BRITISH MEDICAL JOURNAL

LONDON SATURDAY JULY 13 1957

THE ERYTHROMYCIN GROUP OF ANTIBIOTICS

BY

LAWRENCE P. GARROD, M.D., F.R.C.P.

Bacteriologist, St. Bartholomew's Hospital; Professor of Bacteriology, University of London

The comparative value of a new antibiotic depends not only on its therapeutic usefulness but also on its relationship to others already in existence. If it differs little from them its discovery may not be of major importance: if it has entirely new properties it may represent a major advance. The discovery of erythromycin in 1952 must unquestionably be placed in the second of these categories. This antibiotic has a similar range of activity to that of penicillin, but there is no relationship between the two, or between it and the tetracyclines. It can hence be used when these earlier antibiotics are contraindicated either by reason of sensitivity in the patient (particularly to penicillin) or resistance in the organism (notably in staphylococci). Unfortunately staphylococci can also become resistant to erythromycin itself, and where it has been extensively used, particularly in the United States, resistant strains have become prevalent, even causing fatal infections on which no antibiotic had any effect (Martin, Nichols, Wellman, and Geraci, 1954). In the hope of preventing or delaying the development of such a situation in this country, it has been suggested that the use of erythromycin be restricted to really necessary indications. This warning has had an unprecedented effect, and has so far almost completely achieved its purpose.

Since 1952, several other antibiotics have been produced which stand in close relationship to erythromycin: they have a similar "spectrum" of activity and exhibit at least some degree of cross-resistance with it. These therefore require to be considered from two points of view: their therapeutic merits relative to those of erythromycin, and the possible effect of their use on bacterial sensitivity to it. If complete cross-resistance occurred, as between the three tetracyclines, any restrictive policy in the use of erythromycin should clearly also be applied to these others. In the order of their discovery these are:

Carbomycin.—This antibiotic is practically identical in its range of activity with erythromycin, but at a considerably lower level. Cross-resistance is close if not complete. Carbomycin, besides being less active, also attains lower concentrations in the blood (Hewitt and Wood, 1953) and, as would be expected from these two facts, is decidedly inferior in therapeutic action (Finland, Purcell, Wright, and Love, 1953). Hence it is not a serious competitor, at least for the treatment of most bacterial infections.

Spiramycin.—This antibiotic was discovered in France and appears to have been used there more than

anywhere else, although it has also undergone trials in the United States and is commercially available in this country under the name "rovamycin." Its *in vitro* activity is decidedly inferior to that of erythromycin; although the same species are susceptible, the relative degrees of activity differ somewhat: thus the approximate factors by which the activity of erythromycin is superior are $\times 16-32$ for *Staphylococcus pyogenes*, $\times 8$ for *Streptococcus pyogenes*, and $\times 4-8$ for *Streptococcus pneumoniae* (Garrod and Waterworth, 1956). There is cross-resistance with erythromycin when strains of staphylococci are habituated *in vitro* (Chabbert, 1955), but Hudson, Yoshihara, and Kirby (1956), in examining 117 strains of staphylococci, found only two resistant to both of these antibiotics, one resistant to spiramycin only, and 11 resistant to erythromycin but sensitive to spiramycin. It appears from these findings that cross-resistance is the exception rather than the rule in strains which have presumably become resistant *in vivo*.

Oleandomycin.—This antibiotic was first described under the designation P.A.105 by Sobin, English, and Celmer (1954-5), and for two years little more was heard of it. Recently it has appeared on the market under three names and in two forms: as pure oleandomycin ("matromycin," Pfizer; "romicil," Hoffmann-La Roche) and as a mixture with twice its weight of tetracycline ("sigmamycin," Pfizer).

The range of activity of oleandomycin, so far as it has been ascertained, corresponds closely to that of erythromycin, and cross-resistance between them in strains of staphylococci habituated in the laboratory was found by Sobin *et al.* (1954-5), by Fust, Böhni, Zbinden, and Studer (1956), and by Noyes, Nagle, Sanford, and Robbins (1956). On the other hand, according to Ross (1955-6) 22 erythromycin-resistant strains were "either quite sensitive or moderately sensitive" to oleandomycin; and Needham and Geraci (1956) found 54 out of 75 erythromycin-resistant strains to be sensitive to it. There is thus more extensive evidence than that cited in connexion with spiramycin to show that cross-resistance is not the rule in naturally occurring strains of staphylococci.

In trying to adjudicate on the merits of these various preparations, information seems desirable on the two following questions: (1) the activity of oleandomycin, alone and in combination with tetracycline; and (2) the closeness of the relationship between members of the whole group, from which it may be

deduced how far acquired resistance to each involves the others.

The following experiments were aimed at obtaining such information.

Materials and Methods

I am indebted to Charles Pfizer & Co. and to Hoffmann-La Roche Ltd. for supplies of pure oleandomycin. Other antibiotics were pure materials kindly supplied by their manufacturers.

Strains of staphylococci and other bacteria were recently isolated in St. Bartholomew's Hospital, except 44 strains of staphylococci the sources of which are specified later.

Medium, preparation of antibiotic solutions, methods of determining minimum inhibitory concentrations, and procedure for habituation were as described by Garrod and Waterworth (1956) except where otherwise specified.

