

- Imm. Prin. and App. in Med. and Pub. Health*, 1939, Ed. 5, p. 504.
7. Pappenheimer, A. J., Jr., and Williams, C. M., *J. Gen. Physiol.*, 1952, v35, 727.
8. Appel, S. B., Fulton, L. A., and Orr, K. D., *U. S. Armed Forces Med. J.*, 1952, v3, 373.
9. Spector, S., and Mercer, R. D., *J. Inf. Dis.*, 1951, v89, 224.
10. Thompson, I. M., personal communication, unpub. data.
11. Zoger, S., *Yale J. Biol. and Med.*, 1952, v25, 202.
12. Dougherty, T. F., and Schneebeli, G. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v75, 854.
13. Ragan, C., Howes, E. L., Plotz, C. M., Meyer, K., and Blunt, J. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1949, v72, 718.

Received June 4, 1953. P.S.E.B.M., 1953, v83.

Intrinsic Factor Studies I. Paper Electrophoresis of Mixture of Gastric Juice and Radioactive Vitamin B₁₂.^{*} (20398)

ROBERT F. SCHILLING AND WILLIAM P. DEISS.[†]

From the Department of Medicine, University of Wisconsin Medical School.

The simultaneous oral administration of normal human gastric juice and small amounts of vit. B₁₂ to patients with pernicious anemia in relapse results in prompt hematopoiesis, while a similar amount of B₁₂ without gastric juice is ineffective(1). The parenteral injection of microgram quantities of vit. B₁₂ causes an excellent hematopoietic response in such patients(2). The simplest explanation of these observations is that the intrinsic factor activity of gastric juice promotes the intestinal absorption of the vitamin, either by means of a chemical binding of the B₁₂ or by acting on the intestinal mucosa in such a manner as to facilitate the transport of the vitamin across the mucosa(3). Binding of vit. B₁₂ has been studied most extensively by microbiological assay, and it has been shown that gastric juice combines with the vitamin in such a way as to render it unavailable to certain microbes(4). Several other substances, some known not to be active as intrinsic factor, combine with vit. B₁₂ in a similar fashion (5,6).

This paper describes experiments designed to localize by paper electrophoresis the vit. B₁₂ binding fraction in human gastric juice

using radioactive vit. B₁₂ (B₁₂ Co⁶⁰)[‡](7) as a tracer.

Methods. a) Preparation of gastric juice. Gastric juice secreted after histamine injection was collected by a Levine tube from fasting normal subjects or patients with a peptic ulcer or an anxiety state. The juice in the stomach before histamine stimulation was not used. Only specimens showing an acid reaction to Toepfer's reagent were processed. After filtration through gauze to remove some of the mucus the juice was titrated with sodium hydroxide until neutral to brom thymol blue. After suction filtration through coarse sintered glass, the juice was stored at 4° or -10°C. Concentration of gastric juice was effected by dialysis against tap water and freeze-drying. In some experiments B₁₂ Co⁶⁰ was added before freeze-drying, and in others the powdered gastric juice was dissolved in an aqueous solution of the vitamin before application to the paper strip. b) *Paper electrophoresis.* A technic previously described(8) was used in these studies. Schleicher and Schuell No. 598 paper was found satisfactory for this work. Fifty microliters of the concentrated gastric juice-B₁₂ Co⁶⁰ mixture was applied to the paper, and after electrophoresis

^{*} This investigation was supported by a research grant from the National Institutes of Health, U. S. Public Health Service.

[†] Research Fellow of the Arthritis and Rheumatism Foundation.

[‡] Supplied through the generosity of Charles Rosenthal of Merck & Co., Rahway, N. J. The specific activity of the vitamin was approximately 64 microcuries/mg.

