2. Mühlbock, O., Boot, L. M., Cancer Res., 1959, v19, 402.
3. Furth, J., ibid., 1959, v19, 241.
4. DeOme, K. B., Faulkin, L. J., Jr., Bern, H. A., Blair, P. B., ibid., 1959, v19, 515.
5. Busch, H., Hurlbert, R. B., Potter, V. R., J. Biol. Chem., 1952, v196, 717.
6. Smith, L., Chromatographic and Electrophoretic Techniques, Interscience Publishers, Inc., N.Y., 1960.
7. Feigl, F., Spot Tests in Organic Analysis, 6th ed., Elsevier Publishing Co., Inc., N.Y., 1960.
8. Seligson, D., Shapiro, B., Anal. Chem., 1952, v24, 754.
9. Umbreit, W. W., Burris, R. H., Stauffer, J. W., Manometric Techniques, Burgess Publishing Co., Minneapolis, 1957.
10. Keilin, D., Hartree, E. F., Biochem. J., 1945, v39, 239.
11. Moore, S., Stein, W. H., J. Biol. Chem., 1948, v176, 367.
12. Spies, J. R., Methods in Enzymology, v3, Academic Press, N. Y., 1957.
13. Barker, S. B., Summerson, W. H., J. Biol. Chem., 1941, v138, 535.
14. Katsuki, H., Kanayuki, H., Yoshida, T., Kawano, C., Tanaka, S., Anal. Biochem., 1961, v2, 421.
15. Okita, G. T., Kabara, J. J., Richardson, F., LeRoy, G. B., Nucleonics, 1957, v15, 111.
16. Burgers, E. A., Sylven, B., Cancer Res., 1962, v22, 581.
17. Hsiegh, K. M., Mao, S. S., Sasananonth, K., ibid., 1959, v19, 700.
18. Nyhan, W. L., Busch, H., ibid., 1957, v17, 227.
19. Warburg, O., Science, 1956, v123, 309.
20. Burk, D., Laszlo, J., Wright, K., Hunter, J., Woods, M., J. Nat. Cancer Inst., 1960, v24, 57.
21. Dickens, F., Weil-Malherbe, H., Cancer Res., 1943, v3, 73.
22. Meyerhof, O., Canad. J. Med. Sci., 1951, v29, 63.
23. LePage, G. A., Cancer Res., 1950, v10, 77.

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Studies on Therapy of Staphylococcal Infections in Monkeys. I. Comparison of Cloxacillin, Triacetyloleandomycin and Erythromycin.* (32304)

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Appropriate controlled studies for the comparison of antistaphylococcal chemotherapeutic agents in serious infections in humans have been difficult particularly because untreated controls can not and should not be employed. Various problems in the evaluation of therapy in staphylococcal infections have been reviewed recently(1). Previous studies from this laboratory(2) demonstrated that the rhesus monkey was an excellent experimental model for systemic staphylococcal infections. Since the experimental infections simulated those observed in natural infections in man, studies on therapy in monkeys could contribute to a better comparison of the efficacy of various antibiotics *in vivo*. To our knowledge, no studies on therapy of staphylococcal infections in subhuman primates have been described. In this report 2 bacteriostatic macrolide antibiotics are compared with a bactericidal semisynthetic penicillin.

Materials and methods. Thirty-one young adult monkeys *(Macaca mulatta)* weighing 2.8-4.5 kg were employed. Base-line observation included physical examinations, hematologic and bacteriologic studies for 2 weeks prior to challenge with a penicillin-resistant *Staphylococcus aureus,* phage type 80/81; tube dilution sensitivity tests(3) showed that the minimal inhibitory concentrations of cloxacillin, oleandomycin base and erythromycin were 0.25, 1.0 and 0.25 μg/ml, respectively, and bactericidal concentrations were 1.0, 31.3 and 15.6 μg/ml, respectively. Other characteristics of the staphylococcus and technics used in its preparation and intravenous inoculation have been described(2). Therapy with cloxacillin (Tegopen, Bristol,

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oral solution), triacetyloleandomycin (TAO, Roerig, oral suspension), and erythromycin (Erythrocin, Abbott, oral suspension) was initiated 16 hours post-challenge when clinical and laboratory findings(2) consistent with well-established disease were present. Antibiotics were given, intragastrically, through a No. 14 plastic urethral catheter attached to a 10 ml syringe. Daily dose was divided equally and given at 8:00 AM and 5:00 PM; infected controls received only distilled water. Preliminary studies demonstrated that these preparations were all well-tolerated with no anorexia, vomiting or diarrhea following administration of 50 mg/kg/day for 12 days.

