EXPERIENCES WITH DOXYCYCLINE IN THE MANAGEMENT OF ACUTE RESPIRATORY TRACT INFECTIONS*

PETER JACOBS,† M.D. (RAND), F.C.P. (S.A.), *Research Assistant, Department of Medicine, University of the Witwatersrand, Johannesburg,* AND ROY ROBINSON, F.I.M.L.T., *Department of Bacteriology, South African Institute for Medical Research, Johannesburg*

Acute bacterial infection of the respiratory tract, arising either *de novo* or superimposed upon chronic chest disease, constitutes a major cause of morbidity and mortality. This problem is particularly severe in the winter months when an additional burden is placed on practitioners and hospitals alike, and is aggravated by the variety of pathogens encountered and the frequent emergence of resistant strains.

In an attempt to meet the challenge of bacterial mutation and drug resistance, new antibacterial agents are continuously marketed, each accompanied by claims that it possesses one or more advantages over those already in use. The majority of these drugs are carefully tested before their release; however, responsibility remains with those physicians using them, to further evaluate each agent in an attempt to define the incidence and significance of disadvantages and complications.

It is against this background that we have recently administered a new tetracycline antibiotic, doxycycline (Vibramycin), to a group of patients with acute respiratory tract infections, admitted to Johannesburg Hospital.

Doxycycline, chemically known as alpha-6-deoxyoxytetracycline, is a long-acting tetracycline antibiotic which is almost completely absorbed following oral administration,[1] is minimally influenced by the simultaneous administration of food,[2] has a long serum half-life,[2] and is said to attain high tissue levels.[3]

These interesting experimental observations, coupled with previous clinical studies,[4,5] suggested that doxycycline may have a place in the treatment of respiratory tract infection, particularly in those patients who find it inconvenient or who cannot be relied upon to take large numbers of tablets throughout the day. Accordingly, this study was undertaken to further document the clinical response of patients with acute bacterial respiratory tract infection and, in view of the long half-life, to examine the possibility that a build-up of serum antibiotic concentration might be taking place during a full course of therapy. At the same time, laboratory studies were planned to define serum and urine levels and to establish a minimum inhibitory concentration of doxycycline against the pathogens encountered.

MATERIAL AND METHODS

Thirty patients with acute respiratory tract infections were studied. In the majority of these there was underlying chronic chest disease in the form of recurrent bronchitis, with or without associated emphysema. Seven patients had pneumonia arising without any history of preceding chest infection, and 2 further patients suffered from uncomplicated bronchial asthma. The details of these patients are set out in Table I.

*Date received: 8 May 1968.
†Eli Lilly Medical Research Fellow presently Instructor in Medicine and Acting Director Clinical Haematology Service, University Hospital, Seattle, Washington 98105, USA.

On admission, sputum was collected from each patient for culture and sensitivity studies. Immediately thereafter, 200 mg. of doxycycline was administered orally, followed by 100 mg. every 24 hours for 5 days. In each instance the antibiotic was given to the fasting patient. Where necessary, therapy extended beyond the 5 days described in this paper. Minimum inhibitory concentration (MIC) required to prevent growth of bacteria for 24 hours at 37°C was determined by serial tube dilution in 1-ml. amounts of brain-heart infusion broth. All serial dilution tubes were inoculated with 0·02 ml. of an overnight broth culture diluted 1 in 500.

Doxycycline concentrations were determined by the cup-plate biological assay method; *Bacillus cereus* variant mycoides ATCC 9634 served as the test organism. Brain-heart infusion agar seeded with test organisms was poured into large assay plates to give an agar depth of approximately 4 mm. Plugs of agar were removed to give holes of 7 mm. diameter which were filled with the solutions to be assayed. The plates were then incubated overnight at 30°C. To compensate for variation in agar thickness, a mirror technique was used, and all standards and samples to be assayed were tested in duplicate holes diagonally opposed to one another.