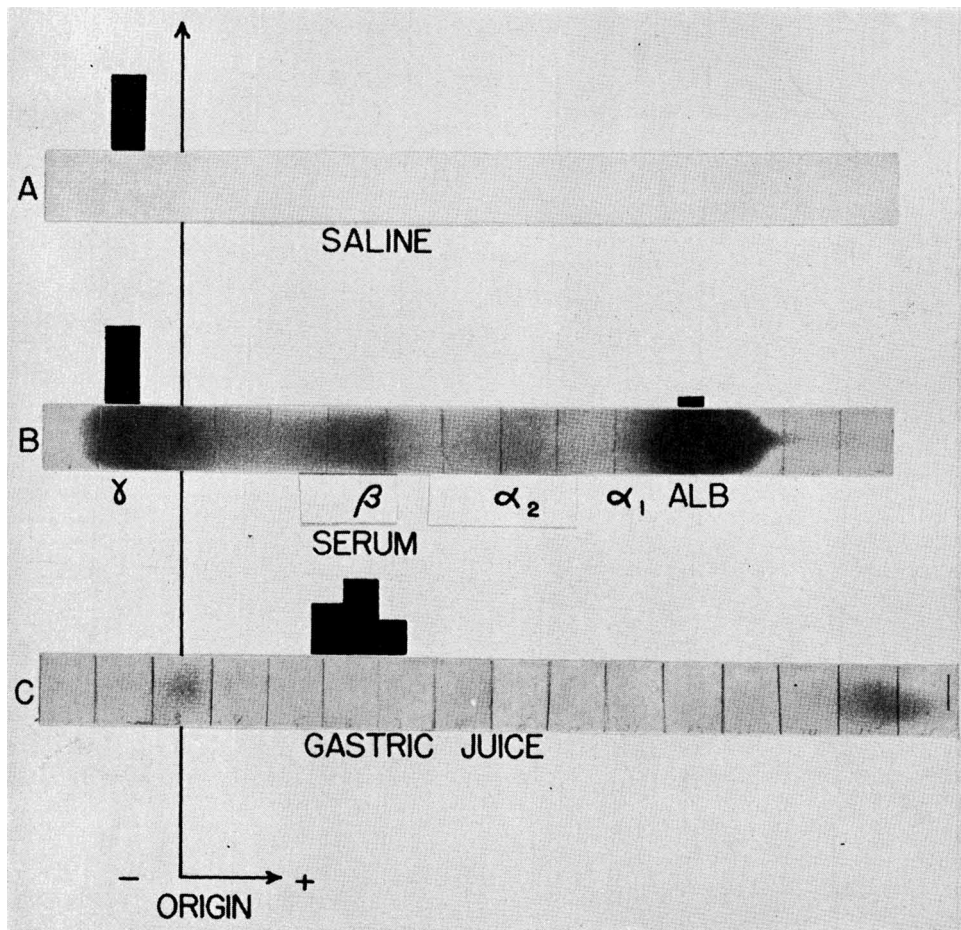


FIG. 1. Localization of radioactivity after simultaneous electrophoresis of $B_{12} Co^{60}$ mixed with 0.9% NaCl (A), serum (B), and gastric juice (C). The bar graphs above the photographs of the stained paper strips represent schematically the localization of radioactivity.

in 0.075 M veronal buffer, pH 8.6, at $4^{\circ}C$ for about 16 hours at 1.5 milliamperes, the paper was dried, cut transversely into 17 segments each 2 cm long, and the radioactivity of each segment determined in a windowless gas flow Geiger counter. These segments were subsequently stained with brom phenol blue to localize the protein spots.

Results. Fig. 1 compares the localization after simultaneous electrophoresis of $B_{12} Co^{60}$ mixed with saline (a), with serum (b), and with gastric juice (c). It will be noted that the radioactive material has moved toward the cathode when mixed with saline or serum. There is also a very small amount of radioactivity associated with the albumin of the

serum, evidence that this fraction bound some $B_{12} Co^{60}$. (This sample of vit. B_{12} contained no inorganic Co^{60} .) In a second experiment, a smaller quantity of $B_{12} Co^{60}$ of higher specific activity was added to serum before electrophoresis, and the majority of the radioactivity was associated with the albumin. Since movement of $B_{12} Co^{60}$ toward the cathode was noted in saline as well as in serum it would seem likely that this movement could best be attributed to electro-osmosis of "unbound" $B_{12} Co^{60}$ in these experiments. The apparent association of radioactivity with the gamma globulin fraction of serum, therefore, is considered fortuitous and does not necessarily represent binding by this fraction.

