

VARIATIONS IN ALIQUOT FRACTIONS OF GASTRIC CONTENTS *

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In a previous paper¹ attention has been directed to the fact that single determinations of gastric acidity by the Rehfuß method are not sufficient on which to base conclusions, because they do not take into account individual variation. Another important question which arises in evaluating results obtained by this method is whether the aliquot fractions aspirated are truly representative of the total gastric contents at the time of withdrawal. Recently, Gorham² has shown that "the gastric chyme is not, in the majority of instances, a homogeneous mixture after a test meal, and that the acidity of different portions may vary widely." He found on the withdrawal in rapid succession of many samples that there was a marked variation in acidity of these samples in subjects having clinical evidence of gastric disease. Such variations were not so apparent in subjects without clinical evidence of gastric disease, although Wheelon³ has reported some interesting variations in normal subjects.

PART I

In view of the important bearing of this observation on the errors involved in the Rehfuß fractional method of gastric analysis,⁴ the following experiment was conducted. A group of subjects showing no clinical evidence of gastric disease were given the standard Ewald meal after removal of the fasting contents. No further samples were aspirated until three-quarters of an hour had elapsed, whereupon the entire gastric contents were removed as rapidly as possible by the successive withdrawal of 10 c.c. samples. This procedure usually required ten or fifteen minutes. The acidity of the samples was then determined in the usual manner by the titration and colorimetric methods.¹ The results are presented in Figures 1, 2, 3, 4, 5, 6 and 7. Figures 1 to 5 represent the graphs of psychotic subjects, while Figures 6 and 7 are of normal individuals.

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1. Kopeloff, N.: Individual Variation as Influencing the Rehfuß Fractional Method of Gastric Analysis, *J. A. M. A.* **78**:404 (Feb. 11) 1922.

2. Gorham, F. D.: Variations of Acid Concentration in Different Portions of the Gastric Chyme, and Its Relation to Clinical Methods of Gastric Analysis, *Arch. Int. Med.* **27**:434 (April) 1921.

3. Wheelon, H.: Relation of the Gastric Content to the Secretory and Motor Functions of the Stomach, *Arch. Int. Med.* **28**:613 (Nov. 15) 1921.

4. Editorial: Critique of Gastric Analysis, *J. A. M. A.* **77**:202 (July 16) 1921.

TABLE 1.—VARIATION IN 10 C.C. FRACTIONS REMOVED IN RAPID SUCCESSION BY THE REHFUSS TUBE THREE QUARTERS OF AN HOUR AFTER THE MEAL

Patient	Number Samples	C.c. N/10 NaOH High Pt.	C.c. N/10 NaOH Low Pt.	Average	Average Deviation
M. Hr.	7	94	11	38	±18.0
M. Kn.	7	82	41	62	±11.5
M. By.	10	74	41	56	± 8.6
I. Sn.	14	51	33	43	± 3.5
E. Kg.	5	41	28	35	± 5.0
Normal Controls:					
M. Ma.	14	63	35	51	± 7.0
C. Te.	9	69	58	64	± 3.0

The data have been further summarized in Table 1. It is evident that there is a wide variation in the acidity of successive samples taken from the same individual. For example, in the first subject (M. Hr.) the variation between the high point (94) and the low point (11) is 83. Such a striking divergence means that there is a gross error involved

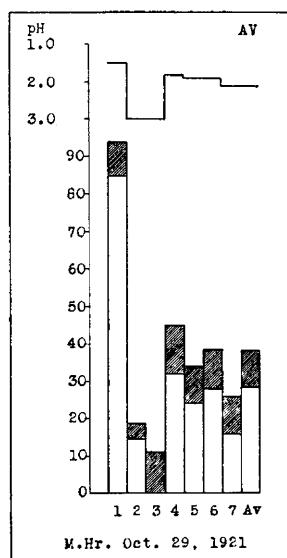


Fig. 1.—The ordinates 0-90 represent cubic centimeters of tenth normal sodium hydroxid per 100 c.c. of gastric contents; p_{H} , from 1.0 to 3.0: the hydrogen-ion concentration measured colorimetrically. The abscissae represent the number of samples taken; with the final column as an average of the preceding acidities. The colorless portion of each column represents the free acidity, while the light and the shaded portions together represent the total acidity.

in assuming any single sample as being representative of the total gastric contents. The last column marked "average deviation" brings this out mathematically. The average deviation is calculated in the following manner. The individual readings, such as 94, 19, 11, 45, 34, 38 and 26, are averaged, giving a mean of 38. The first reading (94)