A standard solution of doxycycline was diluted to a concentration of 8·0 μg./ml. in Sørensen's phosphate buffer solution M/15 at pH 4·5 and then in a diluent consisting of 1 part 7% bovine albumin fraction V and 2 parts of phosphate buffer to obtain a final concentration of 0·8; 0·4; 0·2 and 0·1 μg./ml.

Samples of serum were collected 3 hours after the initial dose on day 1 and an hour before the next dose was due to be administered on day 2. Further samples were collected one hour before the drug was given on day 5. Samples of serum and urine were kept in the deep-freeze for a period not exceeding 5 days before assay. Upon receipt, samples of urine were sterilized by passage through a membrane filter. The sera were initially diluted 1 in 3 with phosphate buffer and further dilutions made with albumin-phosphate buffered diluent to give final dilutions of 1 in 6 and 1 in 12; the albumin content of these dilutions was matched with that present in the standard. All urine samples were diluted 1 in 40 and 1 in 80 with phosphate buffer.

RESULTS

In each patient, response to therapy was assessed clinically, by fall in temperature, by resolution in radiological changes, by reduction in the volume of sputum produced and by alteration in its quality (Table I). In 24 of our patients the response, as judged by these criteria, was either good or excellent. In 3 further subjects the response was good, although somewhat slower than that seen in the majority of the subjects studied. One patient died before he could be assessed.

