

TABLE IV.
Mouse Virulence of Group A, B, and C Beta Hemolytic Streptococci with Acquired Aureomycin Resistance.

Group	Strain	Initial LD ₅₀		LD ₅₀ after 40 transfers on			LD ₅₀ of resistant strains after 12 mouse passages Dilution*
		Dilution*	No. organisms†	Control medium Dilution*	Aureomycin medium		
					Dilution*	No. organisms†	
A	S2	10-4.8	1.6 × 10 ²	10-2.8	10-1.5	3.0 × 10 ⁵	
	S3	10-4.5	2.0 × 10 ²	10-3.7	10-3.0	8.4 × 10 ³	
	S4	10-4.3	3.7 × 10 ²	10-3.2	10-3.0	4.5 × 10 ³	10-6.2
B	T5	10-3.0	1.9 × 10 ⁴	10-4.8	10-2.5	4.3 × 10 ⁴	10-7.0
	T7	10-4.0	6.3 × 10 ²	10-5.3	10-4.0	5.9 × 10 ²	10-8.2
	T8	10-4.0	7.8 × 10 ²	10-6.3	10-4.5	8.5 × 10 ²	10-7.0
C	U1	10-1.5	2.1 × 10 ⁵	10-2.5	10-1.3	1.0 × 10 ⁶	10-3.6
	U2	10-6.0	9.3 × 10 ¹	10-4.0	10-0	2.2 × 10 ⁷	
	U3	10-2.5	5.8 × 10 ⁴	10-2.8	10-1.2	2.0 × 10 ⁶	10-5.7

* The LD₅₀ is expressed in terms of the dilution of the whole broth culture after it had been adjusted in density to that of its corresponding parent strain.

† The LD₅₀ is expressed in terms of the number of viable streptococci inoculated.

green hemolytic characteristics lost their green hemolytic zones when grown on maximum aureomycin concentrations.

8. Reduction in streptokinase production occurred in 2 of 5 group A and C resistant strains and diminished ribonuclease activity in 2 of 7 group A resistant variants. No change in streptolysin S or proteinase activity was observed.

9. The C203 MV strain of group A strep-

tococcus failed to acquire aureomycin resistance after repeated exposures to sublethal amounts of the antibiotic either in mice or embryonated hens' eggs.

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VII. Vitamin B₁₂ and the Intrinsic Factor. (17560)

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Soon after the discovery by Minot and Murphy(1) of the effectiveness of liver in the dietary treatment of pernicious anemia, it was shown that foods such as beef muscle contain a heat-stable substance, designated the extrinsic factor,(2) which produces little or no response when ingested by a pernicious anemia patient unless normal gastric juice is also administered with it. The activity of gastric

juice has been ascribed to the so-called intrinsic factor; gastric juice alone has no oral hematopoietic power.(3) Over a period of years the relationship between the extrinsic and intrinsic factors has been the subject of considerable clinical experimentation by Castle and his associates, and other investigators. Recent findings on this subject are as follows: Vitamin B₁₂, active parenterally at the microgram level, has been found to be essentially inactive when administered orally

1. Minot, G. R., and Murphy, W. P., *J. Am. Med. Assn.*, 1926, v87, 470.

2. Castle, W. B., and Townsend, W. C., *Am. J. Med. Sci.*, 1929, v178, 764.

3. Castle, W. B., and Ham, T. H., *J. Am. Med. Assn.*, 1936, v107, 1456.

at a dosage level of 5 μ g(4,5) daily. However, when a 5- μ g daily dose of vitamin B₁₂ was administered orally together with normal human gastric juice, a satisfactory hematopoietic response was observed. It has been suggested(4) that the food (extrinsic) factor may be identical with or closely related to the antipernicious anemia principle in liver, which is itself presumably identical with vitamin B₁₂. These investigators further suggest that gastric juice is necessary for optimal utilization of the small amounts of vitamin B₁₂ in foods by promoting more effective absorption;(4) a direct reaction between extrinsic and intrinsic factors does not necessarily occur. Similar results and conclusions have been reported by others.(6,7) Prior to this interpretation, much work had been based upon the possibility of a chemical reaction between the two factors to give another substance which was the effective one.(3)

The purpose of the present work was to study the possible chemical reaction between vitamin B₁₂ and normal human gastric juice when incubated under several conditions for such enzyme or other reactions, and to determine whether the extrinsic factor of beef muscle is hematopoietically active when administered by the parenteral route.

Experimental. Samples of residual gastric juice of fasting individuals* were collected in the morning; then, 0.5 mg of histamine acid phosphate was administered intramuscularly and the gastric juice was collected for an additional thirty minutes. The samples were chilled immediately, filtered through gauze, and stored frozen in dry ice. The samples were assayed(8) for LLD activity;(9)† the

TABLE I.
LLD Activity of Gastric Juice.

Individual	Fasting gastric juice, u/ml	Histamine induced gastric juice, u/ml
1	97	23
2	48	42
3	66	52
4	100	68
5	126	81
6	103	100
Avg	108	73

results are in Table I. In their early studies, Castle and Townsend(2) gave to pernicious anemia patients daily oral doses of gastric juice in quantities up to 300 ml without obtaining positive responses. Based on an average value of 100 u./ml, it is evident that this volume of gastric juice probably contained only about 3 μ g of vitamin B₁₂, or its equivalent in microbiological activity. Such an amount of vitamin B₁₂ as a single dose is ineffective orally, but effective parenterally.(10) In 1932, it was shown(11) that a vacuum-concentrate of 450 ml of normal gastric juice produced a striking hematopoietic effect when administered intramuscularly; this result can be interpreted now as due to the presence of an adequate amount of vitamin B₁₂-like activity in the concentrate. It was shown later,(12) that hematopoietic responses were obtained by parenteral injection of similar concentrates of the gastric juice of swine, dogs, and cattle.

