ach tube; in others intravenously as the sodium salt and in a third group both the oral and the intravenous routes were used. Thromboplastin was prepared by the acetone dehydration procedure of Quick.[9] One-tenth of 0.1 M. sodium oxalate per rabbit brain was added before triturating with acetone. By this means any calcium in the brain tissue was removed, since no detectable amount of this element was found by ordinarily chemical methods in 0.2 g of the dried rabbit brain powder. The prothrombin time of oxalated human, dog and rabbit plasma was determined by the procedure of Quick. A standard series of dilutions of $CaCl_2$ solutions was used since the lower concentrations have been shown to be more sensitive to minimum changes of the prothrombin time.

Results and Discussion. It is evident from the results in Table I that the thromboplastin prepared from the brains of rabbits poisoned with dicumarol was less active than that obtained from normal animals. With dogs' and rabbits' plasmas this difference is obtained with the various dilutions of calcium used but becomes more accentuated in the lower concentrations. With human plasma this difference is obtained only in the lower concentrations.

In comparing the thromboplastin from dicumarolized rabbits with that of normal animals on rabbit and dog plasmas in which the prothrombin was depressed by dicumarol, the difference in activity was found to have a wider spread (Table II) but followed essentially the pattern of normal oxalated plasma in regard to the influence of calcium. Occasionally when the prothrombin is presumably normal as measured with fully active thromboplastin, a distinct delay is brought out by dicumarol thromboplastin as shown in the case of dog No. 2 on the 9th day after a single dose of dicumarol intravenously.

It is somewhat surprising that dicumarol should decrease the activity of the thromboplastin in brain tissue and this naturally leads to the question whether the thromboplastin occurring in blood is similarly affected. If this be the case, then the delayed coagulability due to dicumarol would have a 3-fold cause: decrease of prothrombin level, inadequate calcium for optimum prothrombin activity and less active thromboplastin.

What importance the latter factor has is difficult to evaluate especially since the diminution in thromboplastic activity is not particularly marked. In view of the fact that the nature of thromboplastin is poorly understood and the action of dicumarol is not completely known, it would be futile to attempt to offer any possible explanation for the results presented in this paper.

[9] Quick, A. J., *Science*, 1940, **92**, 113.

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Grisein, a New Antibiotic Produced by a Strain of *Streptomyces griseus*.*†

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Streptomyces griseus represents a large and heterogeneous group of actinomycetes, widely distributed in nature. Cultures belonging to this species or species-group have been isolated from a great variety of substrates, largely soils, peats, composts and animal contents. Since the first report[1] on the production of streptomycin by 2 cultures of *S. griseus* obtained from 2 different substrates,

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[1] Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.

TABLE I.
Spectrum of Cultures of *S. griseus* Grown in Nutrient and Corn Steep Broths.
Dilution units per 1 milliliter.

	Nutrient broth	Corn steep broth
Escherichia coli	250	30
Aerobacter aerogenes	0	0
Serratia marcescens	250	50
Pseudomonas aeruginosa	5	0
" *fluorescens*	0	0
Proteus vulgaris	0	0
Mycobacterium phlei	0	0
Bacillus subtilis	200	30
" *mycoides*	0	0
" *megatherium*	100	100
Staphylococcus aureus	200	200
Sarcina lutea	0	0

nearly 100 cultures of this organism or closely related forms have been isolated.[2] However, very few of these were found to be capable of producing streptomycin. Actually, one other culture among the many isolated in our laboratory had the capacity of forming authentic streptomycin.[3] Two other streptomycin-producing strains were reported from another laboratory.[4]

Morphologically, *S. griseus* is characterized by the formation of clusters or tufts of sporulating hyphae in the aerial mycelium. The most characteristic cultural property is the production on suitable media of an abundant, powdery, greyish-green aerial mycelium. No distinct chromogenic (dark brown to black) pigment is produced on organic media; occasionally a greenish to light brownish pigment is observed just below the vegetative growth. The typical cultures may give rise to variants which differ from the original organism both in their morphology and in their physiology. Two variants have already been isolated, one of which is devoid of aerial mycelium[5] and the other showing certain other cultural characteristics which are distinct from those of the mother culture; both variants are unable to produce streptomycin. The ability of other variants to produce streptomycin may be greatly reduced.

