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SPIRAMYCIN: A REAPPRAISAL OF ITS ANTIBACTERIAL ACTIVITY

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Spiramycin was less active than erythromycin *in vitro* against sensitive strains of *Staphylococcus aureus*, but was as effective against staphylococcal infections in mice when the drugs were administered immediately after infection: spiramycin was relatively more effective in prophylactic experiments when the antibiotics were administered at 4 or at 6 hr before infection. Strains of staphylococci which had been habituated to either spiramycin or erythromycin *in vitro* showed complete cross-resistance to both antibiotics *in vitro*, but the majority of a number of erythromycin-resistant strains isolated from patients were sensitive to spiramycin *in vitro*. Spiramycin was effective against a number of such strains *in vivo*, but erythromycin had little effect against these infections. Investigation into the distribution of the antibiotics in the serum and tissues of mice showed that spiramycin was maintained in tissues (lung, liver, kidney, spleen and heart) at higher concentrations and for a longer period than erythromycin, although erythromycin was initially present in serum in higher concentrations. It is suggested that the efficacy of spiramycin against experimental infections is due to its persistence in the animal body.

Soon after the isolation of spiramycin (Rovamycin) in 1954 (Pinnert-Sindico, Ninet, Preud'Homme & Cosar), two years after the discovery of erythromycin (McGuire, Bunch, Anderson, Boaz, Flynn, Powell & Smith, 1952), it became apparent that, although the physical, chemical and biological properties of these two antibiotics were closely related, erythromycin was more active than spiramycin *in vitro*.

Chabbert (1955) demonstrated that the antibacterial spectrum of spiramycin corresponded closely with that of erythromycin, but that the latter was more effective *in vitro* against susceptible organisms. These findings were confirmed by Garrod & Waterworth (1956) and by Garrod (1957), who made extensive studies *in vitro* of the relative antibacterial activities of the erythromycin group of antibiotics. Jones & Finland (1957) found that spiramycin was less effective than erythromycin in tests in which the antibacterial activity of sera was measured *in vitro* after administration of these antibiotics to healthy human volunteers. As it had also been established that some degree of cross-resistance existed between the two substances, it is not surprising that it was concluded that spiramycin was far less effective than erythromycin (Garrod, 1957; Editorial, 1957).

On the other hand, Cosar (1956) reported that spiramycin was as effective as erythromycin against a number of experimental infections. Chabbert, Boyer, Saviard,

Boulingre & Hervé (1957) concluded that spiramycin was more effective in the treatment of experimental staphylococcal infections and, more recently, Benazet & Dubost (1959) reported that spiramycin was more active than erythromycin against streptococci and staphylococci in mice.

The dissimilarity of the two antibiotics was further emphasized by the diversity of results of various studies concerning the nature and extent of the cross-resistance existing between the two antibiotics. Although habituation *in vitro* to either of the antibiotics produced strains of bacteria completely resistant to the other antibiotic, the degree of cross-resistance reported from clinical studies was very variable. For example, while Garrod (1957) reported complete cross-resistance in one-third of the erythromycin-resistant strains examined in his study, Finland (1956) found very little cross-resistance between the two drugs, and Lowbury & Hurst (1959) noted only a slight reduction in sensitivity to spiramycin in about one-quarter of the erythromycin-resistant staphylococci used by them.

The results reported in this paper were derived from experiments designed to compare the relative antibacterial activities of spiramycin and erythromycin *in vitro* and *in vivo* and, if possible, to provide some explanation for the diverse results reported by various workers.

METHODS

In vitro

Bacteriostatic activity. Spiramycin and erythromycin were dissolved in aqueous methanol and serially diluted by 2-fold steps in ox-heart digest broth. Each tube was inoculated with 0.1 ml. of a 6-hr-old broth culture of *Staphylococcus aureus* and incubated at 37° C for 24 hr, when the minimal inhibitory concentrations (M.I.C.) were determined as the lowest concentrations of antibiotic which prevented growth visible to the naked eye.

The effect of serum on the antibacterial activity of the antibiotics was determined by adding horse serum to a final concentration of 10% or 20% to digest broth and by reading the M.I.C. as before. Similarly the effect of inoculum size was determined by inoculating series of antibiotic-broth solutions with varying dilutions of the infecting organism.