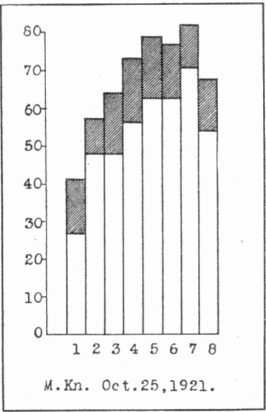


Fig. 2

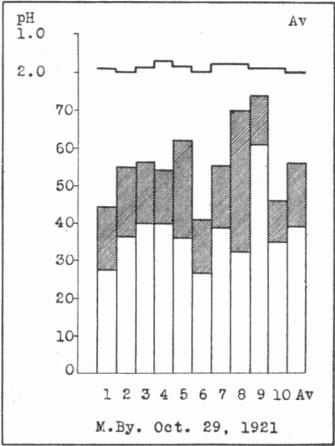


Fig. 3

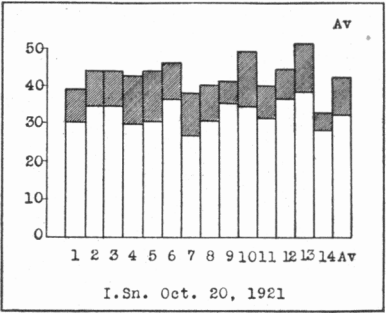


Fig. 4

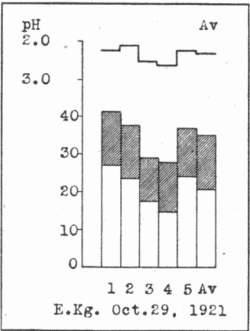


Fig. 5

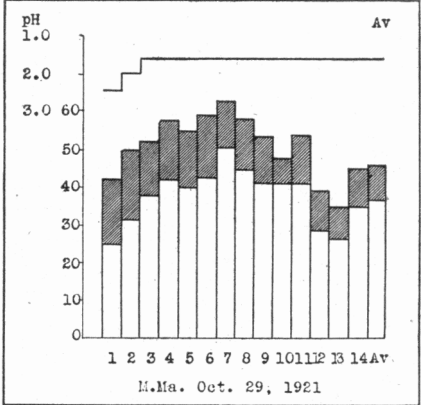


Fig. 6

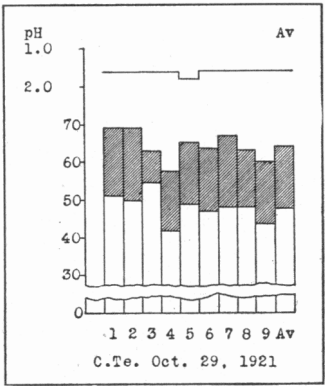


Fig. 7

deviates from the mean by 56; the second reading deviates by 19, and the third by 27—and so on. The sum of these deviations is 131. Dividing by the number of instances (7) one obtains the average deviation, which is ± 18 . In other words, considering the first subject, any single sample might be 18 more or 18 less than the average of all samples—which is virtually a range of uncertainty amounting to 36. Obviously, comparisons or conclusions based on such an uncertain method, when the experimental error is not calculated or taken cognizance of, are open to severe criticism.

It is scarcely necessary to discuss in detail the results obtained with the other subjects, for they merely emphasize in varying degree the points already established. Suffice it to say, that there was little difference between the normal controls and the psychotic patients having a similar acidity. In other words, it seems to be generally true that in subjects showing a fairly high gastric acidity one is more likely to find wider fluctuations than in those having a lower acidity. This may, perhaps, account for Gorham's findings, namely, that subjects with evidence of clinical disease, show wider fluctuations than the normal; and one may really expect that individuals without clinical evidence of disease who have a high acidity will also exhibit wide variations.

PART II

The above experiment explains, in part, the great variety of acidity curves obtainable by the fractional method of gastric analysis, and the fundamental errors involved in single determinations. The fraction obtained at any one moment cannot, therefore, be accepted as representative of the total gastric contents, for it is entirely dependent on the position of the tip of the tube in the stomach at the moment of withdrawal. Obviously, this position "is necessarily a constantly changing one, due, first, to the change of size and position of the stomach while emptying itself through the pylorus, and by aspiration; second, the shortening and lengthening of the stomach from gastric contraction; third, the peristaltic waves that tend to carry the tube toward the pylorus."² The experiment described in Part 1, indicates that the gastric contents are not homogeneous, but the method involved is open to criticism on the ground that some little time is required to rapidly withdraw the successive samples, and that during such lapse of time the gastric acidity might actually be undergoing change. The following experiment, therefore, was designed not only to yield definite information concerning the acidity in different parts of the stomach at the same moment, but also to eliminate the lapse of time required in withdrawing several samples. The following procedure was carried out on three subjects, two psychotic patients and a normal individual. A Rehfuß tube was inserted to a depth of 50 cm. (measured from the teeth).