In Vitro Activity of Oleandomycin

Of 58 strains of *Staph. pyogenes* initially tested for sensitivity to oleandomycin, 54 were inhibited by a concentration of 0.5 but not by 0.25 $\mu\text{g./ml.}$ The usual minimum inhibitory concentration (M.I.C.) of erythromycin as determined by this method is 0.12 $\mu\text{g./ml.}$: oleandomycin thus has one-quarter of the activity of erythromycin against this species. Of the remaining four strains, two were inhibited by 0.25 $\mu\text{g./ml.}$, and two were resistant: these strains were also resistant to erythromycin.

A few tests were done with two other species. Each of six strains of *Str. pyogenes* of group A, and four out of six strains of *Str. pneumoniae* (the remaining two being somewhat more resistant) were inhibited by an oleandomycin concentration of 0.25 $\mu\text{g./ml.}$ These findings again indicate a degree of activity inferior to that of erythromycin, the difference for both species being about eightfold (cf. Garrod and Waterworth, 1956).

Combined Action of Oleandomycin and Tetracycline

The claim that a 2:1 mixture of tetracycline and oleandomycin ("P.A.755," "sigmamycin") exerts a synergic action on staphylococci is made by English, McBride, van Halsema, and Carlozzi (1956). Their *in vitro* tests were made with 22 strains, and for nine of these the M.I.C. of the combination is significantly lower than that of either of its constituents separately. The differences are most striking when the organism is abnormally resistant to one of the two antibiotics.

Several series of tests were made to examine this claim, both on solid and in liquid media. They included initially the 56 strains with normal sensitivity to oleandomycin referred to in the preceding section: 45 of these were also normally tetracycline-sensitive. Fourteen further strains were tested which were abnormally resistant to one or other of these antibiotics. It is unnecessary to present the results in detail, because they are covered by the statement that not in a single instance was the M.I.C. of the combination lower than that of one of its constituents acting alone.

Through the courtesy of Dr. W. Williams, of Chas. Pfizer & Co., and of Drs. van Halsema and English, of the Pfizer Therapeutic Institute, Maywood, New Jersey, I obtained, first, a culture of the strain "Canada 1475," against which synergic action was particularly striking in the findings of English *et al.* (1956), and, later, particulars of how their tests were made. According to these authors the M.I.C. of tetracycline, oleandomycin, and P.A.775 for the strain "Canada 1475" were 8.3, 4.12, and 0.19 $\mu\text{g./ml.}$ respectively. Repetition of this test in my laboratory, using a different broth and a somewhat smaller inoculum, gave M.I.C. of 0.25, 0.5, and 0.25 $\mu\text{g./ml.}$ This strain appears to have lost the resistance to both antibiotics which it possessed when originally tested.

The inoculum used by English *et al.* was 0.5 ml. of a 1 in 1,000 dilution of broth culture, and their medium was Difco brain heart infusion broth, of which they state: "Final pH in our laboratory was 7.0." Some of this medium was obtained: it is labelled as having a pH of 7.4, and in fact had one of 7.6 after being dissolved and autoclaved. It was used both at its natural pH and adjusted to pH 7.0. The above inoculum was used (added to 4.5 ml. medium + 0.5 ml. antibiotic solution), and results were read as presence or absence of turbidity after overnight incubation. The M.I.C. so determined for strains sensitive to both antibiotics and resistant to each and both are stated in Table I.

TABLE I.—Sensitivity to Tetracycline, Oleandomycin, and P.A.775

Strain	Sensitivity Pattern	Minimum Inhibitory Concentration ($\mu\text{g./ml.}$)		
		Tetracycline	Oleandomycin	P.A. 775
Recent isolate ..	Normal	0.12 (0.25)	2	0.25
" " " "	"	0.12	2 (1)	0.25
"Canada 1475"	"	0.12 (0.25)	2 (1)	0.25
Recent isolate ..	Tetracycline-resistant	64	2 (1)	4 (2)
" " " "	"	128	2 (1)	8 (4)
" " " "	"	64	2 (1)	4 (2)
" " " "	"	8	1	2 (1)
" " " "	Oleandomycin-resistant	0.12	16 (32)	0.12
No. 83*	Erythromycin-resistant	0.25	2 (1)	0.12
" 88*	Tetracycline- and oleandomycin-resistant	32	32 (16)	16
" 95*	" "	64 (128)	32 (16)	32

* For source and other characters see Table III.

M.I.C. are those in Difco brain heart infusion broth at pH 7.0; the figures in parentheses are those in same medium of pH 7.6, when the result differed.

As before, the M.I.C. of P.A.775 was usually the same as that of the more active of its two components, or one tube higher. In only two instances, strains 83 and 88, was it lower, and then only by one tube, a result to which no unequivocal significance can be attributed. These experiments have therefore failed to demonstrate the synergic action claimed for the tetracycline-oleandomycin combination.

Habituation to Tetracycline, Oleandomycin, and P.A.775

It is also claimed by English *et al.* (1956) that a proceeding of habituation which causes a large increase in resistance to either tetracycline or oleandomycin has little effect when the two are combined.