TABLE I. ANALYSIS OF RELEVANT CLINICAL AND BACTERIOLOGICAL DATA

Name, Age, Sex	*Clinical diagnosis*	*Radiological findings*	*Bacteriological growth*	*MIC µg./ml.*	*Clinical response*	*Sputum*	*Comments*
EW 72 F	Chronic bronchitis with emphysema. Superimposed acute infection	No abnormality detected	Normal flora	—	Slow, but eventually good. Temperature elevated for approximately 2 months	Purulent sputum gradually reduced over 2 months	Previously no response to Ceporan, ampicillin, Tetralysal or Colistin. Trichomycin necessary because of heavy fungal growth
JHS 63 M	Chronic simple bronchitis. Superimposed acute infection	Slightly increased basal transradiancy only	Haemolytic streptococcus *Streptococcus viridans*	0·2 0·39	Excellent response. Temperature elevated 24 hours	Purulent sputum for 48 hours	Right inguinal hernia. Chronic lymphatic leukaemia
PS 55 M	Simple chronic bronchitis. Superimposed acute infection	Some generalized transradiancy of the lungs	*Kleb. pneumoniae*	0·78	Excellent response. Temperature elevated 24 hours	Purulent sputum for 48 hours	Alcoholic cirrhosis with liver failure
DJB 67 M	Chronic simple bronchitis with emphysema. Right lower lobe broncho-pneumonia	Diminished diaphragmatic movement. Enlarged pulmonary artery. Patchy pneumonic consolidation in the lateral and anterior basic segments of the right lower lobe	*Staph. pyogenes* *E. coli* *Kleb. pneumoniae*	0·075 1·25 1·25	Excellent response. No temperature	Purulent sputum for 24 hours	Smoked 20 cigarettes a day for many years. Previous therapy with ampicillin had failed. Pulmonary thrombo-embolic disease, on anticoagulants
WJL 64 M	Simple chronic bronchitis. Superimposed acute infection	No abnormality detected	*Kleb. pneumoniae*	0·78	Excellent response. Temperature elevated 24 hours	Purulent sputum for 24 hours	—
BPW 16 M	Right lower lobe pneumonia	Consolidation of posterior basic segment of the right lower lobe. Resolution complete in 3 days	*A. wolffii*	0·018	Dramatic response. High temperature for 24 hours	Purulent sputum for 24 hours	Mentally defective
CL 59 F	Chronic mucopurulent bronchitis. Superimposed bilateral bronchopneumonia	Consolidation in the apical segment of the right lower lobe and basal segments of the left lower lobe	*Staph. albus* *Strep. viridans*	1·25 0·6	Good, but slow. High temperature for 36 hours, then settled over one week	Purulent sputum for 1 week. Then clear sputum for 2 further weeks	Ankylosing spondylitis
WJDP 70 M	Chronic simple bronchitis. Superimposed acute infection. Cor pulmonale	Emphysema and cor pulmonale. No evidence of infection noted	*Strep. viridans* *Staph. pyogenes* *Kleb. pneumoniae*	0·07 0·3 2·5	Good response. Temperature elevated 48 hours	Purulent sputum for 48 hours	Maxillary sinusitis. Previous therapy with ampicillin had failed
EH 76 M	Chronic simple bronchitis. Superimposed bilateral bronchopneumonia	Bilateral broncho-pneumonia, left base right base	*Kleb. pneumoniae* *Staph. albus*	0·78 0·075	Good response. Temperature elevated for 24 hours	Purulent sputum for 36 hours	Cor pulmonale
NJP 72 F	Simple chronic bronchitis. Left lower lobe broncho-pneumonia	Apical segment of left lower lobe consolidation	*Kleb. pneumoniae*	1·25	Good response. Temperature elevated 36 hours	Purulent sputum for 48 hours	Maturity onset diabetes. Cor pulmonale
HCA 60 M	Chronic mucopurulent bronchitis. Severe emphysema	Too ill to carry out	*Staph. pyogenes* *Strep. pneumoniae*	0·15 0·075	Died within 24 hours		Previous laryngectomy. Patient taken over by ENT unit and doxycycline stopped. Patient died within 24 hours of admission
ADJ 67 M	Chronic simple bronchitis. Superimposed acute infection	No abnormality detected	Haemolytic streptococcus	2·5	Good response. Temperature elevated 24 hours	Purulent sputum for 48 hours	—
JVDS 70 F	Chronic simple bronchitis. Superimposed acute infection	Patchy loss of trans-radiancy at both bases suggestive of infective changes	*Strep. pneumoniae*	0·15	Excellent response. Temperature elevated 24 hours	Purulent sputum for 24 hours	Cor pulmonale. Systemic hypertension
AJB 55 M	Chronic simple bronchitis	Loss of transradiancy at the right base and elevation of the right leaf of the diaphragm. Small right basal pleural effusion	*Staph. albus* Haemolytic streptococcus	0·075 0·018	Good response. No temperature	Purulent sputum for 24 hours	—
BD 68 F	Severe chronic muco-purulent bronchitis. Severe emphysema. Extensive basal bronchi-ectasis	Extensive radiological findings of basal bronchi-ectasis plus extensive superimposed consolida-tion	*Staph. albus*	5	No response. Temperature persists	Continues to produce large volumes	Resistant to all anti-biotics
MHK 41 F	Bronchial asthma	Mild hyperinflation	*Staph. pyogenes*	10	Excellent response. Temperature elevated 12 hours	Purulent sputum for 48 hours	—
CB 64 M	Bilateral basal broncho-pneumonia	Small right basal pleural effusion plus atelectasis. Hilar lymph nodes en-larged. No radiological evidence of inflammation	*E. coli* *Staph. pyogenes* *E. cloaca* *Strep. faecalis*	1·25 0·15 5 10	Good response. Temperature elevated 24 hours	Purulent sputum for 12 hours	Chronic lymphatic leukaemia. Uraemic

(Cont'd overleaf)

