occurs in diverse types of cells and is probably a widely distributed phenomenon, it is not always readily demonstrable.

Summary. Molecular oxygen is not essential for the photoreactivation of *E. coli* after inactivation by ultraviolet radiation, demonstrating that photodynamic action is not involved.

Vegetative cells from young cultures of *Bacillus cereus* show no appreciable photoreactivation.

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Comparative Action of Aureomycin, Chloromycetin, Neomycin, Q-19, and Polymyxin B Against Gram Negative Bacilli. (17801)

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Although the number of effective antibiotics continues to increase, infections due to gram negative bacilli still pose many therapeutic problems. In the present study, the sensitivities of strains of *Pseudomonas aeruginosa, Aerobacter aerogenes, Escherichia coli* and *Proteus vulgaris* to neomycin, Q-19, polymyxin B, aureomycin, and chloromycetin have been determined. Streptomycin-resistant variants of *E. coli* have been compared with the resistant variants of the same strain appearing with neomycin and Q-19. Studies on the mode of action of neomycin and Q-19 are also presented.

*Materials and Methods:** Neomycin, produced by a member of the genus *Streptomyces* and discovered by Waksman(1,2), has been shown to be active against a wide variety of both gram negative and gram positive organisms. Q-19,† produced by a strain of *Bacillus circulans* and considered to be related to the polymyxin-aerosporin type of antibiotic was discovered by Murray and Tetrault(3). They reported it to be more active against gram negative than gram positive organisms. The activity of preparations from *Bacillus polymyxa* and a related organism, *Bacillus aerosporus,* against gram negative organisms was reported almost simultaneously by 3 groups of workers(5-7). A crude preparation of polymyxin B prepared by the Pfizer laboratory was used in this study. Aureomycin and chloromycetin, whose properties and effective bacterial spectra have been recently reviewed, were used as standards of comparison for the newer antibiotics(8-10).

Neomycin, Q-19, polymyxin B, and aureomycin were stable and readily soluble in concentrated solutions of sterile saline. Fresh stock solutions containing 10 to 25 mg per

* Neomycin and Q-19 supplied by Upjohn Co. Aureomycin supplied by Lederle Laboratories. Polymyxin B supplied by Pfizer Corp.

† Q-19 has also been referred to as circulin. It is a different antibiotic than the one described by McLeod(4), which was originally named circulin but has since been renamed polypetin.

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ml were prepared weekly and appropriate dilutions made on the day of each experiment. Chloromycetin was not as soluble but a stock solution containing 250 μg per ml was made by heating saline solution and slowly adding the drug. All stock solutions were stored in the refrigerator. Twenty strains each of *A. aerogenes, P. aeruginosa, E. coli* and *P. vulgaris* isolated from urines sent for culture to the bacteriology laboratory of the University of Minnesota Hospitals were used as the test organisms. The drug sensitivity of each strain was determined by a broth dilution method. In order to get direct comparisons, simultaneous determinations were made of the sensitivity of each strain to the 5 drugs. One-half ml of a 1:100 dilution of an 18-hour culture of bacteria in tryptose broth was added to tubes (13 x 100 mm) containing 0.5 ml of two-fold serial dilutions of each drug. Since polymyxin B was not available during the initial phases of this study, comparison of its activity with that of the other 4 antibiotics was made on only 11 strains of *E. coli,* 10 strains of *A. aerogenes,* and 10 strains of pseudomonas. The end point of the sensitivity test was determined by selecting the lowest concentration of drug at which no growth was visible after 18 hours of incubation at 37°C. Repeated determinations of the sensitivity of one strain of *E. coli* to neomycin, streptomycin, chloromycetin, aureomycin, and Q-19 revealed a variability of only one tube in the method. While variation in absolute values occurred, the relative sensitivities to the 5 drugs remained almost constant. Those strains whose growth was not inhibited by 31.2 μg per ml of drug were arbitrarily considered to be relatively resistant.