Bactericidal activity. Bactericidal tests were carried out by subculturing from tubes showing no growth in the bacteriostatic tests on to nutrient agar containing no antibiotic. After overnight incubation, the absence or presence of growth was noted.

The effect of the antibiotics on the viable counts of cultures was measured as follows. Three concentrations of spiramycin and erythromycin (equivalent to M.I.C.$\times$4; M.I.C.$\times$1; and M.I.C.$\times$0.25) were prepared in 45 ml. digest broth. For spiramycin these concentrations were 12.5, 3.125, and 0.8 μg/ml., and for erythromycin 1.25, 0.32, and 0.08 μg/ml., respectively. Each bottle of broth, and one containing no antibiotic, were inoculated with 5 ml. of a 24-hr-old broth culture of *Staphylococcus aureus* (Oxford) standardized to contain 10^6 organisms/ml. At intervals of 1, 2, 4, 7 and 24 hr after inoculation 1 ml. aliquots were diluted in 1/4 strength Ringer solution prepared from tablets supplied by Oxoid giving final salt concentrations in g/l. of NaCl 2.25, KCl 0.105, $CaCl_2$ 0.12, $NaHCO_3$ 0.05. Viable counts were made by a drop-plate method, modified from Miles, Misra & Irwin (1938) and readings were made after incubation at 37° C for 48 hr.

Habituation. Serial dilutions of spiramycin and erythromycin were made in digest broth, and the tubes were inoculated with 0.1 ml. of 18-hr broth cultures of *Staph. aureus.* After incubation at 37° C for 24 hr, subcultures were made, from the tubes containing the highest concentration of antibiotic in which there was obvious growth, into a fresh series of antibiotic-broth solutions. Transfers were made daily until resistance developed to the antibiotics.

Coagulase tests. Tubes containing 1 ml. volumes of fresh rabbit plasma, diluted 1/10 with saline, were inoculated with 0.1 ml. of 18-hr broth cultures of *Staph. aureus*, and incubated at 37° C in a water bath. Results were read after 0.5, 1, 3, 6 and 24 hr.

In vivo

Activity in mice

Therapeutic activity. Albino mice were infected intravenously with 0.2 ml. of 18-hr broth cultures of *Staph. aureus*, and dosed orally with 0.5 ml. of suspensions of the antibiotics, ½, 6, 24, 48 and 72 hr after infection. Deaths were noted daily for 10 days, when surviving mice were killed and examined macroscopically for the presence of abscesses on kidneys; all kidneys were cultured for staphylococci.

Prophylactic activity. Groups of mice were given orally a single dose of 0.5 mg/g of the antibiotics at 6, 4 or 2 hr or immediately before intravenous infection with, in the first experiment, 0.1 ml. of an 18-hr broth culture, and in the second experiment 0.5 ml. of a 10^{-1} dilution of an 18-hr broth culture of *Staph. aureus* (CN 491). Deaths were recorded daily for 7 days.

Antibiotic levels in tissues of mice

The methods adopted for the extraction and assay of the tissue antibiotic concentrations were similar to those used by Benazet & Dubost (1959) in their studies on the erythromycin group of antibiotics, except that the French workers used aqueous methanol in their extraction procedures, and preferred a filter-paper technique for the assay of antibiotic concentrations.

Mice were given a single oral dose of 0.5 mg/g of spiramycin or erythromycin, and groups of 10 were killed 2, 6, 24 and 48 hr after injection. Heart blood was collected from the groups of mice killed at 2 hr and 6 hr, and the pooled sera were assayed for antibiotic concentrations. The heart, liver, lungs, kidney and spleen were removed aseptically from the animals killed 6, 24 and 48 hr after dosing, and pools of each of these organs were weighed. Each of the organ pools was triturated with sterile sand (1 g/2 g tissue) and phosphate buffer, *p*H 8.0 (25 ml. for the pooled organs of each group, but 100 ml. for pooled livers), centrifuged, and the supernatant fluids assayed for antibiotic content.

The sera and extracts were assayed for spiramycin and erythromycin as described for erythromycin by Grove & Randall (1955), *Sarcina lutea* (N.C.T.C. 8340) being used as the assay organism. The extracts were added to antibiotic cylinders on Pen-assay agar (Difco), *p*H 8.0, pre-seeded with *Sarcina lutea*. Zone diameters were measured after incubation at 30° C for 24 hr, when the tissue antibiotic concentrations were calculated from standard curves.