TABLE I CONT'D

Name, Age, Sex	*Clinical diagnosis*	*Radiological findings*	*Bacteriological growth*	*MIC µg./ml.*	*Clinical response*	*Sputum*	*Comments*
LDP 70 M	Simple chronic bronchitis plus emphysema. Bilateral basal bronchopneumonia	No abnormality detected	No specimens		Excellent response. Temperature elevated 24 hours	No sputum	Very heavy smoker for many years
JPB 53 M	Chronic simple bronchitis with superimposed acute infection—left basal pneumonia	Extensive left basal consolidation	*Staph. pyogenes* Haemolytic streptococcus *Strep. pneumoniae*	0·009 0·002 0·037	Excellent response. Temperature elevated 72 hours	Purulent sputum for 72 hours	Very heavy smoker. Very heavy alcohol intake
FWD 54 M	Simple chronic bronchitis. Superimposed acute bronchopneumonia	Partial resection right 6th rib, previous right upper lobectomy. Fibrotic changes at right base	*E. coli* *Strep. viridans* Haemolytic streptococcus	10 1·25 0·20	Excellent response. Temperature elevated 24 hours	Purulent sputum for 24 hours	Previously known pulmonary tuberculosis
RG 62 M	Chronic bronchitis plus emphysema. Multiple lung abscesses	Multiple abscesses located around the right hilum	Normal flora		No response. Temperature elevated 3 weeks	Purulent sputum for 3 weeks	No response to tetracycline —organisms insensitive. Following bronchoscopy the patient responded slowly to a long course of Ceporan and gentamicin
LP 92 F	Bilateral broncho-pneumonia	Extensive basal consolidation	Normal flora		Excellent response. Temperature elevated 48 hours	Purulent sputum for 48 hours	Cor pulmonale
JDW 62 M	Chronic mucopurulent bronchitis with emphysema. Superimposed diffuse acute bronchitis	Widespread and frequently changing signs of bilateral pulmonary infection	*Kleb. aerogenes*	6·25	Slow over 2 weeks	Purulent sputum for 2-3 weeks, then 3-4 weeks clear sputum	Myasthenia gravis. Cor pulmonale
HK 72 M	Bronchial asthma	Hyperinflation only	*Staph. albus* *E. coli*	3·125 25	Excellent response. Temperature elevated 24 hours	Purulent sputum for 24 hours	—
PH 46 F	Left lower lobe pneumonia	Left basal consolidation	Normal flora		Excellent response. Temperature elevated 36 hours	Purulent sputum for 48 hours	Alcoholic cirrhosis
IVH 68 F	Chronic simple bronchitis plus emphysema. Superimposed acute infection	Bilateral basal increased bronchopulmonary markings	*Strep. pneumoniae*	0·037	Good response. Temperature elevated 4 days	Purulent sputum for 1 week	—
JK 15 M	Right lower lobe pneumonia	Consolidation of the apical segment of the right lower lobe	No specimen		Excellent response. Temperature elevated 12 hours	No sputum	—
WFK 74 F	Chronic bronchitis with superimposed acute infection	No abnormality detected	*Staph. albus*	5	Good response. Temperature elevated 4 days	Purulent sputum for 4 days	Multiple pulmonary emboli, on anticoagulant therapy
LVB 75 F	Acute right mid-zone pneumonia	Right middle lobe pneumonia	Normal flora		Excellent response. Temperature elevated 48 hours	Purulent sputum for 72 hours	Vertebrobasilar insufficiency
NZ 72 M	Left basal broncho-pneumonia	Apical segment of left lower lobe consolidated	*E. coli*	2·5	Excellent response. No temperature	Purulent sputum for 24 hours	

Two of the 30 patients failed to respond. In the one individual, radiological evidence demonstrated the presence of lung abscesses from which organisms sensitive only to cephaloridine and gentamicin were isolated. Following bronchoscopy and parenteral administration of these agents, the patient made a slow return to health. In the second subject, large numbers of different organisms have been isolated and, despite the use of most of the commonly available antibiotics, either singly or in combination, it has been impossible to influence the course of this patient's disease. At present, she remains chronically ill from persistent chest infection with the continued production of large amounts of purulent sputum.

During the course of study, half of the patients fulfilling the criteria of acute respiratory tract infection were given doxycycline. During the same period of time, a similar number of patients with comparable conditions admitted to the other half of the unit received antibiotics such as ampicillin or combinations of penicillin and streptomycin or other tetracyclines in full therapeutic dosage. Although no direct comparison was made between these groups, clinical evidence and serial radiological studies indicated that there was no appreciable difference in response between the 2 series of patients.