In order to determine whether Q-19 and neomycin induced the penicillin type or the streptomycin type of resistant variant described by Demerec(11), pour plates were prepared of heavy suspensions of *E. coli* in agar containing increasing amounts of streptomycin, neomycin, and Q-19. The strain of *E. coli* used was originally isolated from the urine of a patient. A single colony was seeded on an agar slant which was used as a stock culture for the complete series of experiments. This strain was shown to be most sensitive to Q-19 and equally sensitive to streptomycin and neomycin by the broth dilution method. Large bottles containing agar were inoculated with stock strain of *E. coli* and incubated at 37°C for 18 hours. The organisms were then suspended in saline solution in a concentration of from 1 x 10^{10} to 3 x 10^{10} per ml. One ml of this heavy suspension was placed in Petri dishes and 10 ml of agar containing increasing concentrations of antibiotic was added. After 5 days of incubation at 37° the number of resistant colonies was determined. The results were recorded as the numbers of variants resistant to each concentration of antibiotic per 1 x 10^{10} organisms.

Whether neomycin and Q-19 were relatively bactericidal or bacteriostatic was determined in 2 ways. In the broth dilution method of determining sensitivity, the end point was the first tube showing no turbidity after 18 hours. The presence or absence of micro-organisms in the clear tubes was determined by placing a 0.1 ml aliquot from each of the first 3 clear tubes into 10 ml of tryptose broth. The diluted the drug sufficiently to permit the growth of variable organisms. The rapidity with which Q-19 and neomycin killed organisms was determined by adding 5 ml of a 1:100 dilution of an 18-hour broth culture of the stock strain of *E. coli* to 5 ml of broth containing 20 μg per ml of each of the antibiotics. The number of viable organisms was determined by removing 0.1 ml aliquots immediately, at 40 minutes, 2 hours, 4 hours, 6 hours, and 18 hours, and making colony counts in plain agar plates.

Results of In vitro Sensitivity tests: Escherichia coli—The majority of the strains were sensitive to the 5 antibiotics (Fig. 1). Q-19 was the most effective antibiotic against 13 strains. Five strains were resistant to Q-19, 2 to aureomycin, 1 each to chloromycetin and polymyxin B. None of the 20 strains were resistant to neomycin.

