

Chemical Studies of the Intrinsic Factor in Desiccated Stomach and Normal Human Gastric Juice. I. Separation of the Intrinsic Factor.

S. M. GOLDHAMER AND JEAN KYER. (Introduced by R. Isaacs.)

*From the Thomas Henry Simpson Memorial Institute for Medical Research,
University of Michigan, Ann Arbor.*

In previous reports it was demonstrated that the intrinsic factor of Castle was present in the gastric juice of individuals with pernicious anemia¹ as well as in the gastric contents of normal individuals. It was also shown that desiccated stomach contained both the extrinsic and intrinsic factors.² The object of this paper is to report some results of the fractionation of desiccated stomach and of normal gastric juice in an attempt to isolate the intrinsic factor and determine its nature.

All the subjects used in the experiments were proven to have true pernicious anemia by the history, physical findings and laboratory procedures. To each of these patients either various fractionation products of desiccated stomach or gastric juice incubated with beef steak were fed daily at 8 A. M. No food was permitted for at least 8 hours before this time or for 5 hours afterwards. Blood observations were made daily with U. S. Bureau of Standards equipment. Reticulocyte counts were made from brilliant cresyl blue film preparations; one thousand red cells were counted.

An acidified water extract (pH 4.5) of desiccated hog stomach was prepared² and, after concentration by forced air drying at room temperature, the remaining clear yellow fluid was saturated with ammonium sulfate. The precipitate was removed by filtration and dialyzed in a Parlodion sac against distilled water for several days to remove the ammonium sulfate. The contents of the sac were then removed, dried, and fed daily to a patient with pernicious anemia in relapse (red blood cells 1,700,000 per cu. mm.; hemoglobin 64%). The daily dose was that amount of dried precipitate equivalent to 50 gm. of hog stomach. After 7 days of therapy a slight reticulocyte response was noted, indicative of a subminimal potency.

The same patient was then fed the above described precipitate to-

¹ Goldhamer, S. M., *Am. J. M. Sc.*, 1936, **191**, 405.

² Kyer, J., Brooks, F. P., and Isaacs, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 677.

gether with the boiled acid extract of desiccated stomach which had been shown to contain only the extrinsic factor.² On the eighth day of treatment the reticulocytes reached a peak of 13.7% and the red blood cells on the tenth day increased to 2,500,000 per cu. mm.

The above experiments demonstrated the possibility of the separation of the intrinsic and extrinsic factors of hog stomach by the use of ammonium sulfate and dialysis, and further suggested a method of salting out the intrinsic factor from human gastric juice. The following experiments were performed for this purpose. Our primary intent was to further the isolation of the intrinsic factor and not to prepare a material of any specific degree of potency.

Normal filtered gastric juice was brought to pH of 4.5 with concentrated sodium hydroxide and then saturated with ammonium sulfate. The precipitate was removed, dialyzed for 8 hours, and dried at room temperature. A half gram of the dry powder representing 200 cc. of normal gastric juice was mixed with 200 cc. of water acidified with hydrochloric acid to a pH of 2.0, incubated for 3 hours at 37°C., and fed daily for 7 days to a patient with pernicious anemia in relapse (red blood cells 2,270,000 per cu. mm.; hemoglobin 63%). The subject was permitted no food for at least 8 hours before treatment or 5 hours afterwards. No response occurred during a 12-day period.

This same patient was then fed the identical preparation as described in the previous experiment with the addition of 200 gm. of ground beef steak to supply the extrinsic factor. The mixture was incubated for 3 hours at 37°C. with 200 cc. of water acidified with hydrochloric acid to a pH of 2.0 before administration to the patient. The treatment was continued for 7 days. Although the reticulocytes did not reach the calculated maximum response, a peak of 8.0% was observed on the fifth day after therapy was instituted.

The preceding experiment was repeated to rule out the possibility of a spontaneous remission. A patient with pernicious anemia in relapse (red blood cells 1,740,000 per cu. mm.; hemoglobin 35%) was fed daily a mixture of 0.78 gm. of dry powder (200 cc. normal gastric juice) incubated with 200 gm. of ground beef steak and 200 cc. of acidified water at 37°C. for 3 hours. This treatment was continued for 9 days. A reticulocyte peak of 24% was observed on the sixth day. This experiment adds supportive evidence to the previous one and demonstrates the possibility of removing the intrinsic factor from normal gastric juice by precipitation with ammonium sulfate and dialysis.

*Conclusions.** 1. The intrinsic factor which is present in desiccated hog stomach and normal gastric juice may be salted out from a solution saturated with ammonium sulfate. 2. The intrinsic factor after salting out with ammonium sulfate is not dialyzable.

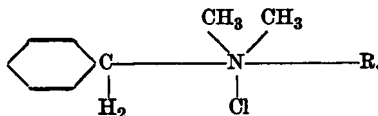
9684

Fungicidal Properties of Alkyl-Dimethyl-Benzyl Ammonium Chlorides.*

CECIL G. DUNN. (Introduced by J. W. Williams.)

From the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge.

The organic compound, alkyl-dimethyl-benzyl ammonium chlorides, is a new type of germicide having the following formula:



R represents a mixture of the alkyl radicals C_8H_{17} , $\text{C}_{10}\text{H}_{21}$, $\text{C}_{12}\text{H}_{25}$, $\text{C}_{14}\text{H}_{29}$, $\text{C}_{16}\text{H}_{33}$, and $\text{C}_{18}\text{H}_{37}$ as derived from the fatty acids of coconut oil.

Some of the germicidal, antiseptic, physical and chemical properties of this rather unusual germicide have been reported by Domagk,¹ Heineman,² and Dunn.^{3, 4}

The test organisms used in the present investigation were secured from the following sources: *Actinomyces gedanensis* and *Cryptococcus hominis* from the American Type Culture Collection, and the others from stock collections maintained by the Department of Biology and Public Health.

The fungi were grown in Sabouraud's dextrose media and subcultured in Sabouraud's dextrose broth, with the exception of *Micro-*

* The conclusions obtained are in agreement with those of Helmer and Fouts³ who published a similar report during the preparation of this paper.

³ Helmer, O. M., and Fouts, P. J., *Am. J. M. Sc.*, 1937, **194**, 399.

*Contribution No. 112 from the Department of Biology and Public Health. Research aided by a grant from the Alba Pharmaceutical Company, Inc.

¹ Domagk, G., *Deut. med. Wochenschr.*, 1935, **21**, 829.

² Heineman, P. G., *J. Am. Pharm. Assn.*, 1937, **26**, 711.

³ Dunn, C. G., *Proc. Soc. Exp. Biol. and Med.*, 1936, **35**, 427.

⁴ Dunn, C. G., *Am. J. Hyg.*, 1937, **26**, 46.