Six strains of *Staph. pyogenes* were habituated to each antibiotic and to the combination by 16 transfers in increasing concentrations in broth, and finally tested for sensitivity to all three. The results, which were broadly consistent among the six strains, are summarized in Table II. The increased resistance obtained to each single antibiotic, which

TABLE II.—Habituation to Tetracycline, Oleandomycin, and P.A.775 of Six Strains of *Staph. pyogenes*

Habituated to	-Fold Increase in Resistance to		
	Tetracycline	Oleandomycin	P.A.775
Tetracycline ..	16-64	0	2-4
Oleandomycin ..	0	32-256	0-2
P.A.775 ..	16-32	0-2	4-8

was somewhat larger to oleandomycin, was unaccompanied by a change in sensitivity to the other, or by much change in sensitivity to the combination, since the second retained its activity. In habituation to P.A.775 the tetracycline component seemed to play the dominant part, and resistance to it increased almost as much as to tetracycline alone: sensitivity to oleandomycin changed comparatively little, and to the combination to an intermediate degree. It seems that the smaller amount of the weaker antibiotic fails to interfere effectively in the reaction of the organism to the major component in the combination.

Relationship between Oleandomycin and Other Antibiotics of the Erythromycin Group

The evidence already cited about cross-resistance between oleandomycin and erythromycin is conflicting. To explain these discrepancies it was necessary to examine a large number of strains, and I am much indebted for cultures of erythromycin-resistant strains of *Staph. pyogenes* to the following: Dr. S. D. Elek, St. George's Hospital; Professor R. Knox, Guy's Hospital; Dr. Mary Barber, St. Thomas's Hospital; Dr. E. J. L. Lowbury, Birmingham Accident Hospital; Dr. W. A. Gillespie, Bristol Royal Hospital; Dr. J. R. B. Williams, Lister Hospital, Hitchin; Professor R. W. Fairbrother, Manchester Royal Infirmary; and Dr. E. S. Duthie, Royal South Hants Hospital, Southampton. I am also much indebted to Dr. R. E. O. Williams, of the Central Public Health Laboratory, for determining the phage types of these strains and for supplying me with other strains of ascertained phage type.

The sources and properties of these strains and of four isolated in this hospital are stated in Table III. That a strain resistant to erythromycin may or may not be resistant to oleandomycin is fully confirmed. The strains isolated in four London hospitals, in Manchester, and in only 1 out of

the 14 of those from Southampton, were resistant to both antibiotics; the remainder from Southampton and those from Birmingham, Bristol, and Hitchin were highly resistant to erythromycin only, the M.I.C. of oleandomycin and spiramycin being either normal or only slightly raised. These two types of resistance are henceforward referred to as "double" and "dissociated."

Distribution of Resistance in Staphylococcus Populations

An indication had been obtained that erythromycin resistance of the dissociated type is not uniform throughout a culture, and experiments such as that in Table IV proved that this is so. When different quantities of a broth culture are cultivated in pour-plates containing different concentrations of erythromycin, a sharp end-point is obtained with a strain of double type: either every cell grows or none. A strain of the dissociated type, on the other hand, appears to contain cells of different degrees of resistance: the higher the concentration of erythromycin, the fewer are the colonies obtained: results with a small inoculum show that the highest degree of resistance is possessed by <1% of the cells. A culture derived from one of this small minority is uniformly resistant (last line of Table IV). It was found in

TABLE III.—Characters of Erythromycin-resistant Strains of *Staph. pyogenes*

No.	Source	M.I.C. (μg./ml.)			Sensitivity to Other Antibiotics*				Phage Pattern		Resistance Type
		Erythromycin	Oleandomycin	Spiramycin	Penicillin	Tetracycline	Streptomycin	Chloramphenicol	Diluted	1,000 × R.T.D.	
51	St. Bart's	16	16	64	0	+	0	+	53/75/77 +		Double
55	"	8	16	64	0	+	+	+	6/47/53/75 +		"
56	"	8	16	64	0	+	+	+	47/53/75/77		"
60	Guy's	16	32	64	0	0	0	+	80 + w	52/52A/80/53/77	"
62	"	8	32	64	0	0	0	+	80 + w	52/52A/80/53 +	"
64	"	8	32	64	0	0	0	+	80 + w	52/52A/80 +	"
65	"	8	32	64	0	0	0	+	80 + w	52/52A/80 +	"
70	St. Thomas's	8	16	64	0	+	0	+	75/77		"
71	"	8	16	128	0	+	+	+	29 +		"
72a	"	8	16	32	0	0	0	+	7/47/54/77 +		"
72b	"	8	16	128	0	+	0	+	6/75/77 +		"
73	"	8	16	64	+	0	+	+	7/42E/54/73 +		"
74	Birmingham	>64	2	4	0	0	0	+	53 +		Dissociated
75	"	>64	1	4	0	0	0	+	75/77		"
76	"	>64	1	4	0	0	0	+	7/54 +		"
77	"	>64	2	4	0	0	0	+	7/54 +		"
78	"	16	1	4	0	0	0	+	29/52/52A/79/80/53/54 +		"
79	"	>64	2	4	0	0	0	+	53w	29/53/73	"
80	"	>64	2	4	0	0	0	0	53 +	29/52/52A/79/80/53/75 +	"
81	"	64	2	4	0	+	0	0	6/47/75		"
82	"	8	1	4	0	0	0	+	53 + w	29/52/52A/79/80/53 +	"
83	"	>64	2	4	0	+	0	+	6/47/75		"
84	"	>64	2	4	0	0	0	0	7/54 +		"
85	"	>64	2	4	0	0	0	+	53 +	29/52/52A/79/80/53 +	"
86	"	>64	2	4	0	0	+	+	80	80 +	"
87	"	1	4	0	0	0	0	+	75/77		"
88	Manchester	4	16	64	0	0	0	0	47/54/73 +		Double
90	Southampton	32	1	8	0	0	0	+	80/53/77v.w.		Dissociated
91	"	32	1	8	0	0	0	+	80/53/77v.w.		"
92	"	32	1	8	0	0	0	+	80v.w.	80/53/77 +	"
93	"	64	1	8	0	0	0	+	80		"
95	"	8	16	64	0	0	+	+	80		Double
96	"	16	0.5	8	0	0	0	+	80/53/77		Dissociated
97	"	>64	1	8	0	0	0	+	—	80/53/77 +	"
98	"	16	1	8	0	0	0	+	80/53/73/77		"
99	"	64	1	8	0	0	0	+	80/53/77		"
101	"	>64	4	8	0	0	0	+	80/53/77		"
102	"	32	1	8	0	0	0	+	80 53/77		"
103	Bristol	4	1	8	0	0	0	+	53 75/77		"
104	"	4	0.5	8	0	+	0	+	53/75		"
105	"	4	0.5	8	0	+	0	+	53/75		"
106	"	4	0.5	8	0	+	0	+	53/75		"
107	St. George's	8	16	32	0	0	0	+	53/77 +		Double
108	Hill End Hospital (St. Bart's)	>64	1	8	0	0	0	+	53w		Dissociated
109	Hitchin	>64	0.5	8	0	0	0	+	75/77 +		"