Pseudomonas aeruginosa—Q-19 and poly-

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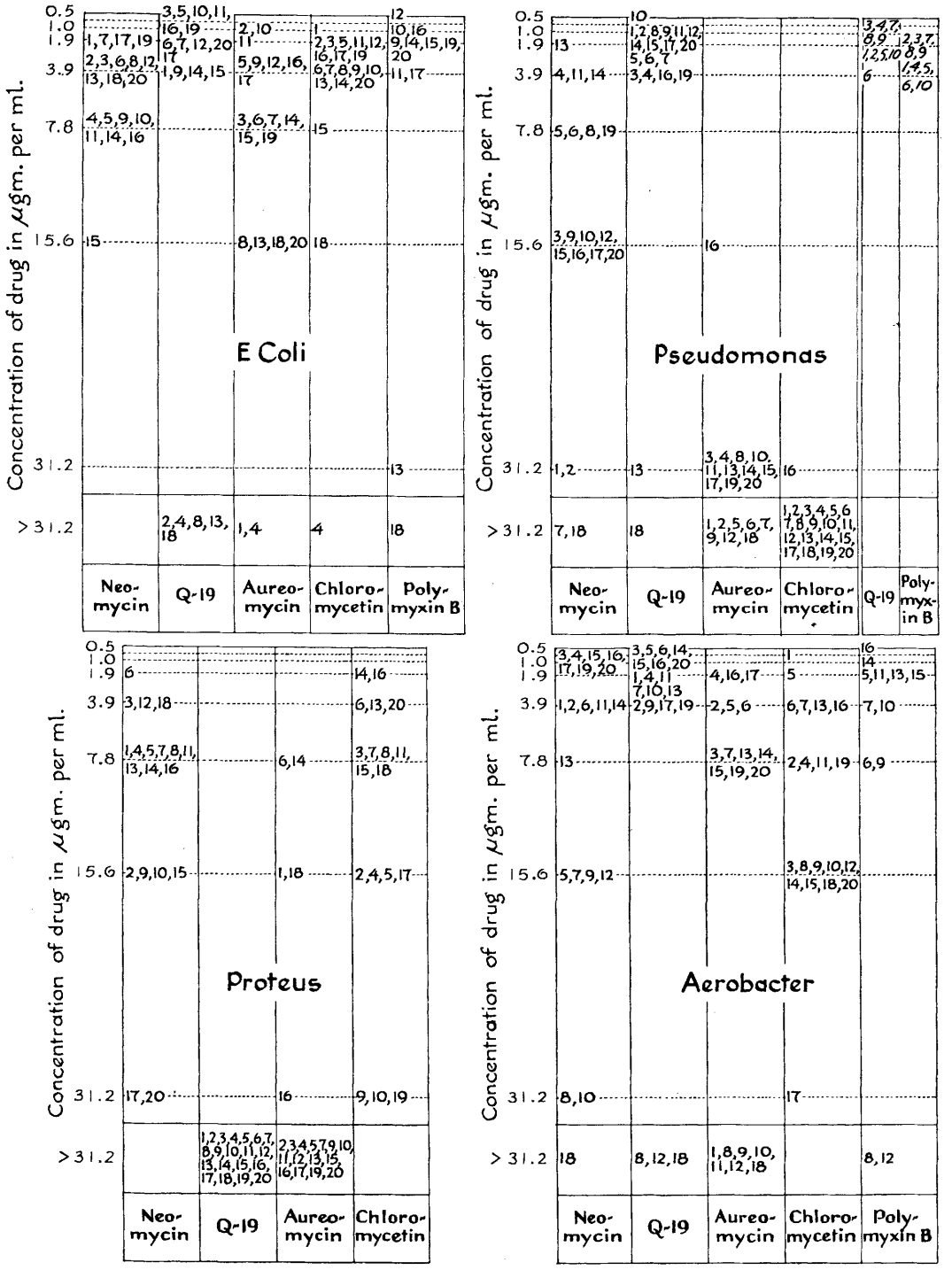

FIG. 1.

Comparative action of neomycin, Q-19, chloromycetin, aureomycin, and polymyxin B against 20 strains each of *E. coli*, *P. aeruginosa*, *P. vulgaris*, and *A. aerogenes*. Strains are numbered from 1 to 20, and the sensitivities of each strain are denoted by the numerals in the columns over the names of the antibiotics.

TABLE I.
Number of Variants of *E. coli* Per 1 × 10^{10} Organisms Resistant to Various Concentrations of Antibiotics.

Antibiotic	Conc. of drug in μg per ml								
	3	5	6	10	12	25	50	100	1000
				Streptomycin					
Exp.									
1 a*			31		55	51	44	31	16
b			9		51	55	40	45	23
2 a		60		109		111	111	94	49
b		54		89			106	111	57
3 a	33	OG		60				83	
b	16	OG		81				81	
4 a	OG		23		139	73	81	42	18
b	OG		42		53	73	63	77	16
				Neomycin					
1 a			46		0	0	0	0	0
b			81		0	0	0	0	0
2 a		103		0		0	0	0	0
b		59		0		0	0	0	0
3 a	OG	20		0				0	
b	OG	24		0				0	
4 a	OG		62		0	0	0	0	0
b	OG		79		0	0	0	0	0
				Q-19					
2 a	OG	OG		OG		0		0	0
b		OG		OG		0		0	0
3 a	OG	63		0				0	
b	OG	87		0		0		0	
4 a	OG		OG		10	0	0	0	0
b	OG		OG				0	0	0

* Two plates were used with every conc. of drug in each exp. They are labeled a and b.
OG = Overgrown.

myxin B were the most effective antibiotics against this species (Fig. 1). Neomycin was less effective and aureomycin and chloromycetin were relatively inactive.