RESULTS

Activity in vitro

The inhibitory concentrations of spiramycin and erythromycin to 45 strains of antibiotic-sensitive staphylococci are shown in Table 1. Erythromycin was more active than spiramycin, the growth of all 45 strains being inhibited by 1.0 μg/ml. or less of erythromycin, whereas only 7 of these strains were affected by similar concentrations of spiramycin. The average inhibitory concentrations of spiramycin

TABLE 1

BACTERIOSTATIC ACTIVITY OF SPIRAMYCIN AND ERYTHROMYCIN AGAINST 45 SENSITIVE STRAINS OF *STAPH. AUREUS*

		No. of strains inhibited. Minimal inhibitory concentrations (μg/ml.)									
Antibiotic	No. of strains tested	0·03	0·06	0·13	0·25	0·5	1·0	2·0	4·0	8·0	16·0
Spiramycin	45	0	0	0	0	2	5	17	16	4	1
Erythromycin	45	4	9	14	11	5	2	0	0	0	0

in this series were 2.0 to 4.0 μg/ml. of antibiotic, about 20-fold greater than those of erythromycin (average 0.1 to 0.2 μg/ml.).

In a series of experiments with antibiotic-resistant staphylococci (naturally resistant and derived from patients), the inhibitory concentrations of spiramycin against 18 out of 20 coagulase-positive, erythromycin-resistant strains were very similar to those found necessary to inhibit sensitive organisms (Table 2). The remaining two strains were inhibited by 25 μg/ml. and 32 μg/ml. of spiramycin.

TABLE 2

BACTERIOSTATIC ACTIVITY OF SPIRAMYCIN AND ERYTHROMYCIN AGAINST 20 ANTIBIOTIC-RESISTANT STRAINS OF *STAPH. AUREUS* DERIVED FROM PATIENTS

The sensitivity of the staphylococci to antibiotics other than spiramycin or erythromycin was determined by a filter paper disc–agar diffusion technique (R=resistant: S=sensitive)

Strain no.	M.I.C. (μg/ml.)		Antibiotic sensitivity			
	Spiramycin	Erythromycin	Penicillin	Streptomycin	Tetracyclines	Chloramphenicol
1	4·0	250	R	R	R	S
2	8·0	250	R	R	R	S
3	32·0	125	R	R	R	S
4	8·0	125	R	R	R	S
5	4·0	125	R	R	R	S
6	8·0	63	R	R	R	S
7	4·0	63	R	R	R	S
8	4·0	8·0	R	R	R	S
9	4·0	63	R	R	R	S
10	2·0	125	R	R	R	S
11	2·0	63	R	R	R	S
12	<1·0	4·0	R	R	R	S
13	3·2	3·2	R	R	R	S
14	12·5	12·5	R	S	R	S
15	12·5	6·3	R	S	S	S
16	25·0	25·0	R	S	R	S
17	2·0	16·0	—	—	—	—
18	8·0	125	R	R	R	S
19	<1·0	1·0	R	R	R	mod. S
20	2·0	125	—	—	—	—
21	2·0	0·1	S	S	S	S

Horse serum added to digest broth at concentrations of 10% and 20% had little or no effect on the activity of spiramycin and erythromycin, there being a 2- or 4-fold change in the M.I.C. at the most. Likewise, altering the inoculum size had little effect on the inhibitory concentration of either antibiotic, there being only a 4-fold difference between series of tubes inoculated with an 18-hr-old culture and those inoculated with a 10^{-6} dilution of the same culture.

The bactericidal effects of both antibiotics were also closely related (Fig. 1), so that equivalent multiples of the M.I.C. of each antibiotic produced similar changes in the viable counts of the Oxford strain of *Staph. aureus*. For example, 3.2 μg/ml. of spiramycin, equivalent to the M.I.C., prevented multiplication of the original inoculum of 10^{-5} organisms/ml. over a period of 24 hr, while 0.32 μg/ml. of erythromycin, also equivalent to the M.I.C., produced a similar effect. Likewise concentrations of M.I.C. × 4 of the antibiotics produced comparable falls in the numbers of organisms (Fig. 1).