Monkeys were examined at least twice daily after challenge. Femoral vein blood was used for blood counts, cultures and C-reactive protein (CRP) tests daily or every other day for 2 weeks, every 3rd or 4th day for 3 additional weeks, and then weekly thereafter through the 10th week. Laboratory procedures used have been described(2,4). Complete autopsies were performed on all fatally-infected animals.

Results. Experiment I. Three groups of 4 monkeys each were treated with cloxacillin, triacetyloleandomycin and erythromycin, respectively, for 9 days beginning 16 hours after intravenous challenge with 1.3×10^{11} staphylococci. Two pairs in each group of 4 received 30 and 50 mg/kg/day, respectively. Three monkeys served as untreated controls (Table I).

All 15 monkeys were moderately to acutely ill when therapy was started; the 4 study groups were similar in this respect. Blood cultures and CRP were positive in all by the 1st post-challenge day. The untreated monkeys became progressively worse, and all 3 died by the 2nd or 3rd day. Similarly, all 4 monkeys receiving erythromycin became progressively worse, and died by the 2nd or 3rd day. Clinical and laboratory findings were comparable in both groups. These included anorexia, lethargy and weakness progressing to prostration and coma, initial fever followed by hypothermia terminally, leukopenia with a marked increase in non-segmented neutrophiles, and anemia. All 7 monkeys exhibited positive blood cultures and CRP tests up to death. At autopsy, hemorrhage and congestion of the lungs, pericardial effusion and splenomegaly were observed uniformly in both groups; one erythromycin-treated animal exhibited numerous myocardial abscesses containing staphylococci. Staphylococci were also isolated from the blood, spleen, liver, kidney, lung, heart and brain from all 7 animals.

With triacetyloleandomycin, 1 of 2 receiv-

TABLE I. Effect of Oral Therapy with Cloxacillin, Triacetyloleandomycin and Erythromycin on Mortality in Rhesus Monkeys After Intravenous Challenge with Staphylococci.

		Exp I				Exp II				Totals—Exp I, II	
		1.3×10^{11} *S. aureus* 80/81 I.V. Treated 9 days				5.2×10^{10} *S. aureus* 80/81 I.V. Treated 12 days					
Antibiotic	Dose,* mg/kg/day	No. monkeys	Survival time, days	Mortality		No. monkeys	Survival time, days	Mortality		Mortality	Totals
Cloxacillin	30	2	2 5	2/2‡						2/2	4/8
"	50	2	7 S†	1/2		4	8 S S S S	1/4		2/6	
Triacetyloleandomycin	30	2	10 S	1/2						1/2	2/8
"	50	2	2 S	1/2		4	S S S S S	0/4		1/6	
Erythromycin	30	2	2 3	2/2						2/2	7/8
"	50	2	2 3	2/2		4	7 10 11 S	3/4		5/6	
Controls	None	3	2 2 3	3/3		4	1 2 5 6	4/4		7/7	7/7

* Divided into 2 doses given orally at 8:00 A.M. and 5:00 P.M. beginning 16 hr post-challenge.
† S = survived.
‡ No. died over total No. challenged.

ing 50 mg/kg died on the 2nd day, and yielded positive cultures at autopsy; the other improved steadily, and was only mildly ill, although still febrile and exhibiting positive blood cultures, CRP, and anemia when therapy was discontinued on the 9th day. Early, transient leukopenia (5-7,000 wbc's as compared to 9-11,000 base-lines) was followed by leukocytosis (18-30,000) with a marked increase in non-segmented neutrophiles (68%) on the 7th to 9th days. This monkey relapsed slightly on the 11th day, had a positive blood culture on the 14th day, but improved rapidly, was afebrile and appeared normal after the 15th day. Blood cultures were negative from the 17th to 24th days, but a positive culture was obtained on the 29th day even though the monkey had appeared normal for 2 weeks. Subsequent blood cultures as late as 10 weeks post-challenge were negative. Positive CRP, leukocytosis and anemia were noted for 29, 35 and 43 days, respectively. Thus, this monkey showed laboratory evidence of persisting infection for 2-3 weeks after apparently complete clinical recovery. Before challenge, it weighed 3.5 kg, but dropped to a low of 2.7 kg on the 23rd day, and at 10 weeks weighed 3.1 kg.

