

ously and of these 17 were injected with 2 mg and 12 with 4 mg of lactogenic hormone. Since the lower dose seemed to be just as efficacious as the 4 mg level, the data are not treated separately. Nor are the rats which received thyroxin considered separately since the results did not differ from those obtained in the other animals. Of the 29 rats, only 5 showed no sign at necropsy of having been pregnant. The other 24 animals succeeded in implanting a total of 175 embryos as judged by the number of normal or resorbing implantation sites. Eighty of these (present in 14 rats) appeared to have been maintained in a healthy condition through day 11. As shown in the table, living fetuses were also found on days 14 and 16. No attempt was made in this experiment to maintain pregnancy until term. The majority of the rats showed lobulo-alveolar mammary

growth equaling that of normal mid-pregnancy. Most of the rats lost weight even in cases where fetal and placental growth were found to have continued normally.

Summary. The same daily doses of purified lactogenic hormone (60 I.U.) and estrone (10 I.U.) capable of inducing beginning lobulo-alveolar mammary growth in the hypophysectomized rat, also ensured successful implantation in rats hypophysectomized and injected from the day of sperm. Since only about one-half of the estimated number of implantations were found to have developed normally through mid-pregnancy it follows that these 2 pure substances in the doses used did not adequately substitute for the intact pituitary of a pregnant rat. When injected alone neither lactogenic hormone nor estrone permitted implantation to occur.

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Antiluminescent Activity of Antibiotic Substances.

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As has been pointed out previously,^{1,2} certain substances prepared from fungi or actinomycetes are capable, in high dilution, of interfering with the bio-luminescence of luminescent bacteria. This observation has been applied to a larger series of antibiotic substances isolated from fungi, actinomycetes, and bacteria, or of simple chemical nature.

The antiluminescent test was carried out as previously described.^{1,2} Although a standard of aspergillilic acid with 256 R. units was included in every test, the results were read in gamma required to produce blacking out per 1.0 ml of a 24-hour culture of *Photobacterium fischeri* without any reference to the standard. This also was true of the antibacterial test which was read in gamma required to inhibit

for 18 hours the growth of 1,500,000 organisms of a culture of *Streptococcus pyogenes* per 1 ml of beef-heart broth. With all substances at least 2 tests were carried out, and with several of them many tests were made. If 2 tests did not give close agreement in the absolute values obtained, enough were made to obtain an average value. The results are shown in Table I, which gives the substances in decreasing order of (1) antiluminescent activity, and (2) antibacterial activity. Also shown is the antiluminescent-antibacterial (AL/AB) ratio.

Certain points of interest arise. Thus, many new antibiotic substances have been shown to have antiluminescent activity. One of these is the chemical compound, tolu-p-quinone;³ 2 of them—pyocyanase and pyocyanin—are bacterial in origin; 2—streptothricin⁴ and actinomycin⁵—are from actinomycetes; and the remainder are from fungi.

¹ Rake, G., McKee, C. M., and Jones, H., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 273.

² Jones, H., Rake, G., and Hamre, D. M., *J. Bact.*, in press.

TABLE I.
Comparison of Antiluminescent and Antibacterial Activities and AL/AB Ratios of Various Antibiotic Substances.

Smallest amount in γ showing activity					
Antiluminescent test		Antibacterial test		AL/AB ratio	
Tolu-p-quinone	0.11	Gramicidin	.002	Tolu-p-quinone	.002
Pyocyanase*	3	Tyrothricin	.008	Pyocyanase	.07
Clavacin* ¹	11	Penicillin ²	.0156	Clavacin ¹	.18
		Penicillin ¹	.06		
		Flavatin	.256		
Aspergillie Acid	15	Gramidinie Acid	.23	Sod. Clavacinate	.18
Gliotoxin	17	AP21	.31	Clavacin ²	.19
Clavacin* ²	22	Actinomycein A	.54	Sulfanilamide	<.56
				Phenol	.5
Pyocyanin*	47	Aspergillie Acid	2	Pyocyanin	1.7
Actinomycein A*	54	Gliotoxin	2.1	Lauryl Sulfate	4.6
Streptothricin* [†]	56	Streptothricin	2.8	Aspergillie Acid	7.5
Sodium Clavacinate* [‡]	94	Fumagacin	13	Gliotoxin	8
Flavatin	256				
Fumagacin*	273	Pyocyanin	27	Streptothricin	20
Lauryl Sulfate	273	Pyocyanase	42	Fumagacin	21
Phenol	1170	Tolu-p-quinone	55	Actinomycein A	100
				Flavatin	1000
Penicillin ^{†1}	1650	Lauryl Sulfate	59	AP21	>1630
Sulfanilamide	3940	Clavacin ¹	63	Gramidinie Acid	>2175
				Penicillin ¹	27,500
Gramicidin [‡]	> 500	Clavacin ²	113	Tyrothricin	>62,500
Gramidinie Acid [‡]	> 500	Sod. Clavacinate	500		
Tyrothricin [‡]	> 500	Phenol	2300	Gramicidin	>250,000
AP21 [‡]	> 500	Sulfanilamide	>7000	Penicillin ²	>325,000
Penicillin ²	>5000				
Egg White	No activity	Egg White	No activity	Egg White	—

*Supplied through the courtesy of Dr. S. A. Waksman of the N. J. Agricultural Experiment Station, Rutgers University.