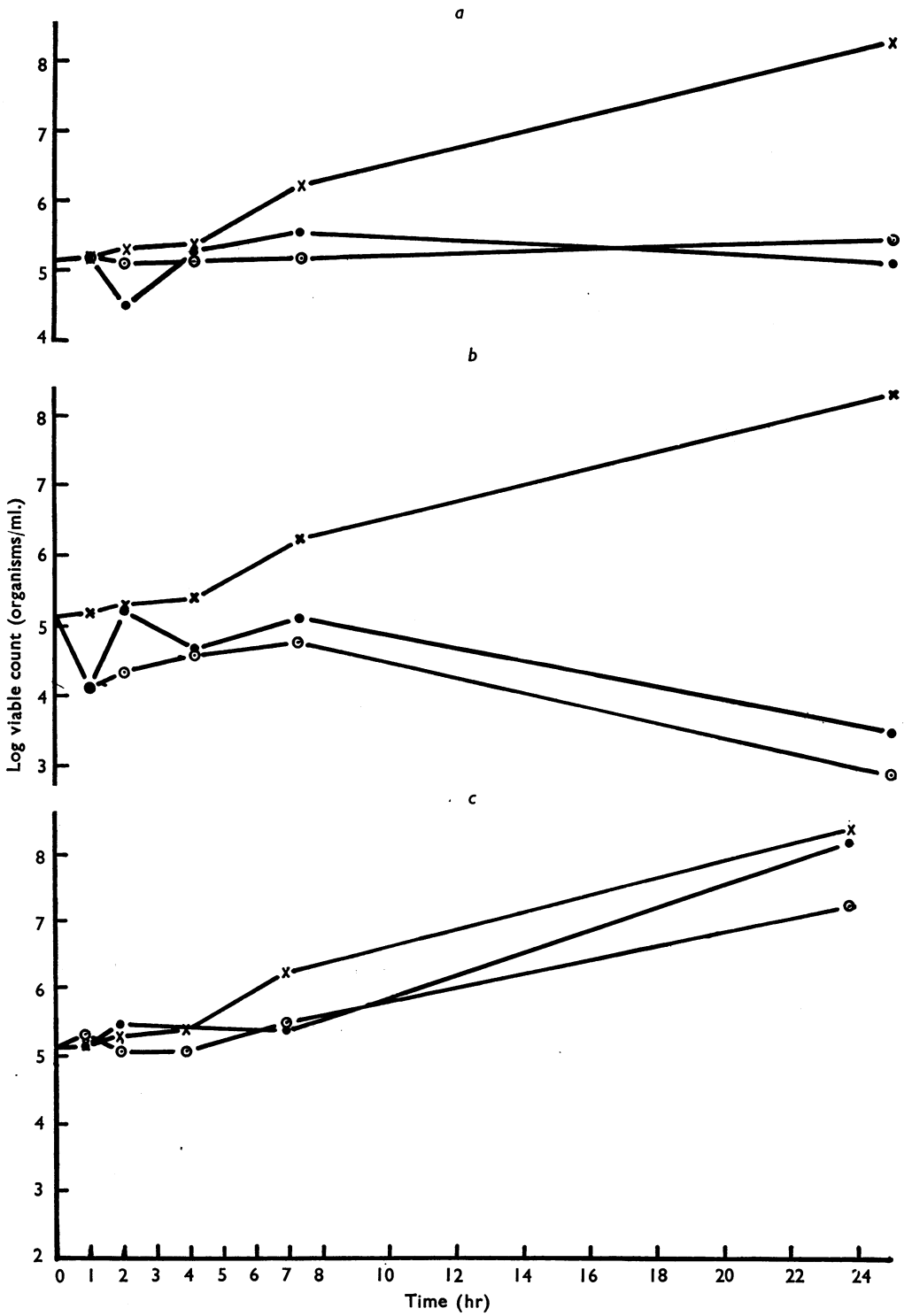

Fig. 1. The bactericidal effects of equivalent inhibitory concentrations of spiramycin (○——○) and erythromycin (●——●) against *Staph. aureus* (Oxford) *in vitro*. The line X——X denotes the growth over 24 hr of the control culture of *Staph. aureus*. (*a*) Minimal inhibitory concentrations (M.I.C.): spiramycin 3.2 μg/ml.; erythromycin 0.32 μg/ml. (*b*) M.I.C.×4: spiramycin 12.5 μg/ml.; erythromycin 1.25 μg/ml. (*c*) M.I.C.×0.25: spiramycin 0.8 μg/ml.; erythromycin 0.08 μg/ml. Ordinates: Log viable count (organisms/ml.). Abscissae: time in hr.