The stability of vitamin B₁₂ when dissolved in gastric juice was examined. Crystalline vitamin B₁₂ was dissolved in filtered gastric juice and the solutions were incubated at 28-30°C. The solutions were examined for

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

5. Hall, B. E., Morgan, E. H., and Campbell, D. C., *Proc. Staff Meet., Mayo Clin.*, 1949, v24, 99.

6. Spies, T. D., Lopez, G. G., Milanes, F., Toca, R. L., and Aramburu, T., *So. Med. J.*, 1949, v42, 528.

7. Bethell, F. H., Swendseid, M. E., Meyers, M. C., Neligh, R. B., and Richards, H. G., *Univ. Hosp. Bull.*, Ann Arbor, Mich., 1949, v15, 49.

* Courtesy of S. M. Hoch, M.D., Plant Health Department, Merck and Co., Inc.

8. Caswell, M. C., Koditschek, L. K., and Hendlin, D., *J. Biol. Chem.*, 1949, v180, 125.

9. Shorb, M. S., *J. Biol. Chem.*, 1947, v169, 455.

† The LLD activity of a given preparation is a measure of its growth-promoting effect for *L. lactis* Dorner. Vitamin B₁₂ has an LLD activity of 11×10^6 units/mg.

10. West, R., *Science*, 1948, v107, 398.

11. Morris, R. S., Schiff, L., Burger, G., and Sherman, J. E., *J. Am. Med. Assn.*, 1932, v98, 1080.

12. Morris, R. S., Schiff, L., Foulger, J. H., Rich, M. L., and Sherman, J. E., *Brit. Med. J.*, 1932, v2, 1050.

TABLE II.
Activity of Solutions of Vitamin B₁₂ in Gastric Juice.

Time in hr	Fasting gastric juice, u × 10 ⁶ /mg		Histamine-induced gastric juice, u × 10 ⁶ /mg
	pH 2	pH 7	pH 3
.25-.50	12	12.5	11
3	13	16	
6	11	10	13
22	9.3	11	13

changes in absorption spectrum and microbiological activity. Both residual gastric juice and histamine-stimulated gastric juice were used. The absorption spectra of the solutions showed no changes during incubation at pH 2. There was no evidence of a change in the microbiological activity of the solution at pH 2, 3, and 7 as summarized in Table II.

A 16.4-mg sample of crystalline vitamin B₁₂ was dissolved in 25 ml of gastric juice obtained from a fasting individual. The solution (pH 2.3) was allowed to stand eighteen hours at 37° then seven hours at pH 7.2. At the end of the incubation period, vitamin B₁₂ was recovered from the mixture. Because of large amounts of solids in gastric juice only a portion of the vitamin B₁₂ was recovered in crystalline form. The refractive indices of the crystals which were obtained were in satisfactory agreement with those reported (13) for crystalline vitamin B₁₂.

An LLD-active concentrate was prepared from 25 pounds of beef muscle by the following sequence of steps: extraction of ground lean chuck with hot water, precipitation of inert matter from the concentrated extract by 70% ethanol, precipitation of an active fraction from aqueous solution by saturation with ammonium sulfate, dialysis, and extraction of the active fraction from the diffusate with o-cresol. The final product (2.28 g), after lyophilizing from aqueous solution, was a nearly white solid having an activity for

L. lactis of about 500 μ/mg. About 40% of the original activity was recovered. This LLD-active concentrate from beef muscle was found by Hall (14) to be hematopoietically active in 3 cases of pernicious anemia when administered parenterally in a daily dose of about 20 mg. The amount given daily had a microbiological activity equivalent to about 1 μg of vitamin B₁₂. Thus, the extrinsic factor in a concentrate from beef muscle elicits a hematopoietic response on a microgram-basis very similar to that of vitamin B₁₂.

Summary. Human gastric juice contains small amounts of vitamin B₁₂ or its equivalent as assayed by *L. lactis*. Crystalline vitamin B₁₂ was added to gastric juice and the solutions were incubated at pH 2, 3 and 7; there were no observed changes in the absorption spectra or in the microbiological activity over a 22-hour period. Again, crystalline vitamin B₁₂ was added to gastric juice, and after incubation at pH 2 and 7 at 37°, much of the vitamin B₁₂ was recovered. No evidence was found for a chemical reaction between vitamin B₁₂ and the intrinsic factor which results in a gross structural change in vitamin B₁₂. A possible reaction between vitamin B₁₂ and the intrinsic factor in which there was a very small structural change of the large vitamin B₁₂ molecule, and which could produce as little as 5 μg of orally active product is being investigated. A concentrate prepared from beef muscle having vitamin B₁₂-like microbiological activity was found to be hematopoietically active when administered by the parenteral route to pernicious anemia patients; thus, the extrinsic factor acts like vitamin B₁₂. These results support the previously derived hypothesis that the effect of the intrinsic factor is due to its ability to promote the absorption of the small amounts of vitamin B₁₂ or its equivalent which occur in natural foods. The mechanism of this enhancement is being studied further.

13. Rickes, E. L., Brink, N. G., Koniuszy, F. R., Wood, T. R., and Folkers, K., *Science*, 1948, v107, 396.

14. Morgan, E. H., Hall, B. E., and Campbell, D. C., *Proc. Staff Meet., Mayo Clin.*, 1949, v24, 594.

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