

amide or sulfapyridine result in sterility, and the colony counts were usually of the order of 12,000,000 per cc for sulfapyridine and 40,000,000 per cc for sulfanilamide at the time when the colony count in the control reached 100,000,000 per cc.³

Studies of alpha hemolytic streptococcus infections made by the same technic showed an even greater effectiveness of neoarsphenamine and an even lesser effectiveness of sulfanilamide and sulfapyridine.

Since the concentration of the drug must be above 3 parts per million for a period of 6 to 24 hours it would seem that frequent small doses or administration of neoarsphenamine by the method of continuous drip⁴ should be more effective than single larger doses at longer intervals. Studies of the clinical effectiveness of this drug in staphylococcic bacteriemia and in subacute bacterial endocarditis and of the comparative effectiveness in marrow cultures of other arsenicals are in progress.

Summary. In cultures of living human marrow inoculated with hemolytic *Staphylococcus aureus* or alpha streptococci (*Streptococcus viridans*) neoarsphenamine in concentrations of 3 to 9 parts per million was far more effective than 1-10,000 concentrations of either sulfanilamide or sulfapyridine, and did not significantly damage marrow cells.

11056 P

Hyaluronic Acid in Pleura Fluid Associated with Malignant Tumor Involving Pleura and Peritoneum.

KARL MEYER AND ELEANOR CHAFFEE.

From the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, and the Institute of Ophthalmology, Presbyterian Hospital, New York.

Hyaluronic acid, a high molecular weight polysaccharide composed of acetylglucosamine and glucuronic acid in equimolar concentrations, has been obtained from the following sources: vitreous humor of cattle and swine,^{1, 2} human umbilical cord,¹ cattle and human synovial

³ Osgood, E. E., Marrow Cultures, Figures 17-19 and 21, Symposium of the Blood and Blood-Forming Organs, University of Wisconsin Press, pp. 219-241, 1939.

⁴ Hyman, H. T., Chargin, L., Rice, J. L., and Leifer, J. A. M. A., 1939, **113**, 1208.

¹ Meyer, K., and Palmer, J. W., *J. Biol. Chem.*, 1936, **114**, 689.

² Meyer, K., Smyth, E. M., and Gallardo, E., *Am. J. Ophthal.*, 1938, **21**, 1083.

fluid,³ and group A hemolytic streptococci in the mucoid phase.⁴ A polysaccharide acid closely resembling hyaluronic acid has further been obtained from tumors caused by a filterable fowl sarcoma.⁵ An immunologically inactive polysaccharide acid resembling hyaluronic acid has likewise been demonstrated in group C hemolytic streptococci.⁶ In the latter a close relationship between the yield of acid polysaccharide on one hand and the degree of encapsulation, virulence, and invasiveness on the other has been pointed out.

Many tumors are mucilaginous and many are stained by "mucin" stains. The nature of the "mucin" constituent, however, has been determined only in the case of the fowl sarcoma.

We have isolated hyaluronic acid from the viscous pleura fluid* of a patient with a malignant tumor of the pleura and peritoneum. Three different samples of pleura fluid were investigated. The procedure used for the isolation of the polysaccharides followed that described earlier.³ The yield per 100 cc of fluid corresponded to 0.174, 0.187, and 0.142% as compared to 0.02-0.025% from bovine synovial fluid, and about 0.04% from bovine vitreous humor. The polysaccharides isolated from the pleural fluids had compositions and physical constants very similar to those reported previously. The identity was further established by enzymatic analysis.⁷

In previous papers from this laboratory it was pointed out that hyaluronic acid occurred free or in salt linkage only and was not chemically bound to protein.^{1, 8, 9} In the present case the same conclusion could be drawn from electrophoretic measurements carried out by Dr. Longworth.† At pH 7.8 in a 0.05 N lithium chloride-lithium diethylbarbiturate buffer and at 0°C a fraction with a mobility of -10.5×10^{-5} was observed, which was apparently the free polysaccharide acid. The concentration of this fraction as deter-

³ Meyer, K., Smyth, E., and Dawson, M. H., *J. Biol. Chem.*, 1939, **128**, 319.

⁴ Kendall, F. E., Heidelberger, M., and Dawson, M. H., *J. Biol. Chem.*, 1937, **118**, 61.

⁵ Kabat, E. A., *J. Biol. Chem.*, 1939, **130**, 143.

⁶ Seastone, C. V., *J. Exp. Med.*, 1939, **70**, 347, 361.

* We obtained the fluid and the clinical and pathological findings from Dr. R. Loeb to whom we are greatly indebted.

⁷ Meyer, K., Hobby, G. L., Chaffee, E., and Dawson, M. H., *J. Exp. Med.*, in press.

⁸ Meyer, K., Palmer, J. W., and Smyth, E. M., *J. Biol. Chem.*, 1937, **119**, 501.

