antidote to the hypoprothrombinemia produced by U-1363.

The authors wish to acknowledge the generous cooperation of the following associates from these laboratories: Drs. M. E. Speeter, D. G. Thomas and Mr. W. C. Anthony for preparing the indandione compounds; Dr. E. S. Feenstra for the histologic examinations; Mr. O. F. Swoap for the toxicity data; and Mrs. Russell Borst for technical assistance. The vit. K_1 emulsion was kindly furnished by Dr. F. J. Stare, Harvard University.

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Received April 16, 1952. P.S.E.B.M., 1952, v80.

Two Dimensional Paper Chromatography of Proteins. II. Application to Blood Plasma Fractions.* (19549)

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Franklin and Quastel(1) have reported that proteins may be separated by means of 2-dimensional filter paper chromatography; aqueous buffers and the ascent principle were used. These investigators converted the proteins into protein-hemin complexes prior to chromatography and identified the position of each protein-hemin complex by treating the paper with benzidine and hydrogen peroxide. All proteins, however, do not combine with hemin. The method is also limited by the intensely colored background which obscures the color given by the protein-hemin complex. In order to overcome this interference the patterns must be immediately photographed. While Hall and Wewalka(2) have criticized the method of Franklin and Quastel, the former authors concluded that proteins of dissimilar nature could be separated by chromatography. Feigl(3), as early as 1937, showed that dyes may be used for the detection of proteins on filter paper. Based on this principle, we have developed a convenient staining reagent containing the fluorescent dye, eosin, and methyl orange and presented other improvements concerning the 2-dimensional procedure(4).

*A preliminary report was presented before the American Society of Biological Chemists at the meeting of the Federation of Amer. Soc. for Exp. Biol., New York City, April 15, 1952.

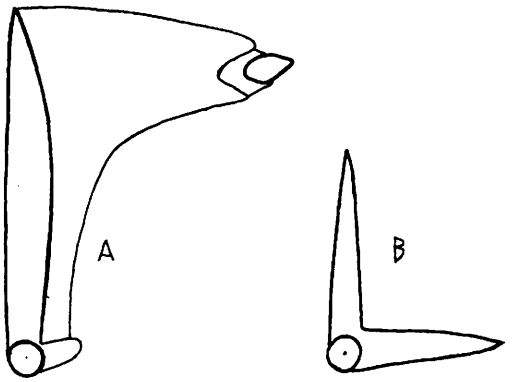

FIG. 1. Tracings of 2-dimensional filter paper chromatograms. A. = albumins, 120; B. = γ-globulins, 120. Quantities are in μg.

In the present paper we wish to describe the results obtained when our technic of 2-dimensional filter paper chromatography and staining was applied to human blood plasma albumins and γ-globulins fractions.

Experimental. The total protein applied in each experiment was 120 μg. The following solution of pH 6.0 was used as the developing agent in the first dimension: trisodium citrate (0.02 M), 9.25 ml (2 N) hydrochloric acid, and 50 g of sodium chloride per 5 l. The same buffer, after adjustment to pH 4.0, was used for dissolving the plasma proteins. Tartaric acid (2%) was employed in the second dimension.

In Fig. 1, "A" represents the tracing of the

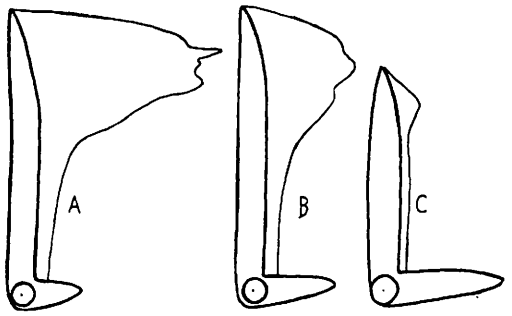

FIG. 2. Tracings of 2-dimensional filter paper chromatograms. A. = albumins, 100 + γ-globulins, 20; B. = albumins, 60 + γ-globulins, 60; C. = albumins, 20 + γ-globulins, 100. Quantities are in μg.

resulting pattern when 120 μg of Cohn's "albumins" of method 10(5), called "Fraction V," was applied to the paper. In "B" 120 μg of Cohn's "γ-globulins" of method 10(5), called Fraction II, was applied. In "A," the upper flag-like portion in the first dimension of the pattern is characteristic, under the conditions of the experiment, for the albumins fraction. In "B" the lower streak, in the second dimension, is typical for the γ-globulins fraction. In both instances there is movement in both dimensions.

These patterns are in harmony with the 3 patterns shown in Fig. 2. "A" in Fig. 2 represents a mixture of 100 μg of albumins and 20 μg of γ-globulins. "B" in Fig. 2 shows a mixture of 60 μg of albumins and 60 μg of γ-globulins. "C" represents 20 μg of albumins and 100 μg of γ-globulins. It may be seen that 20 μg quantities of either albumins or γ-globulins may readily be detected in the presence of 100 μg of the albumin fraction.

Summary. When albumins (Fraction V) were mixed with the γ-globulins (Fraction II) there occurred a typical separation of a portion of each plasma fraction. Our investigations indicate that 2-dimensional filter paper chromatography may have a definite value in protein chemistry, and in clinical research, even if complete separation of proteins is not possible at present.

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Received April 28, 1952. P.S.E.B.M., 1952, v80.

Gastric Ascorbic Acid in the Gastritic Rat.* (19550)

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Ascorbic acid, instrumental in the formation and maintenance of intercellular substance, may also play a role in gastrointestinal homeostasis. It has been demonstrated that ascorbic acid deficiency in guinea pigs results in a high percentage of gastric ulcers(1). Gastric cancer patients utilize more ascorbic acid than patients of other malignant or non-malignant diseases(2).

In the rat, in which abundant biosynthetic ascorbic acid is available, spontaneous and induced gastric disorder is rare(3). A eugenol-induced gastritis(4) is perhaps the only readily reproducible example of a gastric, glandular dyscrasia.

The purpose of the present investigation was to study the ascorbic acid in the tissue of the fore- and glandular stomachs of eugenol-treated, gastritic rats and comparable con-

* Aided by a grant from the Damon Runyon Memorial Fund.

† To be submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Florida.