for the quantitative assay of small quantities of hyaluronidase or hyaluronidase inhibitor in the presence of body fluids is described.

2. Presumably, the conjugates of salicylates as well as salicylate itself in therapeutic concentrations have no inhibitory effect on testicular hyaluronidase *in vitro*.

3. Thermolabile hyaluronidase inhibitor has been found increased in a significant proportion of the sera of human malignancies in a small series studied.

4. Rheumatic states do not differ from the normal with respect to this inhibitor toward testicular hyaluronidase.

16684 P

Calcification of Hypertrophic Epiphyseal Cartilage *in vitro* Following Inactivation of Phosphatase and Other Enzymes.*

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Mineralization of hypertrophic epiphyseal cartilage *in vitro*, first demonstrated by Shipley,[1] requires concentration of calcium ions and phosphate ions in excess of a critical ion product, assumed to approximate the solubility product of a calcium phosphate salt. The concept of calcification solely as a precipitation phenomenon, however, fails to account for the specific localization of the mineral.

Robison[2] demonstrated phosphatase in hypertrophic epiphyseal cartilage and achieved calcification by addition of phosphoric ester in minute amounts to media of subcritical ion product. Robison[3] regarded the local factor as possibly enzymatic since calcification *in vitro* had not been observed in tissues heated beyond 60°C, nor after complete inhibition of phosphatase by various chemical agents.

Gutman and co-workers[4] demonstrated phosphorylative glycogenolysis in hypertrophic epiphyseal cartilage and have contended that calcification *in vitro* utilizes this enzyme system. On the other hand, McLean *et al.*[5] have found little correlation of anaerobic glycolysis with calcification.

The present study was undertaken to determine whether or not enzymes are necessary for *in vitro* calcification.

Procedure. Slices of epiphyseal cartilage were taken from the long bones of young rats made rachitic on a modified diet 2965 of Steenbock and Black,[6] and were subjected to varied preliminary treatment. The slices were then incubated approximately 18 hours at 37°C in isotonic media containing known amounts of calcium and phosphate at pH 7.4. Following incubation the tissues were washed in distilled water and stained with 2% silver nitrate. Experiments were designed to compare calcification in inorganic phosphate with calcification in phosphoric ester (sodium glycerophosphate). In addition, extracts of epiphyseal cartilage were prepared and were

* This work has been supported in part by research grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service; from the Josiah Macy, Jr. Foundation; and from the Williams-Waterman Fund of Research Corporation.

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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.

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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

* Aided by a grant from the United States Public Health Service.

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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.

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