died on the 23rd day after the first injection with a parasitemia of 74%. This does not mean that parasites were able to survive the rigors of being freed from the erythrocyte by the means employed. We have repeatedly seen that small blood clots will protect the erythrocytes from hemolysis and therefore from digestion. Perhaps a single microscopic mass of such material could be infective and result in the death noted. We believe it most probable that the treatment destroys all those parasites freed from red cells.

Summary. By combining saponin hemolysis with enzymatic digestion at low pH avian plasmodia have been freed from the enclosing erythrocytes. These free parasites retain their specific morphology so that in stained smears of the erythrocyte-free parasites *P. lophurae* can be distinguished from *P. cathemerium.* It is probable that the treatment used kills the parasites but the potential value in experimental malariology of this new method is not necessarily reduced thereby.

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An Antibiotic (Anti-Smegmatis Factor) Produced by an Actinomycete, Specifically Inhibiting Species of *Mycobacterium.**

ALBERT KELNER† AND HARRY E. MORTON. (Introduced by A. N. Richards.)

From the William Pepper Laboratory of Clinical Medicine and the Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Penn.

The crude culture filtrate of an actinomycete (designated as A-82) has been observed to show extreme specificity in its action. It inhibited only species within the genus *Mycobacterium,* particularly the saprophytic species of *Mycobacteria,* and especially *Mycobacterium smegmatis.* This antibiotic will be referred to as the antismegmatis factor. The apparent genus specificity of the antismegmatis factor gives it unusual interest.

The actinomycete, A-82, was isolated from potted greenhouse soil by methods to be described elsewhere.[1] Although the organism resembles *Actinomyces lavendulae,* it differs sufficiently to justify a description.

Elliptical spores are borne in chains. Hyphae are coarse. No spirals were seen. Optimum temperature for growth about 28°C; growth at 14°C but not at 37°C. Peptone and gelatin media are blackened, but otherwise no soluble pigment is formed. Abundant grey growth on nutrient agar with white to grey spores. Thin spreading growth on Czapek medium with pink spores. Moderate growth on glucose asparagin agar with lavender colored spores. Good growth on calcium citrate agar; spores white, with trace of pink. Poor growth on calcium malate agar with white spores. Abundant grey-brown growth on potato with spores white, becoming lavender; the potato is blackened. Milk becomes alkaline with rapid clearing. Slow and variable liquefaction of gelatin. Moderate hydrolysis of starch.

The culture was maintained on the starch, potato, tryptone medium described by Kelner and Morton.[1]

Since *Mycobacterium smegmatis* (Univ. of Penn. strain P-49) was the organism most susceptible to the action of the antismegmatis factor, it was used for assay purposes. *M. smegmatis* was grown in Bacto-nutrient broth with daily transfers to stimulate rapid and diffuse growth. An 18-24 hour broth culture was used for streaking plates. The usually aggregated growth was dispersed by

* This investigation has been supported by a grant from the Smith, Kline, and French Laboratories.

† Present address, Carnegie Institute, Cold Spring Harbor, N.Y.

[1] Kelner, A., and Morton, H. E., in press.

TABLE I.
Production of Antismegmatis Factor by Actinomycete A-82.

Type of culture	Days									
	2	3	4	5	6	7	8	9	11	13
Stationary*										
pH of culture				6.4	6.8	6.6	6.2		7.0	
Activity†				400	400 (1000)	1000	1000 (4000)	1000	400 (1000)	
Stationary*										
pH of culture				6.6	6.8	6.8		7.0		
Activity†				4000	2000	2000 (4000)	4000 (10,000)	4000 (10,000)		2000 (4000)
Shake‡										
Activity†	40	40	40			1000 (2000)		2000 (4000)		
Shake‡										
pH of culture	6.2	5.8	6.0		4.8		4.6	4.2		
Activity†	40 (100)	400	2000		1000		200 (400)	400		

* Medium contained 30 ml glycerine/liter.

† *M. smegmatis* dilution units/ml (roughly equivalent to inhibiting dilution); one dilution unit being the smallest amount of crude culture filtrate per ml of assay medium which would inhibit *M. smegmatis* under conditions of the test. Figures in parenthesis indicate dilution of antismegmatis factor allowing only trace of growth of *M. smegmatis*.

‡ Medium contained 40 ml glycerine/liter.

vigorous shaking just before use, until the culture had a uniform, finely granular appearance. The agar dilution method of assay was used employing Bacto-nutrient agar, pH 7.3. *M. smegmatis* was streaked with a loop, and the plates read after incubation at 37°C for 18 to 36 hours, as soon as the control plate showed good growth. If the end-point was not sharp, it was helpful to dilute the culture 1½ to 2 times with sterile broth.

