analyzed for phosphatase activity by the method of Huggins and Talalay.[7]

Results. Incubation of slices in silver nitrate c. 1:1000 for one hour, followed by washing in distilled water for one hour, caused complete inhibition of calcification in phosphoric ester substrate. On the other hand, heavy calcification was observed when similarly treated slices were incubated in inorganic phosphate substrate. Untreated control slices calcified heavily in both media. Phosphatase activity of cartilage treated with silver nitrate was nil.

Similar results were observed when bichloride of mercury (c. 10^{-2} molar) was used as the inhibiting agent.

Incubation of slices in distilled water for one hour at constant temperatures ranging from 25° to 100°C (at 5° intervals) revealed that calcifiability in phosphoric ester substrate was lost above 50° to 55°C as determined by subsequent incubation. Similarly, phosphatase activity decreased as the temperature increased, and was nil in tissues heated above 60°C. On the other hand, when inorganic phosphate substrate was used, calcification occurred in all tissues including those that had been boiled. In tissues heated above 65°C calcification was found to depend on a high concentration of medium: thus it was observed in media containing 1 mM calcium and 20 mM phosphate per liter, but it was absent when the phosphate concentration was less than 10 mM per liter.

Conclusions. 1. Calcification of hypertrophic epiphyseal cartilage *in vitro* is intrinsically non-enzymatic. 2. The phosphorylase and phosphatase systems in hypertrophic epiphyseal cartilage may have supplementary roles in the calcification mechanism, but they are not essential to the localized deposition of bone mineral.

[7] Huggins, C., and Talalay, P., *J. Biol. Chem.*, 1945, **159**, 399.

16685 P

Aureomycin in Treatment of Pneumococcal Pneumonia and Meningococcemia.*

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Aureomycin is a new antibiotic which is active *in vitro* and in experimental animals against gram-positive and gram-negative bacteria and is also effective against experimental infections with rickettsias and with viruses of the psittacosis lymphogranuloma venereum group.‡ The findings in 4 cases of pneumococcal pneumonia and in one case of meningococcemia are presented here briefly. The bacteriologic methods are described elsewhere.[1,2]

The cases of lobar pneumonia were of moderate severity and a single lobe was involved in each instance. All were in males ranging in age from 16 to 65 years. Pneumococci were identified and typed from the

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‡ Data on isolation, pharmacology and activity *in vitro* and in experimental infections have been presented by workers of the Lederle Laboratories Division of the American Cyanamid Company at a Conference on Aureomycin, New York Academy of Sciences, July 21, 1948. Additional clinical and laboratory observations by the authors and by other workers were also presented at that conference.

[1] Paine, T. F., Jr., Collins, H. S., and Finland, M., *J. Bact.*, 1948, **56**, 489.

[2] Collins, H. S., Paine, T. F., Jr., Wells, E. B., and Finland, M., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 174.

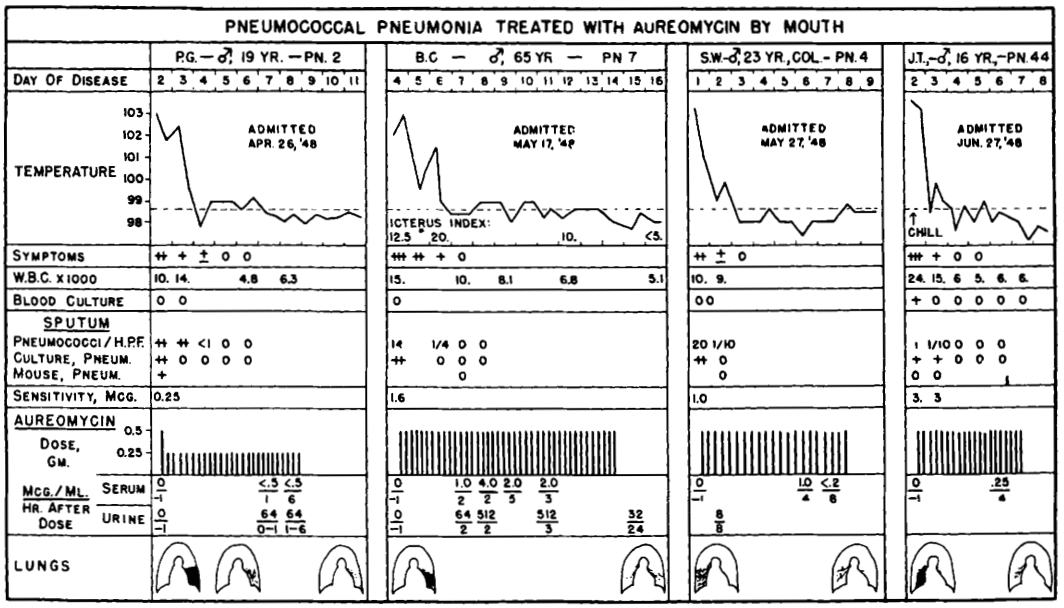

FIG. 1.
Relevant findings in 4 cases of pneumonia.

sputum in each instance and from the blood in one case before oral aureomycin therapy was started. One patient received an initial dose of 0.5 g followed by 0.25 g every 6 hours for a total of 5 g; the others received 0.5 g every 6 or 8 hours for a total of 10 g in 2 cases and 20 g in the oldest patient. No other chemotherapy or antibiotic was used. Some of the relevant findings are shown in Figure 1.