Proteus vulgaris—Neomycin and chloromycetin appeared most active against *P. vulgaris* (Fig. 1). Strain 19, which was resistant to neomycin, was sensitive to chloromycetin and strain 1, which was resistant to chloromycetin, was sensitive to neomycin. None of the 20 strains was affected by Q-19 and only 5 were moderately sensitive to aureomycin.

Aerobacter aerogenes—This species was similar to *E. coli* in that most of the strains were sensitive to the 5 antibiotics. Q-19 was the most effective drug against 14 strains. Seven strains were resistant to aureomycin, 3 to Q-19, 2 to polymyxin B, and 1 to neomycin. Two of the strains (8 and 12) unaffected by Q-19 also were not inhibited by polymyxin B.

Pattern of Resistant Variants. Studies with the stock strain of *E. coli* showed the same pattern of resistance to streptomycin that has been reported(11-14). In populations of *E. coli* greater than 1 x 10^{10}, resistant variants appeared in concentrations of streptomycin as high as 1,000 μg/ml (Table I). The same strain of *E. coli* displayed the "penicillin pattern" of resistance to both Q-19 and neomycin in which resistant variants appear at slightly higher concentrations of drugs than that which inhibits the majority of organisms, but none are found initially resistant to high concentrations of drug. Thus, a considerable number of resistant variants grew out in concentrations as high as 1000 μg/ml of streptomycin while none were found in concentrations higher than

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TABLE II.
Bactericidal Activity of Neomycin and Q-19 Against *E. coli.*

Time after inoculation	Control No. of bacteria	20 μg/ml neomycin No. of bacteria	20 μg/ml Q-19 No. of bacteria
1 min.	2.3×10^6	4.2×10^6	2.0×10^6
40 "	2.1×10^6	5.0×10^5	1.1×10^4
2 hr	3.9×10^6	5.0×10^1	2.4×10^3
4 "	1.7×10^8	No growth	No growth
18 "	3.3×10^8	" "	" "

12 μg/ml of Q-19 or neomycin.

Bactericidal or Bacteriostatic Activity of the Antibiotics: The bactericidal and bacteriostatic results with Q-19 and neomycin were essentially the same in 9 of 11 strains.

When 2 x 10^6 *E. coli* were inoculated into broth containing 20 μg/ml of Q-19 and neomycin, the number of organisms dropped significantly in 40 minutes and the end of 4 hours, no viable bacteria were recovered (Table II). In the concentration of drugs employed, the action of Q-19 and neomycin was primarily bactericidal, whereas the action of aureomycin and chloromycetin is bacteriostatic. Thus, these drugs are similar to streptomycin in their bactericidal action but resemble penicillin and aureomycin in regard to the pattern of resistant variants which appear when a culture of *E. coli* is exposed to them.

Discussion. While *in vitro* results do not always coincide with clinical experience, the foregoing observations with neomycin, Q-19, and polymyxin B suggests that they may be useful in the therapy of some infections caused by *P. aeruginosa, P. vulgaris, A. aerogenes,* and *E. coli.* The great *in vitro* activity of Q-19 and polymyxin B against *P. aeruginosa* suggests that these drugs may be effective against infections caused by this organism. Similarly, neomycin and chloromycetin appear worthy of trial against infections due to *P. vulgaris.* It is encouraging that 79 of 80 strains of gram negative bacilli tested were sensitive to at least 1 of the 5 antibiotics, and the majority of strains relatively resistant to some of the antibiotics were highly sensitive to others. This might warrant the simultaneous use of 2 or more antibiotics when it is impractical to determine specific sensitivities of bacteria. Seventy-five of the 80 strains included in the 4 species were inhibited by 31.2 μg per ml of neomycin, which is a concentration of drug readily obtained in the urine following parenteral administration(15). The failure to demonstrate variants of *E. coli* highly resistant to either Q-19 or neomycin is evidence that the rapid appearance of resistant variants, which is a disadvantage with streptomycin therapy, might not occur during the clinical use of Q-19 or neomycin. These observations confirm the findings of Waksman in regard to the activity of crude neomycin(1).