Titres of 2000 to 4000 (occasionally 10,000) *M. smegmatis* dilution units per ml of culture were formed when A-82 was grown in a medium composed of 5 g Bacto-tryptone, 30 ml glycerine, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 1.2 g Na_2HPO_4, 0.8 g KH_2PO_4, 0.01 g $FeSO_4 \cdot 7H_2O$, and 1000 ml distilled water; final pH, 6.8 to 7.2. For stationary cultures 2.5 g agar were added to the above formula. Incubation temperature was 28°C.

There was little or no antibacterial activity produced in nutrient broth or glucose-nutrient broth. In the tryptone-glycerine medium, the glycerine could not be replaced by dextrose, lactose, or brown sugar.

Table I shows the production of antismegmatis factor in stationary and in shake cultures.

Characteristics of the crude antismegmatis factor. Crude culture filtrates containing the antismegmatis factor, adjusted to pH 7.0, withstood, with little loss of activity, 56°C, for 45 minutes, and 100°C for 30 minutes. The material could be autoclaved at 121°C for 15 minutes with less than 50% loss of activity. Autoclaved material remained stable 6 days, and lost less than 50% activity in 10 days at 37°C. The crude antismegmatis factor was stored satisfactorily in the frozen state.

Partial concentration was easily accomplished by allowing frozen culture filtrates to thaw slowly with no disturbances. The bottom layer of the melted material contained 2 to 4 times more active substance than the top layer.

Intraperitoneal injection of 2 ml of crude culture filtrates (4000 *M. smegmatis* dilution units) had no apparent effect on white mice weighing 17 to 20 g.

The activity of the antismegmatis factor increases with the alkalinity of the assay agar, as can be seen in Table II.

TABLE II.
Relation of Alkalinity of Assay Agar to the Activity of the Antismegmatis Factor Against *M. smegmatis.*

pH	5.4	6.0	6.4	6.8	7.0	7.2	7.4	7.8	8.0	9.0
Activity*	10 (400)	20 (1000)	1000 (2000)	2000 (4000)	2000 (4000)	4000	4000	4000 (10,000)	10,000 (20,000)	20,000

*Dilution units, see footnote, Table I.

In acid media, and sometimes in neutral media, if the culture of *M. smegmatis* contained clumps, the assay plates showed a trace of growth at a wide range of dilutions, *e.g.*, from dilutions of 1:10 to 1:1000. The same phenomenon was shown by other sensitive organisms such as *M. phlei.*

A crude culture filtrate showing nearly complete inhibition of *M. smegmatis* in a dilution of 1:1280 was tested in Bacto-nutrient broth, pH 7.3, against the following microorganisms and found to have no inhibitory action at a dilution of 1:10: various species of *Bacillus, Brucella, Clostridium, Corynebacterium, Diplococcus, Eberthella, Escherichia, Micrococcus, Salmonella, Shigella, Staphylococcus,* and *Streptococcus,* as well as *Alcaligenes faecalis, Chromobacterium violaceum, Gaffkya tetragena, Klebsiella pneumoniae, Neisseria catarrhalis, N. sicca, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Trichophyton interdigitale* and *Vibrio comma.* It had very little if any inhibitory effect on a pathogenic strain of *Mycobacterium tuberculosis,* variety *bovis* (Ravenel strain) by the method described by Wells.[2]

A preparation containing 2000 dilution units failed to inhibit at a dilution of 1:10 in Bacto-nutrient agar *Candida albicans,* or in Czapek agar, *Aspergillus oryzae* or *A. niger.* In another experiment a preparation containing 2000 dilution units inhibited at a dilution of 1:10 but not at 1:20, *Bacillus subtilis,* and an unidentified penicillinase-producing *Bacillus* (No. 569 of the collection at Northern Regional Research Laboratory) at the same range of dilutions. *Mycobacterium phlei* (Univ. of Penn. No. P-50) was inhibited at a dilution of 1:400, with only a trace of growth at a dilution of 1:1000, and *M. tuberculosis* No. 607, a nonpathogenic strain‡ was inhibited at a dilution of 1:40, and showed only a trace of growth at 1:100 dilution, using 3% glycerine nutrient agar for the culture medium. In another experiment, using an antismegmatis factor preparation which inhibited *M. smegmatis* completely at 1:20,000, *M. tuberculosis* No. 607 showed only a trace of growth at dilutions of 1:20 to 1:20,000. The control showed abundant growth.