Serum concentrations of doxycycline 3 hours after an initial dose of 200 mg. ranged from 0·8 to 7·8 µg./ml., with an average of 3·4 µg./ml. (Fig. 1). During the next 20 hours there was a slow decline in the serum concentration, with average levels of 2·5 µg./ml. being present 23 hours after the initial dose. Further assays after 5 days of therapy, using the regimen outlined above, showed a similar range of concentrations, again with an average of 2·5 µg./ml. (Fig. 1).

The average urine concentration of doxycycline 3 hours

after the initial dose was 31·6 μg./ml., and this declined to 21·2 μg./ml. after 23 hours. Further assays after 5 days of therapy gave average concentrations of 13·8 μg./ml.

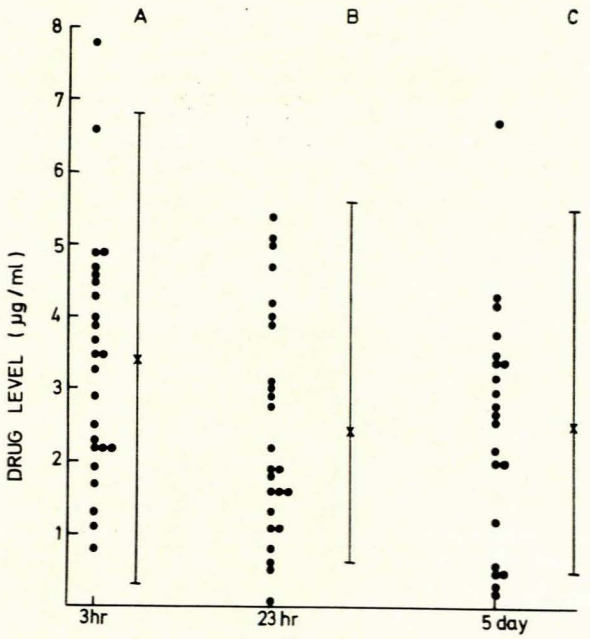

Fig. 1. Doxycyline serum levels (μg./ml.) 3 hours after initial 200-mg. dose (A), 1 hour before second oral dose of 100 mg. (B) and 1 hour before 100-mg. oral dose on day 5.

There were, as expected, instances of individual variation in absorption and excretion. Of interest was the one patient in this series who had a 3-hour level of 0·8 μg./ml. and in whose urine, at this time, doxycycline could not be detected. After 23 hours a concentration of 0·5 μg./ml. was obtained in the urine, while the serum concentration had declined further to 0·1 μg./ml. Serum and urine assays 5 days after therapy showed concentrations of 2·7 and 2·3 μg./ml., respectively. In 4 other patients serum concentrations were higher 23 hours after the initial dose than they were at 3 hours.

The 95% confidence limits, using the Student's t-test, showed a 3-hour serum concentration range of 0·03 - 3·4 - 6·8 μg./ml. After 23 hours this range had narrowed to 0·7 - 2·5 - 5·6 μg./ml., and it remained relatively constant after 5 days of therapy, when the limits were 0·5 - 2·5 - 5·5 μg./ml. These findings suggest that there is not any build-up of doxycycline in the serum after a full course of therapy.

DISCUSSION

Bacterial infections of the respiratory tract, although commonly associated with viral infections,[6] frequently require antibiotic therapy, and before any new agent gains acceptance it must be more effective, more convenient, cheaper or safer than those previously used. Doxycycline, a stable tetracycline derivative, has been demonstrated by this study to be effective in treating this type of respiratory infection. These findings are in agreement with those previously reported by Gilgore and Migliardi[4] and Fabre and Hany.[5] In addition, our experience suggests that doxycycline administered in the dosage defined above controls this type of infection as well as ampicillin or tetracycline, or combinations of penicillin and streptomycin. A further 50 patients have subsequently received doxycycline, and clinical response has, in each case, been good.[7] However, a formal double-blind trial will be required to demonstrate any statistical advantage of one drug over the other. In comparing ampicillin and doxycycline, the higher cost of the latter is offset, to some extent, by the fewer capsules required each day, and, excluding possible discomfort to the patient and inconvenience of administration, little difference in cost is likely to be present.