* Determined by agar diffusion method (cup). 0 = Resistant. ++ = Fully sensitive. + = Moderately sensitive.

TABLE IV.—Distribution of Erythromycin Resistance in Populations of Double and Dissociated Types

Strain	Dilution of Inoculum	Colonies per Plate in Medium Containing Erythromycin (μg./ml.)									
		Nil	0.25	0.5	1	2	4	8	16	32	64
73 (Double type)	1/100,000	++	++	++	++	++	++	0	0	0	0
	1/1,000	+++	++	++	+++	+++	++	++	++	++	++
84 (Dissociated type)	1/10,000	+++			+++	++	±500	±500	348	385	249
	1/100,000	++			248	75	24	18	12	3	7
	1/1,000,000	207	132	108	75	46	5	1	2	0	2
84 (Fully resistant variant)	1/100,000	++						++	++	++	++

++ and +++ = Innumerable colonies.

further experiments that growth in broth containing erythromycin in concentrations from 32 to as low as 0.0015 $\mu\text{g.}/\text{ml.}$ bred a population uniformly resistant to $>64 \mu\text{g.}/\text{ml.}$ Uniformly high resistance so produced was unstable: after six transfers in antibiotic-free broth much of it was lost.

Effect of Adding Erythromycin after Growth has Begun

These findings illustrate the behaviour of a population of the dissociated type in the presence of erythromycin: they afford no clue to its composition *qua* erythromycin resistance in normal medium. It seemed at least possible that so mixed and apparently unstable a population might include fully sensitive cells in the absence of the antibiotic. This was first investigated by adding erythromycin in different amounts at intervals after incubation of a broth culture had begun. The results in Table V typify those of several such experiments, and show that this suspicion was well founded. Whereas the time of addition of antibiotic up to five hours makes no difference to the growth of a strain of the double type, addition after two, three, or four hours enables much lower concentrations to arrest visible growth of one of dissociated type. It therefore seems that this type, growing in the absence of antibiotic, consists largely of more sensitive cells.

The mechanism of this effect was further investigated in a more complex experiment, which after several ineffectual trials of less lengthy proceedings had to be begun at 7 a.m. and terminated at 10 p.m. It comprised three cultures of strain 87 (dissociated), one containing no antibiotic, one having erythromycin (2 $\mu\text{g.}/\text{ml.}$) added at the start, and the third receiving the addition after four hours' incubation. Viable counts were made from each at intervals up to 15 hours on plain medium and medium containing 1 and 8 $\mu\text{g.}$ of erythromycin per ml., a picture being thus obtained not only of the rate of growth (or death) but of the proportion of resistant cells at each stage. Detailed results are omitted for the sake of brevity: they may be summarized as follows. The antibiotic-free cultures showed growth at a normal rate of cells of which only a minority were resistant. With erythromycin added at the start, growth was delayed, but consisted eventually entirely of resistant cells. The addition of erythromycin at four hours caused a progressive fall in the viable count during the ensuing nine hours, showing a 98% mortality in the predominantly sensitive population: the few almost exclusively resistant survivors ultimately resumed growth.

If so low a concentration of erythromycin has a bactericidal effect on them, it seems probable that the bulk of the

TABLE V.—Effect of Adding Erythromycin During Early Stages of Incubation

Strain	Time of Addition	Concentration of Erythromycin Added ($\mu\text{g.}/\text{ml.}$)							
		64	32	16	8	4	2	1	0.5
62 (Double type)	Zero	—	—	—	+	++	++	++	++
	After 2 hours	—	—	—	+	++	++	++	++
	" 3 "	—	—	—	+	++	++	++	++
	" 4 "	—	—	—	++	++	++	++	++
	" 5 "	—	—	—	++	++	++	++	++
87 (Dissociated type)	Zero	++	++	++	++	++	++	++	++
	After 2 hours	—	—	—	—	—	+	++	++
	" 3 "	—	—	—	—	—	++	++	++
	" 4 "	—	—	—	—	—	++	++	++
	" 5 "	—	—	—	—	+	+	++	++

Inoculum: 0.02 ml. of 1 in 500 broth culture in 5 ml. of medium. ++ = Full growth. + = Diminished growth. — = No visible growth after overnight incubation.