Discussion. The apparent genus specificity of the antismegmatis factor is of theoretical interest since it points to the possibility of antibiotics specific for other genera of bacteria or infectious agents. The specificity of the antismegmatis factor is probably not absolute, for more concentrated, chemically purified, preparations would probably inhibit such nonacid-fast organisms as *Bacillus subtilis,* an organism which was occasionally inhibited by very high concentrations of our most active crude culture filtrates. The far greater sensitivity of the *Mycobacteria,* as shown in our experiments, would still make the antismegmatis factor genus-specific. It is of course possible that genera not included in the 60-some strains of nonacid-fast organisms tested by us are sensitive to the antismegmatis factor. An example of great sensitivity of a single group of organisms for a compound is shown by yeasts for 2,3-dichloro-1,4-napthoquinone.[3]

Several *Mycobacterium*-inhibiting agents have been reported as being formed by

2 Wells, W. F., *Science,* 1946, **104**, 254.

‡ We wish to thank Dr. Florence Seibert of the Henry Phipps Institute, Philadelphia, for this strain.

3 Woolley, D. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 225.

Aspergillus (usually *A. fumigatus*)[4-7] and by *Penicillium*.[8,9] Unfortunately little is reported of the bacterial spectra or other characteristics of these agents. However, "aspergillin"[7] may resemble the antismegmatis factor in that preparations inhibiting *M. phlei* at dilutions of 1:50 to 1:100 and *M. tuberculosis* at 1:10 to 1:20 failed to inhibit 2 strains of *Staphylococci*. Both "aspergillin" and "mycoidin"[6] are thermostable, as is the antismegmatis factor. Such *Mycobacterium*-inhibiting agents as streptothricin and streptomycin are clearly differentiated from the antismegmatis factor because they inhibit *Escherchia coli* and *Staphylococcus aureus* to the same degree they inhibit *Mycobacteria*.[10]

Of the older antibacterial agents, the most specific against *Mycobacteria* are the chaulmoogrates.[11] Hesse and Meisser[12] report that activity of several heterocyclic organic compounds against *M. tuberculosis* is correlated with their basic nature. The marked increase in activity of the antismegmatis factor with increasing pH, a property suggestive of a basic compound (see studies on streptothricin and streptomycin[13]) is of interest in this connection.

Although the antismegmatis factor failed to inhibit one strain of pathogenic *M. tuberculosis*, variety *bovis*, the fact that *M. tuberculosis* variety *hominis* No. 607 was inhibited, suggests that pathogenic strains of *M. tuberculosis* may be sensitive to preparations of the antismegmatis factor more concentrated than the crude culture filtrates we used and that there may be variations in sensitivity of strains within the species.

Summary. An actinomycete (A-82) forms an antibiotic which when tested against more than 60 species, including more than 20 genera of bacteria inhibited only *M. smegmatis*, *M. phlei*, and a nonpathogenic, rapidly growing strain of *M. tuberculosis*, variety *hominis* No. 607. A pathogenic *M. tuberculosis*, variety *bovis*, was not inhibited. The specificity of this antibiotic, referred to as the antismegmatis factor (because of the sensitivity of *Mycobacterium smegmatis*), for the genus *Mycobacterium* is discussed. Methods for producing the crude antismegmatis factor are outlined. The antibiotic is thermostable, and is more active in alkaline media than in acid media. No chemical purification of the antismegmatis factor was done.

4 Asheshov, I. N., and Strelitz, F., *Science*, 1945, **101**, 119.

5 Kallos, P., *Nature*, 1945, **155**, 300.

6 Gerber, I. E., and Gross, M., *Science*, 1946, **103**, 167.

7 Soltys, M. A., *Nature*, 1944, **154**, 550.

8 Miller, D. K., and Rekate, A. C., *Science*, 1944, **100**, 172.

9 Smith, M. I., and Emmart, E. W., *Pub. Health Rep.*, 1944, **59**, 417.

10 Schatz, A., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 244.

11 Walker, E. L., and Sweeney, M. A., *J. Inf. Dis.*, 1920, **26**, 238.

12 Hesse, E., and Meissner, G., *Arch. Exp. Path. u. Pharmakol.*, 1931, **159**, 676.

13 Waksman, S. A., *Microbial Antagonisms and Antibiotic Substances*, 1945, The Commonwealth Fund, N.Y.

15558

The Biological Activity of Calciferol (Vitamin D_2).*

AARON ARNOLD. (Introduced by M. L. Tainter.)

From the Sterling-Winthrop Research Institute, Rensselaer, N.Y.

The international unit of vitamin D is the activity of 1 mg of the international Standard of Reference which, in turn, is defined in terms of a solution of irradiated ergosterol in olive oil. Collaborative study[1] in 1940 showed

* The writer wishes to express his appreciation to Mrs. Ellen Miller for technical assistance and to Mrs. Mary Lenz for the statistical treatment of the data.

1 Coward, K. H., *Bull. Health Org., League of Nations*, 1940-1, **9**, 425.