A previous study[2] has compared plasma levels in subjects given 100 mg. oral doxycycline in the fasting state with levels obtained after 300 mg. demethylchlortetracycline and contrasted these with the plasma levels obtained after simultaneous administration of food. This study showed that both drugs administered to the fasting patient resulted in high blood levels, doxycycline reaching a peak of 1·8 μg./ml. in 2 hours, and demethylchlortetracycline a peak of 1·99 μg./ml. at 4 hours. A decline of plasma concentrations in both instances was slow, and a concentration of more than 1 μg./ml. was still present 12 hours after the oral ingestion. Of central importance is the minimal effect on plasma levels of doxycycline produced by food, as opposed to the marked depression on demethylchlortetracycline when studied under similar conditions. Significantly, 15 ml. of antacid containing aluminium hydroxide administered simultaneously with either drug resulted in negligible blood levels.

Serum half-life of doxycycline in adults is 15·1 hours as compared with 12·7 hours for demethylchlortetracycline.[2] Other evidence has been cited[8] in which this value, calculated in premature infants during the first 3 weeks of life, ranged between 14·5 and 23·5 hours with an average of 20·2 hours. The value of 2·5 μg./ml. at 23 hours obtained in this study is of some interest, since it confirms that doxycycline has a long half-life and may well exceed the time reported by other workers.[2,3] However, although individual variation should be expected, it is not clear why the 23-hour levels obtained in this study should show such a degree of variation since the drug was, on each occasion, administered to the patient in a fasting state.

Doxycycline is highly bound to serum protein[2] as are other tetracyclines.[9] Thus, using a modified ultrafiltration method described by Bennett and Kirby,[10] figures of 93% were obtained for doxycycline, 91% for demethylchlortetracycline and 64% for tetracycline.[9] Following 200 mg. of doxycycline as an initial dose and 100 mg. every 24 hours thereafter, crude tissue levels estimated on samples collected during surgery were consistently greater than those present in simultaneously collected blood samples, and this was more obvious in specimens of lungs than in skeletal muscles.[3] Similar observations have been reported in dogs.[11] Although the measurement of tetracycline levels on tissues is difficult, these observations may have significance regarding the effectiveness of doxycycline when used in the treatment of bacterial infections of lung parenchyma.

It has been reported that there is no need to reduce the level at which doxycycline is administered orally in renal insufficiency, since it is not accompanied by elevated

plasma levels or by detectable toxic effect on the liver or the marrow.[5] Although it is not clear why a drug like doxycycline, which is excreted by the kidney and which has a long half-life, should not accumulate when renal function is disturbed, we believe that this feature is an important one and requires further investigation. Confirmation of these observations would suggest that doxycycline has a specific place in the treatment of bacterial infections accompanying renal insufficiency.

No side-effects were encountered in this study following the administration of doxycycline as outlined above. It might be anticipated that, with reduced calcium binding and the low dosage required in the treatment of pulmonary infection, staining of the teeth on long-term therapy may be somewhat less of a problem.

Despite evidence that the spectrum of activity of doxycycline is similar to that of other tetracyclines,[12] our own laboratory findings have demonstrated that an appreciable number of fresh isolates of *Staphylococcus pyogenes* from clinical material were sensitive to doxycycline when examined, using sensitivity discs of 30-μg. content, but less so to tetracycline hydrochloride, using discs of 50-μg. content. Since differing diffusion characteristics of the various tetracyclines make accurate comparisons by the latter method difficult, these results were confirmed by minimum inhibitory concentration studies, where at least an 8 - 10-fold difference in favour of doxycycline was consistently obtained. This trend, in lower minimum inhibitory concentrations obtained with the latter agent, has also been observed with *Staphylococcus pyogenes* strains sensitive to both analogues when determined by the disc method.