For the isolation of *S. griseus* from natural substrates, media enriched with streptomycin have been utilized.[3] Such media prevent the development of most of the bacteria and the great majority of actinomycetes. Among a large number of cultures isolated by this method, one was found to form an antibiotic which at first appeared to be streptomycin-like in nature. This culture (G-25) was isolated from a sample of Huleh peat, obtained from Palestine. The antibiotic was produced on a variety of media, of which peptone-meat extract and starch-tryptone had the highest activity. Culture filtrates of the organism were found to inhibit the growth of various Gram-negative bacteria, notably *Escherichia coli,* as well as certain Gram-positive bacteria, such as *Bacillus subtilis* and *Staphylococcus aureus.*

In spite of certain apparent similarities of this antibiotic to streptomycin and to streptothricin, 4 important differences were noted, suggesting that one is dealing here with a type of substance distinct from the others: 1. The bacteriostatic spectrum of the new agent was much narrower than that of either streptomycin or streptothricin. A typical culture filtrate of G-25 had a titer of 300 *E. coli* and 300 *B. subtilis* dilution units, but,

TABLE II.
Bacteriostatic Spectra of Grisein and Streptomycin.
Units per gram of crude preparations.

	Grisein × 1,000	Streptomycin × 1,000
Escherichia coli W*	25	25
Serratia marcescens	10	25
Proteus vulgaris	<.1	10
Pseudomonas fluorescens	3	2
Ps. aeruginosa	<.1	1
Aerobacter aerogenes	<.1	10
Salmonella schottmülleri	10	15
" *aertryke*	<.1	3
Eberthella typhi	<.1	25
Shigella sp.	30	25
Klebsiella pneumoniae	5	25
Mycobacterium phlei	<.1	100
Bacillus subtilis	10 to 30	125
" *megatherium*	10 to 20	100
" *mycoides*	<.1	20
" *cereus*	<.1	30
Staphylococcus aureus	30 to 100	15
S. lutea	0.5	100
Micrococcus lysodeikticus	200 to 300	150

* Considerable variation in sensitivity of different strains has been observed.