One of 2 monkeys given 30 mg/kg of triacetyloleandomycin became afebrile after 2 days, showed gradual improvement during the first week, and exhibited only mild lethargy when therapy was discontinued on the 9th day. Blood cultures and CRP were positive through the 17th and 24th days, respectively, and leukopenia for the first 2 days was followed by polymorphonuclear leukocytosis from the 4th to 35th days. Anemia (hemoglobin 6-8 mg%, as compared to 12 mg% base-line), first noted on the 2nd day, lasted until the 43rd day. Relapse occurred 2 days after therapy was discontinued and weakness, anorexia and lethargy continued through the 15th day. Improvement was noted during the next 6 days, but the animal became more lethargic between the 22nd and 24th post-challenge days. Subsequent recovery was slow and not complete until the 10th week. Fever was not observed during relapse or subsequently. Initial weight of 3.8 kg dropped to 2.8 kg by the 23rd day and returned to 3.6 kg at 10 weeks.

The other monkey given 30 mg/kg of triacetyloleandomycin remained acutely ill during the 9-day therapy period and died the day after treatment was stopped. Blood cultures and CRP were positive from the 1st day to death. Anemia (7-8 mg%) and leukopenia (3,000 wbc's) on the first 2 days was followed by leukocytosis (15,000 wbc's) and increasing anemia (5-6 mg%) to death. Fever (104.3°F) was noted on the 1st day only. Rectal temperatures were below normal on the 4th through 10th days and ranged from 92.4-98.0°F. Positive cultures were obtained from most organs at autopsy.

Neither of 2 monkeys given 30 mg/kg of cloxacillin responded to therapy, and both died on the 2nd and 5th days, respectively. Both showed daily positive blood cultures and CRP tests, leukopenia, anemia and hypothermia prior to death, and positive cultures at autopsy.

After 50 mg/kg of cloxacillin, 1 of 2 did not respond and exhibited positive blood cultures and CRP tests, polymorphonuclear leukocytosis, anemia and hypothermia until death on the 7th day. The other began to show improvement by the 2nd day, and was afebrile and only mildly ill, but still had positive blood cultures and CRP tests, leukocytosis and anemia when therapy was discontinued on the 9th day. Relapse occurred on the 11th day, with increasing weakness, lethargy and anorexia through the 13th day. Gradual recovery beginning on the 14th day was followed by minor fluctuations in the animal's condition until the 26th day after which recovery was slow, but not complete until the 10th week. Blood cultures and CRP tests were positive through the 17th and 21st days, respectively. Blood counts returned to normal by the 29th day. Initial weight of 3.8 kg dropped to 2.9 kg on the 23rd day and at 10 weeks had returned to 3.5 kg.

In summary (Table I), with the 9-day therapy program, all 4 monkeys receiving erythromycin and all 3 untreated controls died between the 2nd and 3rd day; 3 of 4 receiving cloxacillin died between the 2nd and 7th day, and 2 of 4 receiving triacetyl-

oleandomycin died on the 2nd and 10th days, respectively. Serum obtained from all treated monkeys on days 1 to 4, 7 and 9 in those surviving these periods demonstrated similar inhibitory levels ranging from 1:2 to 1:16 against the staphylococcus in all 3 therapy groups. All cultures obtained during the course of infection and at autopsy showed no acquired resistance to any of the antibiotics.

Experiment II. Four monkeys in each of 3 treatment groups received 50 mg/kg of cloxacillin, triacetyloleandomycin and erythromycin, respectively, for 12 days beginning 16 hours after intravenous challenge with 5.2×10^{10} staphylococci. Four monkeys were similarly challenged, but not treated.

All 16 monkeys were moderately ill when therapy was started. The 4 controls became worse after the 16th hour and died on the 1st, 2nd, 5th and 6th days, respectively, (Table I). Clinical, laboratory and autopsy findings were similar to those in untreated monkeys in Experiment I. Two dying on the 5th and 6th days, respectively, exhibited myocardial abscesses at autopsy. Three of 4 given erythromycin showed no improvement, and died after 7, 10 and 11 days, respectively. All exhibited daily positive blood cultures and CRP tests; 2 of 3 showed leukopenia for the first 2 days followed by polymorphonuclear leukocytosis and anemia up to death while only an increase in non-segmented neutrophiles was observed in the 3rd animal. Initial fever was followed by hypothermia (94-96.8°F) from the 3rd day to death. At autopsy, myocardial abscesses were observed in the monkey that died on the 10th day, and the specific organism was isolated from all 3 monkeys from most organs.

The remaining monkey receiving erythromycin was moderately to acutely ill the first 5 days, began to improve by the 6th day, was febrile on the 7th and 8th days only, and was still very weak and lethargic when therapy was stopped on the 12th day. Blood cultures and CRP tests were positive for the first 11 days. No early leukopenia was detected, and leukocytosis and anemia were noted through the 11th day. The monkey's condition became worse on the 17th day and it was acutely ill for 3 days, recovered slowly, and was still mildly ill 8 weeks post-challenge, and not normal until the 11th week. As seen in Table II, blood cultures were negative after the 11th day, CRP tests were positive through the 21st day, negative the 24th and 28th days, positive again on the 31st and 35th days, and negative thereafter. The blood picture did not return to normal until the 56th day.