TABLE VI.—Effect of Presence of Erythromycin on Sensitivity of Dissociated Type to Oleandomycin and Spiramycin

Strain	Oleandomycin ($\mu\text{g.}/\text{ml.}$)						Oleandomycin ($\mu\text{g.}/\text{ml.}$) + 0.06 $\mu\text{g.}$ Erythromycin per ml.					
	0.5	1	2	4	8	16	0.5	1	2	4	8	16
70	++	++	++	++	+	—	++	++	++	++	+	—
77	++	+	—	—	—	—	++	++	++	+	—	—
80	+	+	—	—	—	—	++	++	++	+	—	—
81	+	—	—	—	—	—	++	++	++	+	—	—
87	+	—	—	—	—	—	++	++	++	+	—	—
103	+	—	—	—	—	—	++	++	+	—	—	—
	Spiramycin ($\mu\text{g.}/\text{ml.}$)						Spiramycin ($\mu\text{g.}/\text{ml.}$) + 0.06 $\mu\text{g.}$ Erythromycin per ml.					
	1	2	4	8	16	32	1	2	4	8	16	32
70	++	++	++	++	++	+	++	++	++	++	++	+
77	++	+	—	—	—	—	++	++	++	++	+	—
80	++	+	—	—	—	—	++	++	++	++	+	—
81	++	+	—	—	—	—	++	++	++	++	+	—
87	++	+	—	—	—	—	++	++	++	++	+	—
103	++	+	—	—	—	—	++	++	++	+	—	—

++ = Full growth. + = Diminished growth. — = No visible growth after overnight incubation on plates inoculated with a 1-mm. loopful of a 1 in 500 dilution of a broth culture.

TABLE VII.—Effect of Different Concentrations of Erythromycin on Sensitivity of Dissociated Type to Oleandomycin and Spiramycin

Strain	Erythromycin ($\mu\text{g.}/\text{ml.}$)	Oleandomycin ($\mu\text{g.}/\text{ml.}$)							Spiramycin ($\mu\text{g.}/\text{ml.}$)						
		0.5	1	2	4	8	16	32	1	2	4	8	16	32	64
81	Nil	+	+	—	—	—	—	—	++	+	—	—	—	—	—
	0.003	++	++	+	+	—	—	—	++	++	—	—	—	—	—
	0.015	++	++	++	++	+	+	—	++	++	++	+	—	—	—
	0.06	++	++	++	++	++	++	+	++	++	++	++	+	+	—
	0.25	++	++	++	++	++	++	++	++	++	++	++	++	++	++
87	Nil	+	—	—	—	—	—	—	++	+	—	—	—	—	—
	0.003	+	—	—	—	—	—	—	++	+	—	—	—	—	—
	0.015	++	+	—	—	—	—	—	++	++	+	—	—	—	—
	0.06	++	++	++	+	—	—	—	++	++	++	+	+	—	—
	0.25	++	++	++	++	+	+	+	++	++	++	++	++	++	—

Inoculum, etc., and symbols as in Table VI.

cells in an antibiotic-free culture of the dissociated type are normally sensitive. If this is so, erythromycin resistance of the dissociated type is conditioned by the presence of the antibiotic.

The examination of many colonies from such a culture failed to yield pure cultures of organisms of normal sensitivity.

Effect of the Presence of Erythromycin on Resistance to Other Antibiotics

Since it appeared that the presence of erythromycin is necessary for organisms of the dissociated type to exhibit resistance to this antibiotic, it became of interest to determine

FIG. 1.—Nutrient agar plate containing 1 $\mu\text{g.}$ oleandomycin per ml., surface-inoculated with strain 87, with three cups containing erythromycin solutions of 100 (top right), 50 (below), and 25 (top left) $\mu\text{g./ml.}$ Growth of all but very few colonies is inhibited within a few millimetres of the cups: in a zone surrounding this the erythromycin permits growth despite the presence of oleandomycin. Elsewhere oleandomycin inhibits it.

whether in these circumstances there is any change in resistance to related antibiotics. The experiment in Table VI shows that there is: in the presence of 0.06 $\mu\text{g.}$ of erythromycin per ml. the concentration of either oleandomycin or spiramycin required to inhibit the growth of each of five strains of the dissociated type is increased fourfold to eightfold; there is no effect on a strain (70) of the double type.

In further experiments the concentration of erythromycin as well as of the other two antibiotics was varied: all the strains in Table VI were so tested with similar results and those for two of them are stated in Table VII. With fourfold increases in concentration of erythromycin from 0.003 to 0.25 $\mu\text{g./ml.}$ there is a progressive increase in resistance to both oleandomycin and spiramycin.

The presence of a low concentration of erythromycin thus confers double resistance on an organism of the dissociated type. This highly paradoxical result has no parallel that I am aware of, and is not fully explicable in the present state of knowledge about this group of antibiotics and their mode of action. On the other hand, it serves to explain further the observations of Chabbert

(1956) on "antagonism" between erythromycin and spiramycin. This can be illustrated more strikingly than in this paper by placing erythromycin solution in cups in a plate containing an inhibitory concentration of oleandomycin: a strain of dissociated type then grows only in a zone surrounding the cups (Fig. 1).

