyclines (May, 1964; Saggars and Lawson, 1968). Saggars and Lawson (1968) found no direct correlation between the serum and sputum levels in 12 children with extensive lung damage due to cystic fibrosis but the mean sputum level was 0.8 µg/ml tetracycline or 25% of the serum level. The sputum levels found in this study also ranged widely but have been found to correlate with the average serum tetracycline level obtained in each patient. Over the serum tetracycline range 1.0-5.0 µg/ml, the mean sputum levels were estimated as 23 to 18% of the serum level in ultracentrifugation samples, and 36 to 19% in samples treated by pronase digestion. These levels do not appear to differ substantially from those reported for saliva (Kraus *et al*, 1951; Maynard *et al*, 1953-54).

The two methods of homogenization of sputum described here were both found to be useful, and close agreement was found in the duplicate tetracycline assays. Despite the fact that up to 25% tetracycline may be sedimented by the ultracentrifugation technique, the level in the non-viscous supernatant was not appreciably lowered. For most purposes this technique would appear to be preferable in any study of biological activity in sputum because of its ease of operation and the avoidance of temperature and pH changes necessitated by the pronase proteolytic method. The substantially higher values found in the pronase-treated samples at the lower range of tetracycline concentration in sputum were probably due to the observed non-specific biological activity of the proteolytic digest.

Gibbons (1959 and 1961), in his studies on bovine cervical mucus, showed that the non-viscous supernatant resulting from ultracentrifugation contained small quantities of protein identical with serum proteins. As the high molecular weight mucopolysaccharides and mucoproteins are readily sedimented, the ultracentrifuged supernatant of sputum in this study, comprising 80-90% whole sputum, may be regarded as a plasma exudate variably contaminated with saliva. The serum protein content is small, but, even discounting this factor, the sputum supernatant tetracycline levels are well below the estimated non-protein-bound serum tetracycline levels, i.e. 64-72% total serum tetracycline levels using Wozniak's (1960) findings. Similarly the whole tetracycline levels, as measured after pronase digestion, are also low, but correspond to values found by Siggers and Lawson (1968). There are several possible explanations for these relatively low tetracycline levels found in sputum, including specific secretion of tetracyclines, inactivation by protein-binding, or degradation within the respiratory tract, but further facts are not available at present.

The minimum inhibitory concentrations of tetracycline strains for *H. influenzae* reported previously have shown considerable variation. Franklin and Garrod (1953) found that only 41% of 22 strains tested were inhibited by 0.5 µg/ml or less but all were sensitive to 1.0 µg/ml. Goslings, Valkenburg, and Los (1961) found that only 76% of 30 strains had an MIC of 1.0 µg/ml. Hirsch and Finland (1960) and May and May (1963) both found a higher MIC; only 54% of the strains they tested were sensitive to 1.6 µg/ml and 1.25 µg/ml respectively. In this study 86% had an MIC of 0.5 µg/ml or less and all but one of the strains were sensitive to 1.0 µg/ml. The *Strep. pneumoniae* strains were all sensitive to 0.5 µg/ml or less, an MIC which corresponds to that found by Rolinson and Stevens (1961). Kislak, Razavi, Daly, and Finland (1965), in a study of 200 recently isolated strains, found that 95% were sensitive to 0.4

µg/ml and all but one to 0.8 µg/ml.

In this study it has been shown that 86% of the *H. influenzae* strains tested, and all of the *Strep. pneumoniae*, were sensitive to 0.5 µg/ml tetracycline, which was the average level found in the sputum of patients receiving 1 g tetracycline daily by mouth. A sputum concentration of 1.0 µg/ml was outside the normal mean limits, and thus organisms with this MIC or higher are probably not controllable by oral tetracycline therapy at this dosage. Thus it can be postulated that if *H. influenzae* is implicated in the morbidity of chronic respiratory disorders, about 80 to 90% of patients should benefit from the oral treatment with tetracycline 1 g daily. For the remainder, or possibly all, to whom oral tetracycline therapy is given, a higher daily dosage should be employed. Conversely, by implication, it would appear that oral tetracycline therapy in dosage less than 1 g daily is unlikely to be effective in a large proportion of respiratory tract infections with *H. influenzae*. These conclusions appear to be borne out clinically by the variable efficacy of tetracycline in chronic bronchitis, and by the ready elimination of *Strep. pneumoniae*, but only partial elimination of *H. influenzae* in clinical trials of tetracycline at various dosage regimes.

I should like to thank Professor R. E. O. Williams and Dr T. D. Brogan for helpful criticism at all stages of this work, and also the consultant medical staff of St. Mary's Hospital, London, for permission to study patients under their care. I am indebted to Dr W. D. Brighton for the production of the fluid medium used in the sensitivity testing of *Strep. pneumoniae*.

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