As seen in Table I, all 4 monkeys receiving triacetyloleandomycin survived. One began to improve on the 3rd day of therapy, became afebrile on the 8th day, and was fully recovered by the 11th day and thereafter. Blood cultures were positive through the 5th day only, and CRP tests negative after 11 days. Total leukocyte counts were normal the first 2 days, but an increase in non-segmented neutrophiles was evident; leukocytosis was observed from the 4th through 11th days only, and slight anemia through the 21st day. Similarly, 1 of the remaining 3 was afebrile and showed improvement beginning on the 5th therapy day, and appeared normal by the 11th day and thereafter. Blood cultures and CRP tests were negative after the 5th and 11th days, respectively. Polymorphonuclear leukocytosis was noted from the 1st through 14th days, and anemia from the 2nd through 24th days. A 3rd monkey exhibited definite improvement beginning on the 2nd day of therapy and appeared normal by the 11th day. However, lethargy became apparent 7 days after therapy was stopped and continued through the 35th day, after which the monkey appeared fully recovered. Blood cultures were positive through the 7th day of therapy, negative on the 9th and 11th days, but positive again from the 14th through 31st days (2-19 days after cessation of therapy) and negative thereafter (Table II). CRP tests were positive through the 21st post-challenge day; the total white cell count was normal with an increase in non-segmented neutrophiles until the 4th day when leukocytosis and anemia were observed which persisted through the 31st day.

The 4th monkey in this group exhibited fever the 1st day only, was moderately ill

TABLE II. Incidence of Positive Blood Cultures in Monkeys Treated with Cloxacillin, Triacetyloleandomycin and Erythromycin After Intravenous Challenge with Staphylococci.

Day post-challenge*	Cloxacillin 50 mg/kg/day†				Triacetyloleandomycin 50 mg/kg/day				Erythromycin 50 mg/kg/day				Controls			
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Died	Died
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
5	+	+	+	+	+	+	+	+	+	+	+	+	+	Died		
7	+	+	+	+	—	—	+	—	+	+	Died	+	Died			
9	Died	—	—	+	—	—	—	—	+	+		+				
11		—	—	—	—	—	—	—	+	Died		Died				
12					Therapy discontinued											
14		—	+	+	—	—	+	—	—							
17		—	—	—	—	—	+	—	—							
21		—	—	—	—	—	+	—	—							
24		—	—	—	—	—	+	—	—							
28		—	—	—	—	—	+	—	—							
31		—	—	—	—	—	+	—	—							
35		—	—	—	—	—	—	—	—							
42		—	—	—	—	—	—	—	—							
49		—	—	—	—	—	—	—	—							
56		—	—	—	—	—	—	—	—							

* 5.2×10^{10} *S. aureus* 80/81 intravenously.

† Divided into 2 doses given orally at 8:00 A.M. and 5:00 P.M. for 12 days. Therapy started 16 hr post-challenge.

through the 6th day of therapy, became more active and alert on the 7th day, and was only slightly lethargic on the 12th day when therapy was discontinued. However, 4 days later (16th post-challenge day), the monkey relapsed, appeared very lethargic and withdrawn for 11 days, then showed gradual recovery which was complete by the 10th week. Blood cultures and CRP were positive through the 5th and 14th days, respectively. Leukopenia was observed on the 1st day only, and leukocytosis from the 4th through the 17th days; anemia was noted between the 4th and 24th days.

One of 4 monkeys given cloxacillin did not respond to therapy, became progressively worse and died on the 8th day. Leukocytosis and positive blood cultures and CRP were observed from the 1st day until death. One of the remaining 3 suddenly became worse on the 2nd day and was acutely ill for 2 days; marked improvement was noted beginning on the 4th day, and it was afebrile on the 6th day, but still lethargic and weak when therapy was discontinued on the 12th day. Blood cultures were negative on the 9th and 11th days, positive on the 14th day but negative thereafter. Leukocytosis and anemia were observed through the 11th day and positive CRP for 17 days. No evidence of clinical relapse was noted after therapy was discontinued, and it appeared normal by the 28th day.