When fully erythromycin-resistant variants (see above, under the heading "Distribution of Resistance in Staphylococcus Populations") of strains 80, 87, and 103 were cultivated in medium containing different concentrations of oleandomycin or spiramycin and much higher concentrations of erythromycin than those used in the preceding tests, it was shown that all concentrations of the latter up to 64 $\mu\text{g./ml.}$ enabled growth to take place despite the presence of concentrations of oleandomycin (16–128 $\mu\text{g./ml.}$) or spiramycin (32–256 $\mu\text{g./ml.}$) which were otherwise inhibitory.

Effects of Habituating Dissociated Type

Strains 77, 80, 81, 87, and 103 were transferred repeatedly in broth containing 64 $\mu\text{g.}$ of erythromycin per ml. After six transfers they acquired partial resistance to oleandomycin but none to spiramycin: after 12 transfers in either this concentration or one of only 0.1 $\mu\text{g./ml.}$ oleandomycin resistance became more pronounced and there was some increase in resistance to spiramycin.

Strains 87, 90, 91, and 92 were habituated to oleandomycin, and all became resistant to 512 $\mu\text{g./ml.}$ in eight transfers: sensitivity to spiramycin was unaffected. The same strains were habituated to spiramycin: all became resistant to 512 $\mu\text{g./ml.}$ both of spiramycin and of oleandomycin (Fig. 2). Thus habituation of this type to oleandomycin still leaves a dissociated form of resistance: habituation to spiramycin makes the organism resistant to all three antibiotics.

Attempts to Produce Dissociated Resistance Artificially

All authors are agreed that when staphylococci are habituated to one of these antibiotics *in vitro* by the usual proceedings, resistance also develops to the others. Naturally occurring strains may either have this type of resistance ("double") or be resistant to erythromycin only. What determines this difference is unknown, but the factors operative *in vivo* can presumably be imitated *in vitro*. The following fruitless attempts were made to identify them. Six normally sensitive strains having phage patterns corresponding to those of certain strains in Table III, kindly furnished by Dr. R. E. O. Williams, were habituated to erythromycin. Two of these had the phage patterns of strains 72, 80, 82, and 85, all of which had dissociated resistance. All six developed double resistance. It seems unlikely from this and from the distribution of phage

FIG. 2.—Nutrient agar plates containing (left) 512 $\mu\text{g.}$ oleandomycin per ml. and (right) the same concentration of spiramycin. Both inoculated with strains 87, 90, 91, and 92: those on the left half of the plate had been habituated to spiramycin by eight transfers in increasing concentrations, those on the right half to oleandomycin by the same process. When a strain of this type is habituated to this extent, exposure to oleandomycin produces resistance to that antibiotic only; exposure to spiramycin produces resistance to both.

patterns among strains of the two resistant types (Table III) that phage type is a factor.

The same six sensitive strains were transferred repeatedly in or on the following media with added erythromycin: on nutrient agar aerobically; on nutrient agar anaerobically; in broth at 20° C.; in broth at pH 6.0; in Ringer's solution at 30° C.; in serum; in plasma. In so far as resistance was developed, it was of the double type. So also was that of variants appearing after six days in massively inoculated pour-plates containing up to 8 µg. of the antibiotic per ml. Thus various conditions less favourable to growth *in vitro* and corresponding to some which may exist *in vivo*, do not alter the result of artificial habituation.

Sensitivity to E 129

Antibiotic E 129 shows a minor degree of cross-resistance with erythromycin and spiramycin in artificially habituated strains (Garrod and Waterworth, 1956). The 45 strains in Table III were tested against it: 30 were inhibited by 0.25 µg./ml. (the normal concentration), 14 by 0.5, and 1 by 2 µg./ml. (this last being the double type). The inclusion of erythromycin in the medium did not alter these results. These findings suggest an even more remote relationship between E 129 and the erythromycin group than those reported earlier.

Discussion

The Problem of Cross-resistance

Relationships in this group are evidently less close and more complex than those between the tetracyclines. This would not be supposed if only the effects of habituation in the laboratory were considered: authors cited earlier are unanimous in finding that artificially produced resistance involves other members of the group, and Jones, Nichols, and Finland (1956) have amply confirmed this. By producing a high degree of resistance to erythromycin, oleandomycin, spiramycin, or carbomycin, and studying resistance to all four during the process of habituation to each, they showed that resistance to all four increased to the same extent and at approximately the same rate.

In striking contrast with this uniform behaviour, it has been shown that erythromycin-resistant staphylococci recovered as such from patients may or may not be resistant to other antibiotics of this group. An extraordinary feature of the strains is that with a single exception all the strains from each source behaved in the same way. Disregarding for this purpose sources from which only a single strain was obtained, all those from three London hospitals were fully cross-resistant, while 14 from Birmingham, 4 from Bristol, and 10 out of 11 from Southampton showed resistance of the dissociated type. Even the single exception is accounted for, since it came from a patient who had been treated with spiramycin.

Inquiries which the donors of some of these strains have kindly answered for me about the mode of therapeutic use of these antibiotics in their hospitals have revealed no differences in such use or in the types of case treated that can be held accountable for the difference in effect on the organism. That all staphylococci from one hospital should behave alike would be expected if in fact they were of a single strain spreading by cross-infection, but some diversities in phage pattern show that this was not always so. Whatever the explanation of this consistency of behaviour within one hospital, an explanation of why resistance should sometimes embrace all three antibiotics and sometimes involve only erythromycin has yet to be found. Attempts to induce the dissociated type of resistance by various manoeuvres *in vitro* have failed.