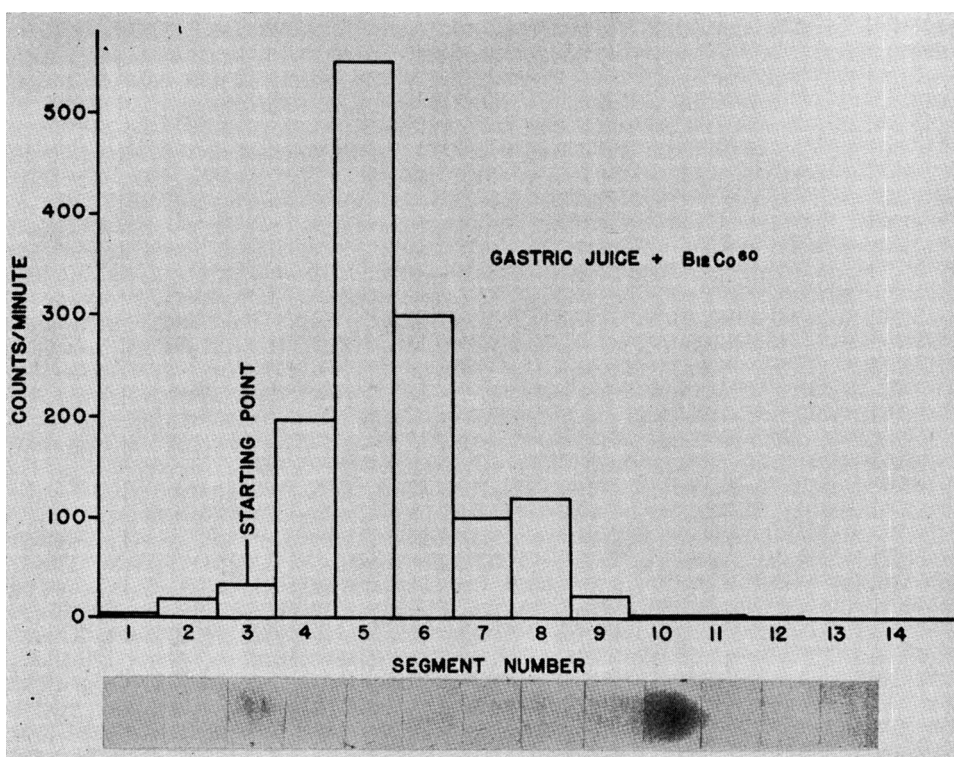


FIG. 2. Localization and quantitation of radioactivity after paper electrophoresis of a mixture of gastric juice and $B_{12} Co^{60}$. Current was applied for a shorter time here than in experiment pictured in Fig. 1.

When the $B_{12} Co^{60}$ was mixed with gastric juice, it was bound to some component moving toward the anode. This behavior was noted in all eight of the experiments performed using 6 different gastric juices. That this component was not one of the 2 major protein peaks is evident from Fig. 1C and Fig. 2. One of the protein peaks remained at the starting point, and the other moved faster than the serum albumin. The $B_{12} Co^{60}$ was not bound to either of the two detected protein peaks in gastric juice.

The effect of boiling the mixture of gastric juice and $B_{12} Co^{60}$ on this binding is demonstrated in Fig. 3. It will be noted that, whereas definite localization of the radioactivity occurs when the vitamin and unboiled gastric juice are subjected to electrophoresis, the radioactivity is not well localized when the mixture was boiled.

Discussion. The data reported herein demonstrate that gastric juice contains a com-

ponent, distinct from the major protein components, capable of binding vit. B_{12} . These observations of themselves are no evidence that this vit. B_{12} binding fraction is intrinsic factor. Using paper electrophoresis under different conditions of technic and buffer than those utilized in our experiments, Latner and Ungley(9) found at least two fractions of gastric juice which acted as intrinsic factor and were capable of binding vit. B_{12} as determined by microbiological assay. Differences in technic may explain the discrepancy between results reported here and those of Latner and Ungley *et al.*(9).