Similarly, subcultures taken at 24 hr from the bacteriostatic tests showed both antibiotics to be bactericidal at concentrations within one or two tubes of the bacteriostatic inhibitory concentrations.

Two sensitive strains of *Staph. aureus*, cultured in subinhibitory concentrations of either spiramycin or erythromycin, soon developed resistance to the antibiotics in which they were cultured, and highly resistant strains were obtained after 15 transfers in increasing concentrations of the antibiotics. These strains (made resistant in the laboratory) showed complete cross-resistance to both antibiotics, and it was noted that some of the characteristics of the resistant variants differed from those of the parent (sensitive) strains. In general (Table 3) these strains did not ferment mannitol, the coagulase reaction was less marked, and their pathogenicity to mice was reduced.

TABLE 3

CHARACTERISTICS OF STRAINS OF *STAPH. AUREUS* HABITUATED *IN VITRO* TO EITHER SPIRAMYCIN OR ERYTHROMYCIN

Mannitol fermentation was determined on mannitol salt agar (Oxoid) and pathogenicity to mice was estimated by a comparison of the effect of graded dilutions of cultures of the respective strains injected intravenously

Strain	Habituated to	M.I.C. (μg/ml.) Spiramycin	M.I.C. (μg/ml.) Erythromycin	Coagulase	Mannitol fermentation	Pathogenicity to mice
CN 491	—	2·0	0·1	+++	+	+++
CN 491 SR	Spiramycin	>50	>50	+	—	+
CN 491 ER	Erythromycin	>50	>50	+	—	—
133	—	2·0	0·1	++	+	+++
133 SR	Spiramycin	>50	>50	+	—	—
133 ER	Erythromycin	50	>50	+	—	+
Oxford	Spiramycin	>100	>100	Not tested		

Activity in mice

In these experiments, the intravenous infection of untreated mice normally caused the death of most of the animals within 2 to 5 days; after 2 days, abscesses visible to the naked eye could be observed in the kidneys of most of the infected mice, and staphylococci could be cultured from the kidneys of surviving mice in nearly all cases.

Therapeutic activity. Spiramycin was as active as erythromycin in *in vivo* experiments against a mouse-virulent laboratory strain, *Staph. aureus* (133), although erythromycin was considerably more active *in vitro* against this organism (Table 4). Post-mortem examination of surviving mice, 10 days after infection, showed that there was a lower incidence of kidney infection in the groups of mice treated with spiramycin, as judged by the presence of macroscopic abscesses and by the numbers of healthy-looking kidneys from which staphylococci were cultured.

Spiramycin was also highly effective against mouse infections caused by recently isolated strains of erythromycin-resistant staphylococci from patients (Table 5). In contrast, erythromycin was inactive, or relatively so, against these experimental infections.

TABLE 4

COMPARATIVE ACTIVITIES OF SPIRAMYCIN AND ERYTHROMYCIN AGAINST AN INTRAVENOUS STAPHYLOCOCCAL INFECTION IN MICE

Organism: *Staph. aureus* 133. The drugs were administered orally 0·5, 6, 24, 48 and 72 hr after infection. Numbers in parentheses are fiducial limits of error. $P=0·05$

Antibiotic	M.I.C. (μg/ml.)	Single oral dose (mg/g)	No. surviving after 10 days	ED50 mg/g
Spiramycin	2·0	0·5	10/10	
		0·25	10/10	
		0·13	8/10	0·075
		0·06	4/10	(0·05–0·11)
Erythromycin	0·1	0·5	10/10	
		0·25	8/10	0·11
		0·13	6/10	(0·07–0·17)
		0·06	3/10	
Untreated	—	—	0/10	—