It is generally accepted that the emergence of bacterial drug resistance is due to mutants which have the ability to grow in the presence of antibiotics and so confer upon these emergent strains a selective advantage over the sensitive organisms. If, during treatment of a patient, the concentration of an antibiotic is allowed to drop below its minimum inhibitory concentration, it may be speculated that the organism will multiply, with the possibility that drug-resistant mutants will occur. When therapeutic levels are again achieved, the mutants will have developed a selective advantage and so be able to maintain the infection. However, in any theoretical consideration of this nature, it must be appreciated that bacteria have a well-defined recovery phase before multiplication again takes place, and for this reason further sophisticated studies will be necessary before specifically attributing to doxycycline, with its extended half-life and slow decline in serum levels, the added role of reducing the emergence of antibiotic-resistant mutants.

SUMMARY

Doxycycline has been administered to 30 patients with acute pulmonary infection over a period of approximately one year. In all but 2 of the patients the clinical response to a loading dose of 200 mg. a day and 100 mg. orally each day for 5 days thereafter has proved eminently satisfactory. The clinical response was paralleled by a fall in temperature and reduction in the amount of sputum produced, and was supported in each case by improvement shown on serial radiological studies. Bacteriological studies characterized the offending organism and defined minimum inhibitory concentrations of doxycycline required to prevent growth. A long half-life is confirmed, yet no evidence was obtained to suggest that this antibiotic accumulates significantly in the plasma. No side-effects were encountered in this study and it is concluded that, under these conditions, doxycycline is an efficient and convenient antibiotic, particularly in those patients where the number of tablets required daily is a significant factor.

We wish to thank Dr M. M. Suzman for allowing us to study patients admitted under his care; Drs A. Bass and N. R. Strachan for their assistance in the management of these patients; the nursing staff for their cooperation; and Dr H. van Wyk, Medical Superintendent of Johannesburg Hospital, for permission to publish. We should also like to thank Mr A. Edwards of Chas. Pfizer for kindly making available laboratory standards, for supplies of doxycycline and for personal and secretarial assistance.

REFERENCES

1. Fabre, J., Pitton, J. S. and Kunz, J. P. (1966): Chemotherapia (Basel), **11**, 73.
2. Rosenblatt, J. E., Barrett, J. E., Brodie, J. L. and Kirby, W. M. M. (1966): Antimicrob. Agents Chemother., p. 134.
3. Williamson, G. M. (1967): *International Symposium on New Resource in Antibiotic Therapy*. Buenos Aires: National Academy of Medicine.
4. Gilgore, S. and Migliardi, J. R. (1967): *Ibid.*
5. Fabre, J. and Hany, A. (1967): *Ibid.*
6. Edwards, G. (1966): Brit. Med. J., **1**, 963.
7. Morris, C. W. (1967): Personal communication.
8. Simon, H. J., Yaffe, S. J., Fontana, V. J. and Axline, S. G. (1966): Antimicrob. Agents Chemother., p. 121.
9. Bennett, J. V., Mickelwait, J. S., Barrett, J. E., Brodie, J. L. and Kirby, W. M. M. (1965): *Ibid.*, p. 180.
10. Bennett, J. V. and Kirby, W. M. M. (1965): J. Lab. Clin. Med., **66**, 721.
11. Schach von Wittenau, M. and Delahunt, C. A. (1966): J. Pharmacol. Exp. Ther., **152**, 164.
12. Barrett, J. E., Brodie, J. L. and Kirby, W. M. M. (1966): Papers read at the Second Inter-Science Conference on Antimicrobial Agents and Chemotherapy, Philadelphia, Pennsylvania.