Summary. 1. Results of paper electrophoresis of mixtures of radioactive vit. B_{12} and concentrated human gastric juice demonstrate that the radioactive B_{12} is bound to a component of gastric juice which is not one of the major protein peaks and moves toward the anode. This behavior of the vit. B_{12} was not noted when it was mixed with saline or

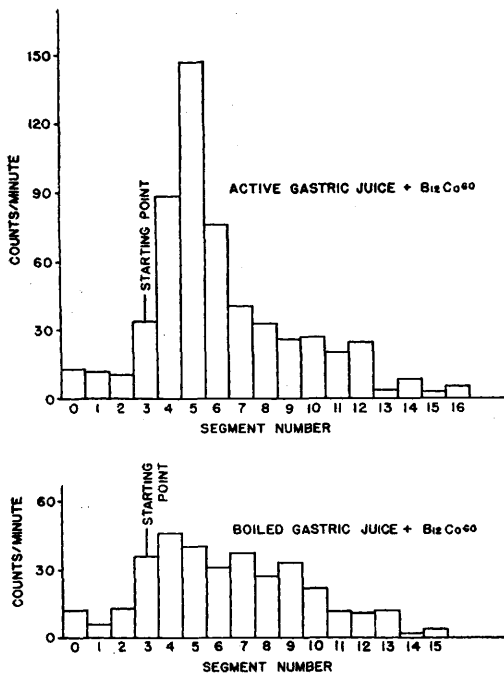


FIG. 3. Comparison of localization of radioactivity after simultaneous paper electrophoresis of boiled and unboiled aliquots of a mixture of gastric juice and $B_{12} Co^{60}$.

serum. 2. This localization of vit. B_{12} to a specific portion of gastric juice by paper electrophoresis may be a clue to the further identification of the intrinsic factor of Castle.

1. Berk, L., Castle, W. B., Welch, A. D., Heinle, R. W., Anker, R., and Epstein, M., *New Eng. J. Med.*, 1948, v239, 911.

2. Schilling, R. F., Harris, J. W., and Castle, W. B., *Blood*, 1951, v6, 228.

3. Wallerstein, R. O., Harris, J. W., Schilling, R. F., Castle, W. B., *J. Lab. and Clin. Med.*, 1953, v41, 363.

4. Ternberg, J. L., and Eakin, R. E., *J. Am. Chem. Soc.*, 1949, v71, 3858.

5. Beerstecher, E., and Altgelt, S., *J. Biol. Chem.*, 1951, v189, 31.

6. Bird, O. D., and Hoebet, B., *J. Biol. Chem.*, 1951, v190, 181.

7. Chalet, L., Rosenblum, C., Woodbury, D., *Science*, 1950, v111, 601.

8. Deiss, W. P., Albright, E. C., and Larson, F. C., *J. Clin. Invest.*, 1952, v31, 1000.

9. Latner, A. L., Ungley, C. C., Cox, E. V., McEvoy-Bewe, E., and Raine, L., *Br. Med. J.*, 1953, v1, 4808.

Received June 4, 1953. P.S.E.B.M., 1953, v83.

Growth of Deciduumata in Adrenalectomized Rats. (20399)

JOSEPH T. VELARDO, FREDERICK L. HISAW,* AND ALDEN B. DAWSON.†

From the Biological Laboratories, Harvard University, Cambridge, Mass.

Recent reports by Pomeau-Delille(1), Swingle *et al.*(2), and Hisaw *et al.*(3) have clearly shown that decidual development can proceed in bilaterally adrenalectomized rats. The paper by Hisaw *et al.*(3) further indicated that the antimesometrial and mesometrial decidual tissue were histologically comparable in intact and adrenalectomized rats, and that the metrial glands were indistinguishable in the two groups as well. The present study is chiefly concerned with a series of experiments devised to ascertain the differ-

ence in the weights of uteri containing decidual tissue from the fifth full day after uterine traumatization to the twenty-first day of prolonged pseudopregnancy.

Procedures. Estrous rats were bilaterally adrenalectomized 12 hours after cervical stimulation(4) and were then divided into 2 groups. One was given 1% NaCl drinking solution and the other tap water. Vaginal smears were taken daily, fixed in methyl alcohol, and were then stained in Giemsa stain. Five full days after electrical stimulation of the uterine cervixes, both uterine horns were traumatized throughout their entire length by scratching the antimesometrial surface with a burred needle. Laparotomies were performed 72 hours after uterine traumatization to es-

* This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

† Aided by a grant from the William F. Milton Fund, Harvard University.