TABLE 5

COMPARATIVE ACTIVITIES OF SPIRAMYCIN AND ERYTHROMYCIN AGAINST EXPERIMENTAL INFECTIONS CAUSED BY RECENTLY ISOLATED ERYTHROMYCIN-RESISTANT STRAINS OF *STAPH. AUREUS*

The drugs were administered orally 0·5, 6, 24, 48 and 72 hr after infection

Strain	M.I.C. (μg/ml.) Spiramycin	M.I.C. (μg/ml.) Erythromycin	Treatment	Single oral dose (mg/g)	No. surviving after 10 days
MB 1	4·0	250	Untreated	—	1/10
			Spiramycin	0·5	10/10
			Erythromycin	0·5	2/10
MB 2	8·0	250	Untreated	—	0/10
			Spiramycin	0·5	10/10
			Erythromycin	0·5	1/10
MB 7	4·0	63·0	Untreated	—	1/10
			Spiramycin	0·5	8/10
			Erythromycin	0·5	4/10
MB 9	4·0	63·0	Untreated	—	0/10
			Spiramycin	0·5	9/10
			Erythromycin	0·5	2/10
MB 11	2·0	63·0	Untreated	—	1/10
			Spiramycin	0·5	10/10
			Erythromycin	0·5	4/10
MB 12	<1·0	4·0	Untreated	—	1/10
			Spiramycin	0·5	10/10
			Erythromycin	0·5	4/10

On the other hand, neither antibiotic was active *in vivo* against a strain of *Staph. aureus* (133 ER), which had been habituated to erythromycin *in vitro*, although both were effective *in vivo* against a strain (CN 491 SR) which had been habituated to spiramycin (Table 6). These strains were completely resistant *in vitro* to both antibiotics.

Prophylactic activity. Spiramycin was more effective than erythromycin when the antibiotics were administered to mice at intervals before infection with *Staph. aureus* (CN 491). The results of two experiments are given in Table 7. Thus, in the first

TABLE 6

EFFECT OF SPIRAMYCIN AND ERYTHROMYCIN *IN VIVO* AGAINST STRAINS OF *STAPH. AUREUS* HABITUATED *IN VITRO* TO THESE ANTIBIOTICS

The compounds were administered orally 0·5, 6, 24, 48 and 72 hr after infection

Strain	Habituated to	Antibiotic	M.I.C. (μg/ml.)	Dose (mg/g)	No. surviving after 10 days
133 ER	Erythromycin	Spiramycin	50	0·5	3/8
				0·25	2/8
				0·13	1/8
		Erythromycin	>50	0·5	3/8
				0·25	2/8
				0·13	0/8
		—	—	Untreated	0/15
CN 491 SR	Spiramycin	Spiramycin	>50	0·5	8/8
				0·25	6/8
				0·13	2/8
		Erythromycin	>50	0·5	8/8
				0·25	7/8
				0·13	4/8
		—	—	Untreated	0/6

TABLE 7

PROPHYLACTIC EFFECT OF ONE ORAL DOSE OF SPIRAMYCIN AND ERYTHROMYCIN ADMINISTERED TO MICE 0, 2, 4 OR 6 HR BEFORE INTRAVENOUS INFECTION WITH *STAPH. AUREUS* CN 491

Experiment 1: infecting dose, 0·1 ml. of 18-hr culture. Experiment 2: infecting dose, 0·5 ml. of 1/10 dilution of 18-hr culture

Treatment	Dose (mg/g)	Time of administration before infection (hr)	No. surviving after 7 days		Mean survival time (days)	
			Expt. 1	Expt. 2	Expt. 1	Expt. 2
Untreated	—	—	1/5	0/10	2·8	2·5
Spiramycin	0·5	0	4/5	10/10	6·8	7·0
		2	4/5	3/10	6·8	6·0
		4	4/5	8/10	6·6	6·8
		6	3/5	6/10	6·0	6·1
Erythromycin	0·5	0	3/5	3/10	5·8	5·5
		2	3/5	2/10	5·8	5·4
		4	0/5	2/10	3·0	4·9
		6	0/5	0/10	2·0	2·9

experiment, erythromycin afforded some protection to animals treated 2 hr before, and immediately before, infection, but none of the mice dosed with this drug 4 hr and 6 hr beforehand survived the duration of the experiment. Spiramycin, on the other hand, was effective at all four intervals of time.