The patients became afebrile, and both subjective and objective improvement occurred in each instance between 18 and 36 hours after the first dose and was slowest in the oldest patients who had the severest illness and jaundice. The number of pneumococci decreased rapidly in the sputum following treatment and none could be demonstrated by any of the available methods, including mouse inoculation, after the second day of treatment. The pneumococci were completely inhibited by aureomycin in concentrations of 0.25 to 3.0 μg per ml.

The relevant findings in the case of meningococcemia are shown in Figure 2. In this patient a tentative diagnosis of Rocky Mountain Spotted Fever was made on the basis of a history of tick bites, high fever, headache, slightly stiff neck and a rapidly spreading

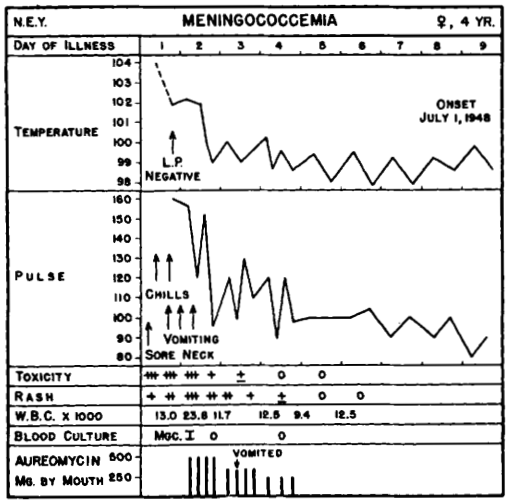

FIG. 2.
Relevant findings in a case of meningococcemia treated with aureomycin.

maculopapular eruption involving the trunk and extremities. Lumbar puncture yielded normal spinal fluid. Treatment was begun 12 hours after the onset of symptoms. The patient received 4 doses of 500 mg followed by 4 doses of 350 mg and then 3 of 250 mg, at 6-hour intervals. The patient was afebrile and much improved symptomatically and the rash had almost cleared within 18 hours after

first dose. A blood culture obtained before treatment was started yielded meningococcus, group I and subsequent blood cultures were negative.

The results in these cases appear to be comparable to those obtained in similar cases treated with penicillin or with effective sulfonamides. There were no toxic effects attributable to aureomycin in any of these cases.

Further experience is needed to determine the optimum dosage and duration of treatment as well as the effect in more severe cases. The present findings, however, suggest that aureomycin may be an effective agent in pneumococcal and meningococcal infections and warrants further clinical trial in these and other types of infections.

16686

Excretion of Androgens and 17-Ketosteroids in the Cebus Monkey Following the Administration of Testosterone Propionate.

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An increase in urinary 17-ketosteroids and androgens after administration of testosterone has been noted in man,[1] chimpanzee,[2] Rhesus monkey,[3] guinea pig[4] and rat.[5] Normally Cebus monkeys excrete minimal amounts of androgens as compared with other primates,[6] hence we wished to examine their 17-ketosteroids and androgen excretion after administration of testosterone propionate.*

Material and Methods. Six adult male Cebus monkeys, weighing 2.8 - 3.3 kg, were used. After a control period of 6 days, each animal received daily 50 mg of testosterone propionate subcutaneously, in 1 ml of peanut oil solution, for 12 days followed by 5 days during which no treatment was given. Throughout the period of observation (23 days) the urine of each monkey was continuously collected under toluene. Twice daily the urine specimen of all monkeys were pooled, acidified with 1% hydrochloric acid, placed in the refrigerator and extracted the following day. Hydrolysis, extraction and fractionation by Girard's reagent for chemical and biological assays was done as previously described.[7] The urinary extracts from the control period of 6 days (I), the treatment period of 12 days (II) and the post-treatment period of 5 days (III) were kept separate for chemical and biological assays. Colorimetric estimations were done by the Zimmermann method as modified by Callow *et al.*[8] and by the antimon trichloride technic.[9] Assays of androgenic activity were made using the chick comb method.[6]

Results. A. *Androgenic activity.* In a preliminary test we verified that the androgenic activity of the urine collected during periods I, II, and III was approximately the same. Therefore the final assay was done only on

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* Testosterone propionate was furnished through the courtesy of the Ciba Pharmaceutical Company of S. Paulo.

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9 Pincus, G., *Endocrinology*, 1943, **32**, 176.