The 3rd monkey given cloxacillin became afebrile by the 3rd day but showed no other clinical improvement until the 7th day, and was only mildly ill when therapy was discontinued on the 12th day. Blood cultures became negative on the 11th day, but the monkey showed positive CRP tests, leukocytosis and anemia for 21, 28 and 28 days, respectively. It continued to improve after treatment was stopped and was essentially normal by the 14th day, although blood culture was again positive on this day, but not thereafter. Slight clinical relapse was observed between the 17th to 22nd days, but it appeared normal thereafter.

The remaining monkey treated with cloxacillin became afebrile on the 2nd day, active and alert by the 5th day, and showed only mild symptoms when therapy was discontinued on the 12th day. Continued improvement was noted until the 19th to 21st days when lethargy reoccurred, but the animal appeared normal thereafter. Blood cultures and CRP were negative after the 7th and 14th days, respectively, and leukocytosis and

anemia persisted for 17 and 21 days, respectively.

In summary (Table I), with the 12-day therapy program, the 4 controls died 1 to 6 days after challenge, 3 of 4 receiving erythromycin died 7 to 11 days post-challenge, and 1 of 4 receiving cloxacillin died on the 8th day. All of 4 treated with triacetyloleandomycin survived. As in Experiment I, no differences in serial serum inhibitory levels were demonstrated and no evidence of acquired resistance was detected in any of the antemortem or postmortem cultures.

Discussion. In clinical situations it is generally agreed that chemotherapeutic agents which are primarily bactericidal are preferable to bacteriostatic agents in serious staphylococcal infections, as recently reviewed(1). In the present study this concept was supported, in part, by the obvious superiority of cloxacillin over erythromycin. On the other hand, triacetyloleandomycin not only exhibited effects far superior to erythromycin but comparable or even superior in most respects to cloxacillin. The *in vitro* studies suggested that cloxacillin would be more effective than erythromycin and triacetyloleandomycin, and that the latter would be the least active of the 3 agents against the penicillin-resistant strain of staphylococcus employed. Under the conditions of this study, employing the same dose regime, and using oral preparations, the efficacy of triacetyloleandomycin, a bacteriostatic drug, in the treatment of severe staphylococcal infections was of particular interest. That these differences were not related to absorption was demonstrated by comparable inhibitory levels of the sera of all animals under treatment.

The question of extrapolation of results from monkey to man is a pertinent one, and variation may occur with the infecting agent employed. However, previous studies from this laboratory have shown that the response of man to infection and therapy in tularemia (5,6) was similar to that observed in antecedent monkey experiments. Response to therapy in experimental Rocky Mountain spotted fever(7) was also analogous to that observed in naturally-occurring infections in man.

Therapy in these studies was not instituted until the day after challenge with a lethal dose, and at a time when blood cultures were positive and the monkeys obviously ill. This offered an opportunity to compare therapeutic agents in monkeys severely stressed, and destined not to survive without adequate and specific therapy. It is apparent that additional controlled studies are indicated and necessary to compare these and other antibiotics in severe staphylococcal infection. This would allow comparisons, not only of chemotherapeutic agents, but also the factors of dose, duration of therapy, route of administration, and effect against other strains of staphylococci. Such studies are now in progress in this laboratory.

Conclusions. Intravenous inoculation of a penicillin-resistant, phage type 80/81 staphylococcus caused lethal infection of all 7 untreated monkeys. Daily intragastric administration of 30-50 mg/kg of erythromycin, triacetyloleandomycin and cloxacillin was followed by mortalities in 7 of 8, 2 of 8 and 4 of 8, respectively. Thus, of the 2 bacteriostatic agents, triacetyloleandomycin was far superior to erythromycin as was cloxacillin, a bactericidal drug. Of particular interest was the observation that the bacteriostatic triacetyloleandomycin was as effective or perhaps superior to cloxacillin in preventing lethal infection.

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1. Kirby, W. M., Ann. N. Y. Acad. Sci., 1965, v128, 443.

2. Saslaw, S., Carlisle, H. N., Proc. Soc. Exp. Biol. & Med., 1966, 123, 565.

3. Kavanagh, F., in Analytical Microbiology, F. Kavanagh, ed., Academic Press, Inc., New York, 1963, 126.

4. Saslaw, S., Carlisle, H. N., Proc. Soc. Exp. Biol. & Med., 1964, v117, 420.

5. Saslaw, S., Eigelsbach, H. T., Wilson, H. E., Prior, J. A., Carhart, S., Arch. Int. Med., 1961, v107, 689.

6. Saslaw, S., Eigelsbach, H. T., Prior, J. A., Wilson, H. E., Carhart, S., ibid., 1961, v107, 702.

7. Saslaw, S., Carlisle, H. N., Bact. Rev., 1966, 30, 636.

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