2 Waksman, S. A., Schatz, A., and Reynolds, D. M., *Ann. N. Y. Acad. Sci.,* 1946, **48**, 73.

3 Waksman, S. A., Reilly, H. C., and Johnstone, D. B., *J. Bact.,* 1946, **52**, 393.

4 Carvajal, F., *Mycologia,* 1946, **38**, 596.

5 Waksman, S. A., Reilly, H. C., and Schatz, A., *Proc. Nat. Acad. Sci.,* 1945, **31**, 157.

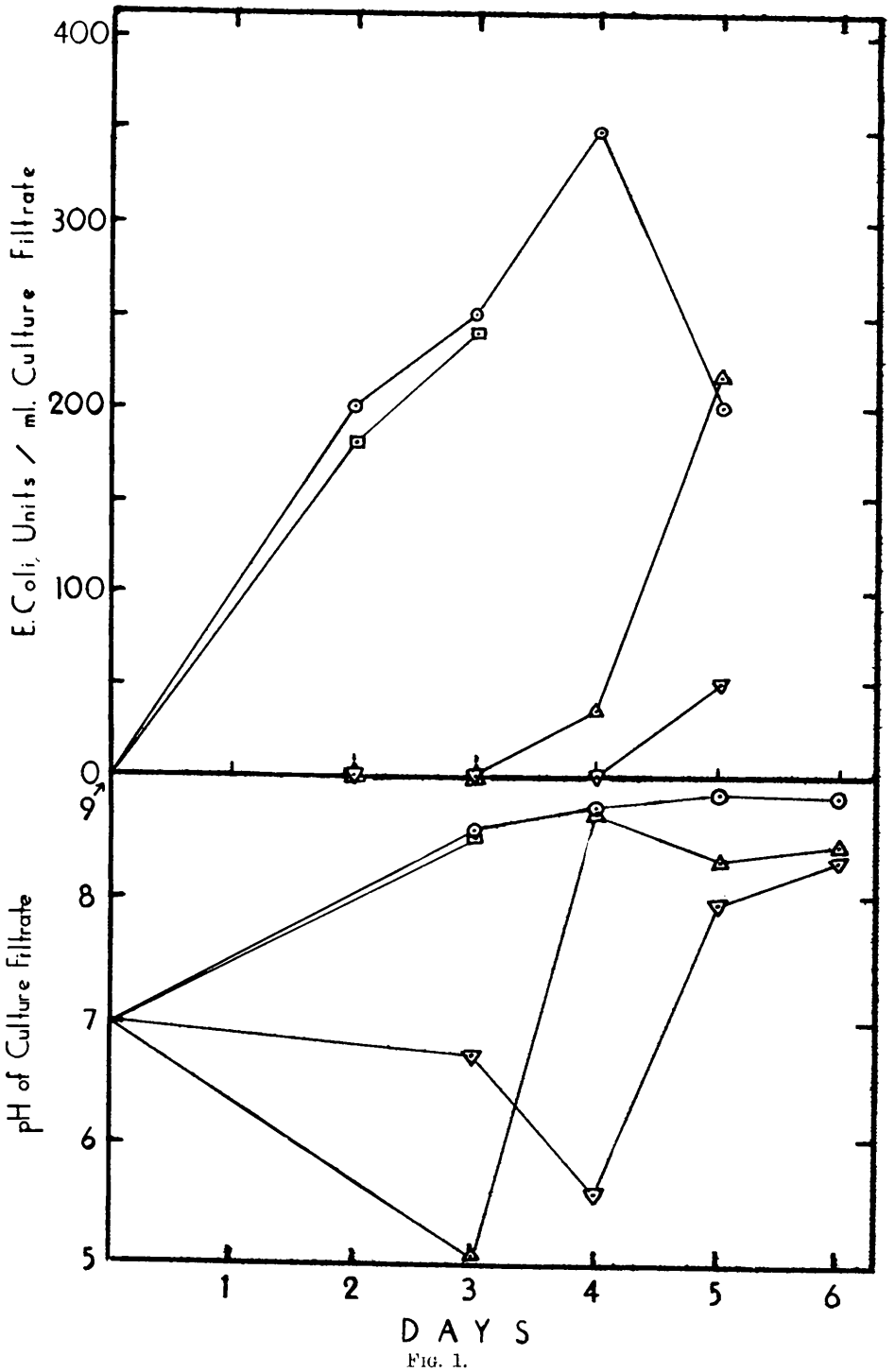

FIG. 1.

The Effect of Various Supplements on the Production of Grisein.
○ Basal medium—5 g peptone, 5 g NaCl, 10 ml neutralized corn steep liquor per liter. Supplements added—10 g/lit.; △, glucose; inverted △, glycerol; □, lactose.

unlike streptomycin, had no activity against either *B. mycoides* or *Aerobacter aerogenes*. Likewise, it had no activity against fungi, a characteristic which distinguishes it from streptothricin. 2. The chemical nature of the antibiotic produced by G-25 is different from that of the other 2 antibiotics, since, while readily adsorbed on charcoal, it could not be removed with acid alcohol. 3. Glucose did not bring about the inactivation of this antibiotic, though it usually inactivates both streptothricin and streptomycin. 4. Streptomycin-resistant strains of *E. coli* remained sensitive to this antibiotic.

Culture G-25 was grown both in stationary and in submerged cultures. The submerged culture underwent rapid, even if incomplete, lysis in 3 to 5 days. Media containing meat extract or corn steep, but free from glucose, gave the best activity. The bacteriostatic spectrum of a typical culture filtrate of this organism is brought out in Table I. The agar streak method[6] was used. The results point to a very narrow antibiotic spectrum, which is not limited either to the Gram-positive or to the Gram-negative bacteria (Table II).

When the culture filtrate was treated with activated charcoal (5 g per liter), all the antibiotic was removed. On treatment of the charcoal with 9% ethanol, about half of the total activity was recovered. The alcohol eluate was concentrated to a syrup *in vacuo*, and absolute methanol was added until a precipitate began to form. The final preparation was precipitated with acetone, washed with ether, and desiccated. A yield of 400-600 mg was obtained per liter of culture. This preparation showed an activity of about 15,000-30,000 *E. coli* dilution units per gram. More active preparations have often been obtained, ranging up to 100,000 *E. coli* units per gram. Unfortunately, only a small part, usually about 20%, of the total active substance present in the culture was thus recovered. The antibiotic spectrum of the isolated fraction was, however, exactly the same as that of the crude culture filtrate, thus pointing to the identity of the substance in the medium and of the isolated preparation.

It is proposed to designate this antibiotic substance as *grisein*. It is insoluble in ether, chloroform, absolute acetone, absolute ethanol, or benzene. It is slightly soluble in ordinary acetone and in 95% ethanol. It is readily soluble in water. It is heat-stable, since heating for 10 minutes at 100°C does not reduce greatly its activity. It is not affected if kept for short periods at pH levels ranging from 4.0 to 10.5.