The nature of the dissociated type of resistance is peculiar. The cells forming such a culture are evidently not stable mutants: it has been shown that in erythromycin-free medium the majority are quite sensitive, whereas a colony on medium containing the antibiotic yields descendants which are uniformly resistant. The presence of erythromycin, even in very low concentration, also converts these organisms

immediately to the cross-resistant state. This observation explains the findings of Chabbert (1956), which he interprets as indicating "antagonism" between erythromycin and spiramycin. This is not antagonism in the ordinary sense, but a kind of interaction of which, so far as I am aware, no previous example has been seen.

To what extent the clinical use of oleandomycin or spiramycin may produce bacterial resistance to erythromycin remains uncertain. In so far as resistance in the strains studied was attributable to a course of treatment, erythromycin was the antibiotic which had been used in almost all the patients: systematic study of the effects of administering either of the other two has been quite inadequate to provide the necessary information. It seems probable, and it would certainly be safer to assume, that cross-resistance may sometimes follow the use of any of the three. Granting that they can be placed in an order of merit, we thus have the unusual and unsatisfactory position that the use of an inferior drug can deprive a better one of its usefulness.

Merits of Individual Members of the Group

Spiramycin has a considerably lower activity *in vitro* than erythromycin, and oleandomycin one intermediate between them. In tests reported here, the activity of oleandomycin against the three perhaps most important species of bacteria was about one-quarter of that of erythromycin: Jones and Finland (1957), in tests of which no details are given, find this proportion to vary from one-quarter to one-thirty-second. These authors also report unfavourably on the antibacterial activity of the plasma of subjects to whom oleandomycin was administered. It is well known that *in vitro* and *in vivo* activity do not always correspond, but confirmation of *in vitro* results by the method used by Jones and Finland gives them added significance.

It is notoriously difficult to assess therapeutic value with any exactitude on the basis of clinical reports unless these deal with comparative trials. The considerable literature on the clinical use of spiramycin includes, so far as I am aware, no account of any adequate comparison with another antibiotic, and in particular with erythromycin. Reports on the clinical use of oleandomycin are few: apart from that of Ross (1955-6), already quoted, there are two emanating from Switzerland. Essellier and Keith (1956) and Siegenthaler, Keiser, and Hegglin (1956) treated 412 and 216 patients respectively suffering from a wide variety of conditions and are enthusiastic about the drug. The latter report success in treating urinary-tract infections caused by demonstrably resistant coliform species, and the former in diverticulitis and inoculation hepatitis: the improbability of any specific effect in such conditions suggests that the credit awarded to the antibiotic in connexion with other patients may sometimes have been misplaced. It can reasonably be concluded that neither spiramycin nor oleandomycin has been shown to be superior in therapeutic action to erythromycin: by the only criterion susceptible to accurate measurement, which is *in vitro* activity, they are both inferior to it, and the probability is that this inferiority has at least some counterpart in their action in the body.

The claim that a 2:1 mixture of tetracycline and oleandomycin (sigmamycin) has a synergic action on staphylococci *in vitro* has been examined and not confirmed. This finding is by no means put forward as final, but only as suggesting that more searching investigations of the combined action of these two antibiotics is called for if this claim is to be sustained, and might indeed well have been undertaken before the claim was made originally. The method used by English *et al.* (1956), and followed in my repetitions of their tests, is a very simple one: more information might well be obtained by one employing serial viable counts to determine the rate of bactericidal action. These authors also report therapeutic tests in mice in which the effect of a given dose of P.A.775 was compared with that of the amounts of tetracycline and oleandomycin contained in that dose. It

would be interesting to know the results of such tests in which the total dose, whether of individual components or of the mixture, was the same.

Synergic action between antibiotics of this type has been thought not to occur, but one other example has been reported by Oskam (1956): using a very unusual *in vitro* method, he claims to have demonstrated synergy between tetracycline and erythromycin. Substantiation of this finding by other methods might raise the question whether a mixture of tetracycline and erythromycin could perhaps be therapeutically superior to one of tetracycline and oleandomycin.

Summary

The *in vitro* antibacterial activity of oleandomycin has been found to be somewhat less than that of erythromycin. The merits of these two antibiotics and of spiramycin are considered, and the conclusion reached is that neither oleandomycin nor spiramycin has been shown to be therapeutically superior to erythromycin: it is probable that the ratios of *in vitro* activity are reflected to some extent in their action in the body.

The repetition of tests showing that a mixture of tetracycline and oleandomycin exerts a synergic action *in vitro* did not confirm the claims made.

Studies of cross-resistance among antibiotics of the erythromycin group showed that, whereas complete cross-resistance develops in strains of staphylococci habituated *in vitro*, erythromycin-resistant strains isolated from patients may or may not be resistant to oleandomycin and spiramycin. Efforts to identify the factors determining which type of resistance is developed were fruitless. The behaviour of strains resistant to erythromycin only was peculiar in several ways. The presence of this antibiotic in a culture medium conferred on them resistance to each of the others.

For not only the execution of much of this work, but for the conception of many of the experiments, I am indebted to my assistant, Miss Pamela M. Waterworth, whose experience in the study of antibiotics has been extensive and continuous since she first worked with penicillin in my laboratory in 1943.