In the second experiment the protection afforded by spiramycin was repeated except for the group of animals dosed 2 hr before infection, where 5 mice died suddenly on day 6. The pattern of survival in the case of erythromycin-dosed animals was similar to that of the first experiment, except for some slight extension of protection to the group treated 4 hr before infection.

Antibiotic concentrations in the organs of uninfected mice

The results of these experiments are summarized in Table 8.

Both antibiotics were present in high concentrations in the serum within 2 hr after administration (15.0 μg/ml. of erythromycin, 9.4 μg/ml. of spiramycin), but the antibiotic concentrations in the serum fell to fairly low values (3.5 μg/ml. of erythromycin, 1.8 μg/ml. of spiramycin) 6 hr after administration. The concentrations of erythromycin in the serum were almost twice those of spiramycin at both periods.

TABLE 8

TISSUE CONCENTRATIONS OF SPIRAMYCIN AND ERYTHROMYCIN AFTER ONE ORAL DOSE OF 0.5 MG/G TO MICE

Expt.	Antibiotic	Time after administration (hr)	Serum concn. (μg/ml.)	Tissue concentration (μg/g)				
				Heart	Kidney	Liver	Lung	Spleen
1	Spiramycin	2	9·4	—	—	—	—	—
		6	1·8	34	95	110	82	111
		24	—	9·0	16·0	7·0	18·0	18·0
		48	—	0 (<5·0)	8·0	2·5	Trace (<3·0)	Trace (<2·0)
	Erythromycin	2	15·0	—	—	—	—	—
		6	3·5	16	59	120	46	51
		24	—	<1·5	6·0	<0·5	1·0	5·0
		48	—	—	—	—	—	—
2	Spiramycin	24	—	10	16	7·0	17	18
	Erythromycin	24	—	<2·0	<1·0	<1·0	<2·0	<2·0

Six hours after administration of the antibiotics, high concentrations of spiramycin and erythromycin were present in the organs. In general, erythromycin produced lower concentrations than spiramycin except in the liver. After 24 hr, however, while substantial amounts of spiramycin (about 1/5 to 1/10 of the 6-hr values) were still present in all the tissues, low concentrations only of erythromycin could be detected in some organs. Spiramycin could still be detected in the organs, except the heart, 48 hr after administration. In a further experiment to confirm these results, spiramycin and erythromycin were administered at the same time and the 24-hr tissue extracts were assayed. The concentrations of spiramycin found in the tissues in this experiment were very similar to those found previously, but erythromycin could not be detected in any of the extracts.

DISCUSSION

Spiramycin is 1/10 to 1/20 as active *in vitro* as erythromycin against antibiotic-sensitive strains of staphylococci. This fact has already been demonstrated in Great Britain (Garrod & Waterworth, 1956), in France (Chabbert, 1955) and in the United States (Jones & Finland, 1957). Likewise, although the results of the bactericidal experiments indicate that the mode of action on one antibiotic is very

similar to that of the other, the equivalent bactericidal concentrations of each differ to about the same extent as do the bacteriostatic concentrations.

Spiramycin, however, is undoubtedly much more active against experimental infections than might be expected from the activity of the compound *in vitro*. Garrod (1957) suggested that the ratio of the *in vitro* activity of spiramycin to that of erythromycin would be reflected in the body, but this is not so. In this series of experiments spiramycin was as effective *in vivo* as erythromycin against the laboratory strain of *Staph. aureus* (133), in spite of the fact that the latter antibiotic was 20 times as active *in vitro* against this strain. Spiramycin, too, was active against naturally occurring antibiotic-resistant staphylococci *in vivo*, whereas erythromycin had little or no effect against these infections. Finally, the extent and duration of prophylactic activity of spiramycin was greater than that of erythromycin. Our experiments have been limited to a study of staphylococcal infections, but others, Benazet & Dubost (1959), Cosar (1956) and Freeman (personal communication), have demonstrated similar anomalous results against other micro-organisms, such as streptococci or pneumococci.

