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.

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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.

for an ideal dilution indicator (Gorham,[2] Bulger, *et al.*,[3] Wilhelmj, *et al.*[4]). A method for determining P.R. in the presence of bile and protein suspensions is described by Wilhelmj. His procedure is unsatisfactory because: (1) it involves a subjective color correction previous to a colorimetric determination, (2) in the presence of dark green bile even this correction is impossible, (3) it necessitates the preparation of a different standard of comparison for each bile-containing specimen, (4) it does not include a standardized technique for adding the (variable) volume of color-correcting solution to the colorimetric standard without introducing an indeterminate change in the P.R. concentration. Furthermore, no data are cited to indicate its quantitative reliability.

In order to eliminate these faults, we have developed a simple and reliable method for the simultaneous removal of bile pigment and protein without loss of P.R. by means of freshly precipitated $Zn(OH)_2$. Following preliminary centrifuging, 5 ml. of the P.R. solution are treated with finely powdered CaO, sufficient to neutralize the gastric HCl and make the solution distinctly alkaline; then one ml. each of NaOH (0.5 N) and $ZnSO_4$ (0.55 N) are added (the latter dropwise), mixed thoroughly, and centrifuged after standing for 15 minutes; finally, 5 ml. of the supernatant fluid are treated with 2 ml. of Na_3PO_4 (0.5 N) to remove excess Zn and simultaneously to adjust the pH to a value suitable for colorimetry. After centrifuging, the supernatant liquid is filtered with suction and compared in the colorimeter with a standard P.R. solution (the test meal itself) similarly treated. Color differences between unknown and standard were practically non-existent. Comparison of treated with untreated standard solutions showed that the Zn treatment causes no significant loss of P.R. We have already run several dozen determinations on P.R. solutions in egg albumin, gastric pouch secretion from dogs, and human stomach contents; the concentration of gastric fluid varied from 25% to 95% and of P.R. from 0.2 mg. to 4.0 mg. per 100 ml. of mixture. Each solution was prepared from a different specimen of gastric contents; at least half of these were thick with dark green bile. In practically every case observed and calculated results agreed to within 0.04 mg. per 100 ml.; this corresponds to 1% of the initial concentration of the indicator in the test meal, which is accurate enough for all clinical and experimental purposes.

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2 Gorham, F. D., *J. A. M. A.*, 1923, **81**, 1738.

3 Bulger, H. A., Stroud, C. M., and Heideman, M. L., *J. Clin. Invest.*, 1928, **5**, 547.

4 Wilhelmj, C. M., Neigus, I., and Hill, F. C., *Am. J. Physiol.*, 1933, **106**, 381.

Concerning the pH to which the P.R. must be adjusted for colorimetry: Wilhelmj uses one ml. of saturated NaOH to 6.5 ml. of P.R. solution, which is equivalent to a final concentration of about 1.8 N NaOH. It is generally known, however, that P.R. will lose its color very rapidly in the presence of strong alkali (Thiel[5]) and we have found that this color loss may be appreciable even in one N NaOH (pH about 14) after one-half hour. In Wilhelmj's procedure, therefore, unless the solutions are alkalinized only a short time before being read and fresh portions of standard solution are prepared at short intervals, there is a great likelihood of error due to this progressive loss of color. On the other hand, we have found that at pH 11-12 no appreciable loss of color occurs, even after 4 hours; also, by colorimetric comparison of P.R. solutions at pH's 11 and 12 and by examination of the pH-dissociation curve (Clark,[6] p. 58), we have shown that the indicator attains its maximum degree of color in this same pH range. Since the pH of Na_3PO_4 solutions is likewise around 12, it follows that our P.R. solutions require no further alkali after the addition of excess phosphate for removal of residual Zn.

Finally, we have investigated the applicability of Beer's law in this situation. Using a standard solution of 1.0 mg. per 100 ml. (instead of the uppermost value of 4.0 mg.) we have found a direct proportionality between concentration and colorimeter reading throughout the entire concentration range of 4.0 to 0.2 mg. per 100 ml. In all cases, calculated and observed readings agreed to 1% or better. Thus, in spite of extensive variations in the scale reading, it is unnecessary to employ standards of different concentration for the upper and lower ranges of P.R. concentration in the unknowns. The scale reading corresponding to the standard solution may be 5.0, 10.0, 20.0 or 40.0 as conditions dictate.

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[5] Thiel, A., *Monatsh. Chemie.*, 1929, **53**, 1008.

[6] Clark, W. M., *The Determination of Hydrogen Ions*, 2nd ed., Baltimore, 1923.