on each adjacent carbon atom. The secondary alcohol group in glycerin only, meets these requirements; therefore, it is concluded that the *Acetobacter suboxydans* exhibits an oxidative specificity for the trihydric alcohol, glycerin.

9310 P

Correlation of *in vitro* Activity of Normal Human Gastric Juice on Casein at pH 7.4 with Gastric Intrinsic Factor.

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The administration of mixtures of normal human gastric juice (intrinsic factor) and beef muscle (extrinsic factor) at pH 5 or 7 to patients with pernicious anemia results in increased blood production and clinical improvement.[1, 2]

In 1930, in association with Dr. C. W. Heath, unsuccessful attempts were made to show that gastric juice at pH 7.4 caused the production of amino acid from beef muscle. Griffiths[3] showed that gastric juice incubated with beef muscle globulin at pH 6 produced certain chemical changes. Emerson and Helmer[4] could not confirm his results. However, the present observations show that when casein was substituted for beef muscle, gastric juice at pH 7.4 did produce progressive changes in the casein.

Normal human gastric juice was obtained free from bile after injection of histamine, filtered through gauze and placed in the ice box. A one percent solution of A. H. Thomas & Company washed casein was prepared at pH 7.4, avoiding excess of acid or alkali. To 50 ml. of this solution were added 50 ml. of normal human gastric juice at pH 7.4 and 2 ml. of toluol. The mixture was incubated at pH 7.4 for 24 hours at 37.5°C., the pH remaining essentially constant.

Ten ml. samples were removed at 4 hours and 24 hours for formol

[1] Castle, W. B., Townsend, W. C., and Heath, C. W., *Am. J. Med. Sc.*, 1930, **180**, 305.

[2] Castle, W. B., *Science*, 1935, **82**, 159.

[3] Griffiths, W. J., *Biochem. J.*, 1934, **28**, 671.

[4] Emerson, C. P., and Helmer, O. M., *Am. J. Digestive Dis. and Nutrition*, 1936, **3**, 735.

titration. At the same time intervals 5 ml. samples were removed and the proteins precipitated with 20 ml. of 10% trichloracetic acid, and after standing at room temperature for 15 minutes, filtered through No. 12 Whatman folded filter paper. Total nitrogen determinations were made on the filtrates, using a micro-Kjeldahl digestion with subsequent distillation and nesslerization.

Eleven samples of normal gastric juice produced a progressive increase in a colloidal suspension in the filtrates which paralleled an increase in the total soluble nitrogen. This colloidal material could not be removed by centrifuging at 2500 r.p.m. There was, however, no corresponding increase in the amino nitrogen as determined by the formol titration of the digests.

Gastric juice was then subjected to certain procedures before mixing with the casein solution. The effect of the manipulations was compared with their known effect on the gastric intrinsic factor as shown by clinical observations in pernicious anemia. The intrinsic factor in gastric juice is not destroyed by passing through a Berkefeld V filter,[5] by exposure to pH 10 for 30 minutes at room temperature[6] or by removal of pepsin by isoelectric precipitation with casein at pH 4.7.[7, 8] Similarly when gastric juice was treated by these procedures its subsequent activity on casein at pH 7.4 *was not destroyed.*

When gastric juice is boiled for 5 minutes, heated at 70° to 80°C. for 30 minutes,[1] or incubated at pH 1.5 for 72 hours at 40°C. no subsequent hematopoietic activity with beef muscle appears clinically.[7] Likewise, as a result of such procedures, the activity of gastric juice on casein at pH 7.4 *was destroyed.*

Clinical observations have shown that saliva, pepsin U.S.P. and normal duodenal contents collected without admixture with gastric juice[1] are not sources of intrinsic factor. Saliva and pepsin solution had no action on casein at pH 7.4. A mixture of gastric juice and duodenal contents produced on incubation with casein at pH 7.4 increases both in total soluble nitrogen and in amino nitrogen as determined by formol titration. However, the increase in amino nitrogen was greatly reduced if the mixture was exposed to pH 10 for 2 hours at 37.5°C., although the mixture, like gastric juice after this treatment, retained its ability to cause an increase in total soluble nitrogen from casein at pH 7.4. On the other hand, 2 samples of

[5] Castle, W. B., Heath, C. W., Strauss, M. B., and Heinle, R. W., *Am. J. Med. Sc.*, in press.

[6] Flood, C., and West, R., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 542.

[7] Castle, W. B., Townsend, W. C., and Heath, C. W., *J. Clin. Invest.*, 1930, **9**, 2.

[8] Helmer, O. M., Fouts, P. J., and Zerfas, L. G., *Am. J. Med. Sc.*, 1934, **188**, 184.

gastric secretion obtained from patients with pernicious anemia and containing regurgitated duodenal contents showed an increase in the formol titration; but after exposure to pH 10 showed no activity of any sort on casein at pH 7.4. The consistent failure of normal gastric juice on casein at pH 7.4 to produce significant increases in amino nitrogen confirms the apparent absence of contamination with duodenal contents. This fact, together with the persistence of increase in total soluble nitrogen after exposure to pH 10,[9] suggests that trypsin and erepsin-like enzymes of gastric or duodenal origin were not responsible for the increases in total soluble nitrogen.

Clinical observations show that mixtures of beef muscle and gastric juice administered at pH 1.8 or 2.5 have no hematopoietic activity.[5] When pepsin was removed from gastric juice by exposure to pH 10 for 2 hours at 37.5°C.,[6] though the *in vitro* activity of the resulting gastric juice on casein at pH 7.4 was retained, there was no activity at pH 2.5. This is in agreement with the negative clinical result and also indicates the absence of pepsinogen in the gastric juice.

The above correlations suggest, but obviously do not constitute final proof, that the action on casein is due to intrinsic factor. They also do not necessarily imply that casein is a clinically effective extrinsic factor.

9311 P

Determination of Phenol Red in Gastric Contents.*

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A dilution indicator in gastric analysis is a substance employed for determining quantitatively the proportion of residual test meal present in a sample of gastric contents. Of the numerous substances so employed, phenolphthalein and phenol red (P.R.) are the most common. The former, however, is not suitable for the purpose because of its low solubility in water (Hollander, Penner, and Saltzman[1]), but the latter possesses most of the characteristics requisite

[9] Northrup, J. H., *J. Gen. Physiol.*, 1921-22, **4**, 261.

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[1] Hollander, F., Penner, A., and Saltzman, M., *Am. J. Dig. Dis. and Nutr.*, in press.