The instability of aureomycin in dilute solutions makes it difficult to compare *in vitro* results over a period of 18 hours with those of neomycin, Q-19, and chloromycetin, which form stable solutions. The data show, however, that aureomycin is not as active against *P. vulgaris* and *P. aeruginosa* as it is against *E. coli* and *A. aerogenes,* and that it is not equally active against different strains of the 2 latter species.

The suggested chemical relationship between Q-19 and the group of antibiotics, which include polymyxin and aerosporin, seems to be borne out in the data presented (3). Two strains of *A. aerogenes* resistant to Q-19 were also resistant to polymyxin B and, in general, there was close agreement between the sensitivities of the strains to these 2 antibiotics. Clinical studies based on these findings are now in progress.

Summary and conclusions. Twenty strains each of *E. coli, Aerobacter aerogenes, Pseudomonas,* and *Proteus vulgaris* were tested for sensitivity to neomycin, Q-19, aureomycin, chloromycetin, and polymyxin B. Q-19 and polymyxin B were particularly effective against Pseudomonas. Neomycin and chloromycetin were most effective against Proteus.

15. Unpublished data.

Only 5 of the 80 strains tested were not sensitive to 31.2 μg or less of neomycin.

The degree of resistance possessed by variants exposed to Q-19 and neomycin is low. These variants display the "penicillin" rather than the "streptomycin" type of drug resistance.

Q-19 and neomycin are bactericidal in a concentration equal to or only slightly higher than that in which they are bacteriostatic.

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Content of Desoxyribo and Ribonucleic Acids of Retina Under Various Conditions.* (17802)

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Reports from various sources indicate(1) that the cells of cortical centers show considerable loss of ribonucleic acid after intense physiological stimulation, the level of ribonucleic acid returning to normal only slowly. In these experiments the ribonucleic acid was determined by microscopic measurement of the ultraviolet absorption of individual cells. The interpretation of results of such measurements has recently been criticized(2). The retina consists of several layers of ganglion cells and their neurites and nerve cells differentiated to cones and rods. All these nerve cells are readily accessible, can be obtained in considerable amount and can easily be stimulated by physiological means and in varied intensity. We have attempted to study, by chemical methods, the influence of light stimuli on the ribonucleic acid content of the retina of the rabbit, but observed no significant effect. However, it was found that the retina differs with respect to the composition of its nucleic acid fraction considerably from other tissues (including the cortex of the brain.) As this research cannot be continued in the near future we report here our experimental material so far obtained.

Experimental preparation of materials. The eyes were enucleated immediately after death of the animal. To obtain easy and complete separation of the retina from the choroidea the eye was immediately placed in solid CO_2 and either was kept frozen for several days or, after being frozen, immersed in absolute alcohol at 0° and kept there for 5 days. Neither the freezing nor the treatment with alcohol interfered with the determination of the nucleic acids. The retina could be separated from the choroidea equally readily after being thawed and after fixation in alcohol.

Analytical methods. The nucleic acids were extracted according to the procedure of Schneider(3). Desoxyribonucleic acid (DNA) was determined as a rule by the diphenylamine reaction(4). Readings were carried out at 610 mμ with the Beckman spectrophotometer. To ascertain that the substance determined by the diphenylamine reaction was identical with desoxyribonucleic acid, the determination of the latter was carried out in a few cases by the cysteine reaction according to the procedure recommended by Stumpf (5). These completely different procedures gave practically identical results. The ribonucleic acid (RNA) was in all cases determined by the orcinol reaction according to

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