[†]Crude preparations.

[‡]Supplied through the courtesy of Dr. J. C. Hoogerheide of the Biological Laboratories of E. R. Squibb and Sons. AP21 is the active material obtained from a spore-forming anaerobe.

Two substances, namely, lauryl sulfate and fumagacin from a mold,⁶ show slight antiluminescence while phenol, sulfanilamide and flavatin,⁷ a newly discovered product of *Aspergillus flavus*, join penicillin and the products from certain soil bacteria in showing little antiluminescent activity under the condition of the test.

In the case of such substances as the chemicals tolu-p-quinone, lauryl sulfate, phenol and sulfanilamide, or substances which have

been obtained in crystalline form such as pyocyanin, actinomycin, aspergillie acid or gliotoxin, one can be sure that the antiluminescent activity is inherent in the substance itself and is not due to contaminating impurities. In other cases—clavacin,⁶ pyocyanase, streptothricin, flavatin, and penicillin—the substances tested were of varying degrees of purity. In these cases the actual figures given for the antiluminescent activity have little significance since they vary with the preparation. Moreover, there is no evidence from the examination of one preparation whether the antiluminescence is an inherent property of the substance or is due to an impurity. In the case of flavatin, for example, it may be that the slight antiluminescent activity is due to small amounts of aspergillie acid which substance is formed in large quantity by the mold *Aspergillus flavus* from which flavatin is ob-

³ Waksman, S. A., and Woodruff, H. B., *J. Bact.*, 1942, **44**, 373.

⁴ Waksman, S. A., and Woodruff, H. B., *Proc. Soc. Exp. Biol. and Med.*, 1942, **49**, 207.

⁵ Waksman, S. A., and Woodruff, H. B., *J. Bact.*, 1941, **42**, 231.

⁶ Waksman, S. A., Horning, E. S., and Spencer, E. L., *Science*, 1942, **96**, 202.

⁷ McKee, C. M., to be published.

tained, under different conditions of growth. If, however, several preparations of the same substance are examined, and the AL/AB ratios compared, definite evidence may be forthcoming. Thus in Table I the activities of 3 preparations of clavacin or sodium clavacinate are shown. These show antibacterial activities varying nearly 10-fold, but the AL/AB ratios are identical. This would indicate that the antiluminescent activity of clavacin might be due to a constantly present impurity, but is more probably inherent in the substance itself. Decrease in the AL/AB ratio with increasing purification would indicate that the antiluminescence was due to an impurity; increase in the ratio, that the antibacterial activity was due to an impurity.

It is clear that the antibiotic substances divide themselves into groups on the basis of their AL/AB ratios (Table I). While no clear cut divisions occur there is marked difference between penicillin or the products from soil bacteria with their very high ratios on the one hand, and tolu-p-quinone or pyocyanase with very low ratios on the other.

The results obtained with penicillin will be considered more fully elsewhere.⁸ It is clear from Table I, however, that penicillin in purified form has no antiluminescent activity under the conditions of the test as described here. Because of its high antibacterial activity, however, it does affect the *Photobacterium fischeri* itself and causes gradual disappearance of luminescence over a long period of observation.

Summary. The power to inhibit the bioluminescence of luminescent bacteria has been shown to be possessed by antibiotic agents other than those derived from fungi and actinomycetes. Amongst the antibiotic substances newly shown to have such activity are some obtained from bacteria as well as others which are derived from fungi, and still others which are pure chemicals. An antiluminescent-antibacterial ratio has been obtained for all of the substances tested and its significance is discussed.

⁸ McKee, C. M., and Rake, G., to be published.

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Germicidal Action of Aliphatic Alcohols.

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Several investigators have compared the bactericidal properties of the aliphatic alcohols. Wirgin¹ and Stadler² reported that the disinfecting action of the alcohols corresponded to an increase in their molecular weights, with the exception of the tertiary alcohols. None of the alcohols could kill spores at room temperature. Kokko³ confirmed the previous reports and observed that among isomers toxicity showed the following order: primary > iso- > secondary > tertiary. Preliminary

experiments to determine the action of 12 different concentrations of ethyl alcohol on *Staphylococcus aureus* (2 strains), *Aerobacter aerogenes* (2 strains), *Serratia marcescens* (2 strains), *Eberthella typhosa* (2 strains), *Escherichia coli* (2 strains), *Bacillus megatherium*, *Bacillus subtilis* (2 strains), *Saccharomyces cerevisiae* and *Monilia albicans* indicated that all nonsporeforming bacteria tested were destroyed in any percentage of the alcohol between 100% and 45% in 5 minutes, while the yeasts and 2 strains of *Eberthella typhosa* were destroyed by 35% alcohol and above. The sporeforming organisms were not affected.

The next set of experiments was concerned

¹ Wirgin, G., *Z. Hyg. und Infekt.*, 1904, **46**, 149.

² Stadler, H., *Arch. Hyg. und Bakt.*, 1911, **73**, 195.

³ Kokko, U. P., *Arch. Hyg. und Bakt.*, 1939, **122**, 44.