The antibiotic activity of grisein can be measured by the dilution agar-streak and cup assay methods. With the cup method, *B. subtilis*, *S. aureus* and *E. coli* can be used as test organisms; the zone of inhibition, for the first organism, is usually narrower than that for comparable concentrations of either streptomycin or streptothricin, but it is wider and more distinct for the second and third organisms. The broth dilution method cannot be used very effectively for the study of grisein, since there are always a few cells in the culture of the test bacterium that are originally resistant or become readily resistant to this antibiotic; these cells begin to multiply rapidly, making impossible accurate readings by the dilution method. The rapid development of resistant strains from originally sensitive cultures makes studies of the bactericidal action of grisein upon bacteria in broth cultures rather difficult.

The presence of glucose or glycerol in the culture medium for the production of grisein results in very low yields of this antibiotic due to a lowering of the pH. However, as soon as the pH begins to rise, the antibiotic is formed, as shown in Fig. 1. Lactose, however, has no such effect.

Samples of crude grisein preparations were tested in mice infected with *Salmonella schottmülleri* and *S. aureus*. A single dose of 800 units (dilution units against *E. coli*) per mouse, administered subcutaneously immediately after infection, protected 100% of the animals infected with the first, and 1,600 units/mouse gave complete protection against the second. The antibiotic showed only little toxicity to experimental animals and was excreted readily and in an active state in the urine. Because of its low toxicity, its activity *in vivo* and also its activity upon

[6] Waksman, S. A., and Reilly, H. C., *Anal. Ed., Ind. Eng. Chem.*, 1945, **17**, 556.

streptomycin-resistant strains point to the possibility of utilizing grisein for the control of infections caused by bacteria resistant to other antibiotics. However, its narrow antibiotic spectrum would limit its practical application.

Summary. *Streptomyces griseus* is widely distributed in soils, peats and in composts. Only very few strains of this organism are capable of producing streptomycin. Most of the strains produce no antibiotic at all, whereas certain strains produce antibiotics that are distinctly different from streptomycin. One such new antibiotic was isolated and designated as grisein.

Grisein is active against certain Gram-positive and Gram-negative bacteria. Its antibacterial spectrum is much more limited than that of either streptomycin or streptothricin. Cultures of bacteria that are made resistant, by serial passage, to streptomycin still remain sensitive to grisein. Cultures of bacteria originally sensitive to grisein give rise easily to strains resistant to this antibiotic.

Grisein shows a rather low toxicity to experimental animals and is rapidly excreted in the urine. It was found capable of protecting experimental animals against infections with *S. schottmülleri* and *S. aureus*.‡

‡ The authors are indebted to Mr. Otto Graessle, of the Merck Institute, for testing the animal toxicity of grisein, and to Dr. H. Boyd Woodruff, of the Merck Laboratories, for checking the sensitivity of streptomycin-resistant strains of different bacteria.

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Determination of Oxygen Consumption in the Albino Rat.*

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Technical aspects of the measurement of gaseous metabolism in the albino rat would appear, from a cursory survey of the literature, to be almost a closed chapter in biologic technology. Yet the determinations, as executed, have proved inaccurate, inconsistent, and time-consuming. The method to be described below appears to offer several distinct advances over those currently in use.

The machine devised for these studies is of the closed-circuit type, measuring only oxygen consumption. Its major structural features are illustrated in Fig. 1.

Animal chamber, spirometer box, and water bath are all constructed of heavy sheet brass, which is adequate for the rapid equalization of temperature throughout the system. The temperature is maintained within narrow limits by means of a tubular model heating element controlled through a suitable relay by a thermoregulator immersed in the water bath. The water is kept circulating by a noiseless electric stirrer. Sensitivity of the thermoregulator is assayed by a blank test, measuring the variations in volume of the inclosed oxygen that are induced by the automatic activation and deactivation of the heating element; such variations have not exceeded 0.5 cc. The temperature within the animal chamber is maintained at 28°C, stated by Benedict and MacLeod[1] to be the temperature of thermic neutrality for the albino rat.

Dimensions of the animal chamber are 9″ x 6″ x 5½″. Its lid is provided with a large window, composed of 2 panes of glass separated by an air space to limit condensa-

* Paper No. 831, Research Series, the University of Arkansas.

1 Benedict, F. G., and MacLeod, G., *J. Nutrition*, 1929, **1**, 367.