REFERENCES

- Chabbert, Y. (1955). *Ann. Inst. Pasteur*, **89**, 434.
 — (1956). *Ibid.*, **90**, 787.
 English, A. R., McBride, T. J., van Halsema, G., and Carlozzi, M. (1956). *Antibiot. and Chemother.*, **6**, 511.
 Essellier, A. F., and Keith, J. (1956). *Schweiz. med. Wschr.*, **86**, 1311.
 Finland, M., Purcell, E. M., Wright, S. S., and Love, B. D., jun. (1953). *New Engl. J. Med.*, **249**, 310.
 Fust, V. B., Böhm, E., Zbinden, G., and Studer, A. (1956). *Schweiz. med. Wschr.*, **86**, 1245.
 Garrod, L. P., and Waterworth, Pamela M. (1956). *British Medical Journal*, **2**, 61.
 Hewitt, W. L., and Wood, J. P., jun. (1953). *New Engl. J. Med.*, **249**, 261.
 Hudson, D. G., Yoshihara, G. M., and Kirby, W. M. M. (1956). *A.M.A. Arch. Intern. Med.*, **97**, 57.
 Jones, W. F., jun., and Finland, M. (1957). *New Engl. J. Med.*, **256**, 115.
 — Nichols, R. L., and Finland, M. (1956). *Proc. Soc. exp. Biol. (N.Y.)*, **93**, 388.
 Martin, W. J., Nichols, D. R., Wellman, W. E., and Geraci, J. E. (1954). *Proc. Mayo Clin.*, **29**, 379.
 Needham, G. M., and Geraci, J. E. (1956). *Antibiot. Med.*, **3**, 334.
 Noyes, H. E., Nagle, S. C., jun., Sanford, J. P., and Robbins, Mary L. (1956). *Antibiot. and Chemother.*, **6**, 450.
 Oskam, H. J. (1956). *Acta med. scand.*, **155**, 335.
 Ross, S. (1955-6). *Antibiot. Ann.*, p. 600.
 Siegenthaler, W., Keiser, G., and Hegglin, R. (1956). *Dtsch. med. Wschr.*, **81**, 2074.
 Sobin, B. A., English, A. R., and Celmer, W. D. (1954-5). *Antibiot. Ann.*, p. 827.

The Wellcome Trustees have made a grant of £5,000 to McGill University, Montreal, for the purchase of books related to the history of medicine needed to fill important gaps in the Osler Library. The Trustees have also agreed to provide £1,200 annually for twenty-five years to assist the further development of this library. A new extension and reading-room for the library, to be known as "The Wellcome Camera," is to be provided by McGill University.

A TRIAL OF FIVE TRANQUILLIZING DRUGS IN PSYCHONEUROSIS

BY

M. J. RAYMOND, M.R.C.P.Ed., D.P.M.

C. J. LUCAS, M.B., M.R.C.P.

M. L. BEESLEY, B.M., D.P.M.

B. A. O'CONNELL, M.B., D.P.M.

Department of Psychiatry, St. George's Hospital, London

AND

J. A. FRASER ROBERTS, M.D., D.Sc., F.R.C.P.

Clinical Genetics Research Unit, Institute of Child Health,
The Hospital for Sick Children, Great Ormond Street, London

Prolonged or disabling anxiety and tension are non-specific accompaniments of many psychiatric disorders. Conflict and frustration may be important contributory factors, and where they can be defined and resolved symptomatic relief may follow. Hence the most effective approach may often be by psychotherapy, especially if the symptoms are of recent origin. As an additional measure, where distress is severe or prolonged drugs are often prescribed. Recently there has been a striking increase of available tranquillizers, as the new preparations are termed. In this paper we report the results of a controlled trial of certain drugs claimed to reduce tension. We are not here concerned with the use of any of these drugs in the treatment of psychotic conditions, for which purpose they are given in much larger dosage to patients under strict hospital supervision.

Among clinical trials which have been reported chlorpromazine in daily dosage ranging from 75 to 300 mg. has been claimed to be of value for the relief of tension in psychoneurotics (Garmany *et al.*, 1954; Winkelman, 1954; Azima and Ogle, 1954). Selling (1955) and Borrus (1955) report favourably on the use of meprobamate in tension states. The usual dose in Selling's series was four tablets a day (each tablet = 400 mg.) and the maximum dose he used was eight tablets a day. Lasagna (1956) found its hypnotic effect equal to that of phenobarbitone, while Hollister *et al.* (1956) found that meprobamate provided effective relief of anxiety symptoms in half or more of the patients treated. Encouraging results with benactyzine in treating psychoneurosis have been reported by Jensen (1955), Munkvad (1955), Davies (1956), Raymond and Lucas (1956), and Hargreaves *et al.* (1957). Seager and Leitch (1956), however, reported unfavourably on its use on a series of 13 patients. A dosage of 2 mg. thrice daily is in line with the recommendations of most workers.

Method

The patients were selected from those attending the psychiatric out-patient department of St. George's Hospital. They comprise a heterogeneous group of psychoneurotic disorders, the criterion of selection being the presence in the symptomatology of a tension component. They were patients who, in the opinion of the referring consultant, had a definite load of symptoms, and they were comparable to those patients for whom sedation is commonly and properly prescribed in general practice. During the trial period we attempted to include all such patients who attended the department. Each patient served as his own control, and was given six medicaments (five tranquillizers and a placebo) for successive two-week periods. He was told that he was taking part in a trial to find out which of several tablets was best for him. He was asked to record his judgment of