⁹ Meyer, K., *Cold Spring Harbor Symposia on Quantitative Biology*, 1938, **6**, 91.

† We are greatly indebted to Drs. D. A. MacInnes and L. G. Longworth of the Rockefeller Institute for Medical Research for the electrophoretic measurements and for their permission to quote the results in this paper.

mined from the electrophoretic measurements corresponded closely to the concentration of free polysaccharide as determined by isolation. Furthermore the mobility of the polysaccharides isolated from the tumor fluid as well as from umbilical cord were very similar to the mobility of the fast component in the tumor fluid.

From the clinical and pathological findings the present tumor was diagnosed as a meso- or endothelioma, a tumor apparently characterized in these locations by the mucilaginous nature of its effusates.¹⁰ The following is part of the histological report from the Dept. of Pathology (Dr. E. Sproul):

"The sediment is a compact sheet of cells of uniform character. They are very large, rounded or polygonal mononuclear forms, usually with ovoid nucleus. The latter has a distinct membrane and delicate chromatin network. There are but rare mitotic figures. The cytoplasm is very abundant and vacuolated so that a delicate network of cytoplasm separates small vacuoles. The latter may be empty or contain slightly basophilic granular material with peripheral concentration of the homogeneous cytoplasm. The cells occur singly or in groups. There is no evidence of gland formation, but in one area the arrangement of the cells suggests a papillary structure. Despite the thick mucoïd character of the fluid it has been impossible to stain mucin within cells or in the fluid by use of the mucicarmin stain. Many of the vacuoles seen in the tumor cells of the Zenker fixed material are shown to represent fat globules when formalin fixed material is stained with Scharlach R. The fat is very abundant and is present in large and small globules. That the presence of the visible fat does not represent an autolytic process is attested by the fact that the nucleus in the fat-laden cell is well preserved.

"Diagnosis: The large cells are those of a malignant tumor. It is felt that the uniformity of morphological features, the absence of mucin and glandular structures, the suggestion of papillary structure and the evidence of phagocytic activity strongly suggest that they are derived from the lining cells of the serosal cavity. The tumor would be classified as an endothelioma or mesothelioma of the peritoneum and pleura."

In regard to the failure of the mucicarmin stain of the tumor cells, one is reminded of a similar situation in the synovial cells which likewise produce hyaluronic acid. According to Kling¹¹ no granules indicating a specific function or secretion could be demonstrated with

¹⁰ Ewing, James, *Neoplastic Diseases*, Philadelphia and London, 1928, p. 350.

¹¹ Kling, D. H., *The Synovial Membrane and the Synovial Fluid*, Los Angeles, 1938.

mucicarmin or mucihematin in the cells. Using a special technic of fixation the same author demonstrated metachromatic granules in synovial cells with Toluidin blue. Our own attempts to stain "mucin" in vitreous humor were all futile. Furthermore the capsule of group A hemolytic streptococci, which at least to a large extent is composed of hyaluronic acid, can only be stained by mucin stains with special technics.¹²

It is not known whether the normal endothelium produces hyaluronic acid in small amounts. However, the usual exudates and transudates of the serous cavities are not viscous.

Conclusion. Hyaluronic acid in high concentration has been isolated from the chest fluid of a patient with a malignant tumor, probably an endothelioma. The polysaccharide acid in the original fluid migrates in an electric field at pH 7.8 at essentially the same speed as the isolated pure polysaccharide acid, indicating the existence in the fluid of the free acid and not of a protein complex.

11057 P

Non-Osseous Origins of Serum Phosphatase.

AARON BODANSKY.

From the Laboratory Division, Hospital for Joint Diseases, New York.

Associated increases of serum cholesterol, serum bilirubin and serum phosphatase have been observed in diseases of the liver with various degrees of obstruction of the biliary passages; *dissociated effects* have also been observed in other liver diseases.^{1,2} We have sought to reproduce analogous conditions and effects in experimental animals, in order to be able better to relate these dissociated effects to the underlying tissue changes.³⁻⁶

The effects of intravenous injections of gum acacia were of interest in this connection. We have obtained a marked *increase* of serum

¹² Hobby, G. L., and Dawson, M. H., *Brit. J. Exp. Path.*, 1937, **18**, 212.

¹ Bodansky, A., and Jaffe, H. L., *Proc. Soc. Exp. Biol. and Med.*, 1933, **31**, 107.

² Flood, C. A., Gutman, E. B., and Gutman, A. B., *Arch. Int. Med.*, 1937, **59**, 981; this paper reviews some earlier work.

³ Bodansky, A., and Jaffe, H. L., *Proc. Soc. Exp. Biol. and Med.*, 1934, **31**, 1179.

⁴ Bodansky, A., *Proc. International Physiol. Cong.*, Leningrad, 1935, 253.

⁵ Bodansky, A., *Enzymologia*, 1937, **3**, 258.

⁶ Bodansky, A., *Proc. International Physiol. Cong.*, Geneva, 1938.