An explanation for this phenomenon would be apparent if spiramycin were metabolized *in vivo* to a highly active form, but extensive studies in France have produced no evidence of the existence of such a metabolite (Benazet, personal communication). An alternative hypothesis put forward by Chabbert *et al.* (1957) is that spiramycin is active *in vivo* because it is retained in the body in high concentrations for a considerable period. To confirm this, these workers carried out an elegant series of experiments, which demonstrated that the activity of spiramycin in mouse infections was related to the tissue concentrations of the antibiotic. More recently, Benazet & Dubost (1959) reported similar findings in an independent study.

The experiments reported here confirm that spiramycin is retained in the body of the mouse longer, and in higher concentrations, than is the case with erythromycin, and it seems reasonable to suggest that the pronounced *in vivo* activity of spiramycin against mouse infections may be due to this factor. The same explanation would account for the superiority of spiramycin over erythromycin in prophylactic experiments. Recently, too, Maniar, Eidus & Greenberg (1960) have reported that, after subcutaneous administration of a single dose of antibiotic to mice, spiramycin was present in various organs at higher concentrations than erythromycin over a period of 24 hr ; after prolonged treatment spiramycin accumulated in the tissues whereas erythromycin did not. These workers also demonstrated that spiramycin was more effective against a pneumococcal infection in mice, although erythromycin was 4 times more active *in vitro*, and concluded, accordingly, that the *in vivo* activity of spiramycin was related to its affinity for animal tissues (Greenberg, Maniar & Eidus, 1960).

It is important to note that in these experiments the serum concentrations of erythromycin were almost twice as great as those of spiramycin after administration of equivalent doses of the antibiotics. If, then, therapeutic activity were related directly to antibiotic serum concentrations, as is the rationale of the *in vivo–in vitro* type of experiment used by Jones & Finland (1957) in the evaluation of these two antibiotics, and which is favoured by many workers for the comparison of related

antibiotics, erythromycin might be expected to be more active *in vivo* than spiramycin—but this is not so. This work therefore appears to support the doubts which have been expressed recently (Editorials, 1961a, 1961b) as to the validity of the assessment of antibiotics by measurement of the antibacterial activity of sera.

The nature of the cross-resistance which exists between these two antibiotics is more obscure, and it is possible that at least two mechanisms are involved. Garrod (1957) and Jones, Nichols & Finland (1956) have demonstrated that strains habituated *in vitro* show complete cross-resistance to each other, but that cross-resistance between strains isolated from clinical infections is variable. For example, Lowbury & Hurst (1959) have shown that, of 305 strains of staphylococci fully resistant to erythromycin, none was resistant to spiramycin *in vitro*.

Our experiments with a limited number (20) of typical antibiotic-resistant strains of staphylococci derived from clinical sources show that not only is spiramycin active against most naturally occurring erythromycin-resistant strains *in vitro*, but also that it is very effective *in vivo* against these organisms. The therapeutic activity of spiramycin against these staphylococci is of considerable practical importance in view of the increasing numbers of infections caused by antibiotic-resistant staphylococci.

A possible explanation for the divergent results reported from various laboratories might be that strains habituated in the laboratory to spiramycin and erythromycin bear as little relationship to recently isolated resistant staphylococci as penicillin-resistant strains produced in the laboratory bear to resistant penicillinase-producing staphylococci. This conclusion might appear to be borne out not only by the fact that the biochemical characteristics of the habituated strains differed from those of the parent strain but also from the unexpected results derived from experimental infections caused by these organisms.

It may be concluded that spiramycin is an active antistaphylococcal antibiotic, effective against many erythromycin-resistant strains of staphylococci. For the purpose of antibiotic-sensitivity tests, and for use in clinical practice, spiramycin, because of its effectiveness against antibiotic-resistant organisms, and by virtue of its high and prolonged tissue concentrations, should be considered as an antibiotic in its own right, rather than as a member of the erythromycin group of antibiotics. It is also suggested that the evaluation of the therapeutic activity of antibiotics from data based largely on *in vitro* studies—the method in common use to-day—may be misleading. Discrepancies between *in vivo* and *in vitro* results in the evaluation of antibiotics should be explored. Light may be shed on these by estimates of tissue concentrations of an antibiotic as well as serum concentrations. Moreover, the assessment of cross-resistance solely by the use of organisms habituated to antibiotics *in vitro* may also be misleading; results should be checked by the use of naturally occurring resistant strains, if any are available.

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