Then a second Rehfuss tube was inserted to a depth of 45 cm.; and, finally, a third Rehfuss tube was inserted to a depth of 40 cm. The gastric contents of each subject were removed simultaneously through all three tubes, by having the operators aspirate the contents at the same moment. The tubes were labeled and special care was taken to have each tube at the proper level before any gastric contents were removed. The standard Ewald meal was given and as usual, at fifteen minute intervals, samples were withdrawn, except that during these fractional analyses, three samples were aspirated simultaneously at the designated time. At the end of the analyses, each subject was given a glass of milk containing a small amount of bismuth subnitrate (15 grains) and a roentgenogram was taken to determine the relative posi-

TABLE 2.—VARIATION IN ACIDITY OF THREE ALIQUOT FRACTIONS OF GASTRIC CONTENTS WITHDRAWN SIMULTANEOUSLY (M. MCA.)

Time, Hours F. C.	Total Acid	Average \pm Average Deviation	Free Acid	Average \pm Average Deviation	pH	Average \pm Average Deviation
	45		33		1.6	
	22	34 \pm 12	9	21 \pm 12	1.6	1.6 \pm 0
¼	22		12		3.4	
	30		18		2.5	
	7	20 \pm 8	0	10 \pm 7	5.4	3.8 \pm 1.1
½	39		18		3.0	
	39		27		2.9	
	22	33 \pm 8	14	20 \pm 5	2.9	2.9 \pm 0
¾	69		38		2.2	
	56		30		2.4	
	28	51 \pm 15	18	29 \pm 7	2.1	2.2 \pm 0.1
1	74		51		1.7	
	63		42		...	
	21	53 \pm 21	14	36 \pm 14	2.5	2.1 \pm 0.4
1¼	73		49		1.8	
	68		46		...	
	37	59 \pm 15	29	41 \pm 8	1.8	1.8 \pm 0
1½	47		35		2.1	
	63		42		1.8	
	36	49 \pm 10	22	33 \pm 7	2.1	2.0 \pm 0.1
1¾	42		28		2.1	
	43		29		1.9	
	8	31 \pm 15	0	19 \pm 12	4.5	2.8 \pm 1.1
2	35		22		1.9	
	27	31 \pm 4	19	21 \pm 1	2.0	2.0 \pm 0

tions of the Rehfuss tubes maintained at a constant level. The acidity of the samples was determined in the usual manner by titration and colorimetric methods. The results of total acidity are graphically presented in Figures 8, 9 and 10, and summarized in Tables 2, 3 and 4.

In Table 2, may be found several points of interest. Chief among these are the wide variations occurring in the total or free acidity of the three fractions aspirated at any one time. Glancing down the second column marked "total acid" it is seen that at the one hour period there is a variation from 21 to 74, or an actual difference of 53. This is, perhaps, larger than the differences found at other periods, but since the one hour period is ordinarily of special interest, these figures are significant. In the third column is given the average of the three

TABLE 3.—VARIATION IN ACIDITY OF THREE ALIQUOT FRACTIONS OF GASTRIC CONTENTS WITHDRAWN SIMULTANEOUSLY (M. HR.)

Time, Hours	Total Acid	Average \pm Average Deviation	Free Acid	Average \pm Average Deviation	pH	Average \pm Average Deviation
F. C.	19		5		5.0	
	25	22 \pm 3	11	8 \pm 3	4.0	4.5 \pm 0.5
$\frac{1}{4}$	17		13		...	
	25		15		3.0	
	22	21 \pm 3	10	13 \pm 2	3.0	3.0 \pm 0
$\frac{1}{2}$	42		26		2.3	
	40		29		2.3	
	41	41 \pm 1	31	29 \pm 2	2.1	2.2 \pm 0.1
$\frac{3}{4}$	61		44		2.1	
	72		54		1.8	
	56	63 \pm 6	43	47 \pm 5	1.7	1.9 \pm 0.2
1	70		48		1.9	
	82		66		1.6	
	65	72 \pm 6	51	55 \pm 7	1.6	1.7 \pm 0.1
$1\frac{1}{4}$	79		60		1.7	
	71		56		1.7	
	62	71 \pm 6	48	55 \pm 4	1.7	1.7 \pm 0
$1\frac{1}{2}$	65		56		1.7	
	65		53		1.6	
	48	59 \pm 7	38	49 \pm 7	1.7	1.7 \pm 0
$1\frac{3}{4}$	80		71		...	
	58		47		1.7	
	51	63 \pm 11	43	54 \pm 12	1.7	1.7 \pm 0
2	74		64		1.6	
	61		53		1.6	
	61	65 \pm 6	54	57 \pm 5	1.7	1.6 \pm 0

TABLE 4.—VARIATION IN ACIDITY OF THREE ALIQUOT FRACTIONS OF GASTRIC CONTENTS WITHDRAWN SIMULTANEOUSLY (M. BY.)

Time, Hours	Total Acid	Average \pm Average Deviation	Free Acid	Average \pm Average Deviation	pH	Average \pm Average Deviation
F. C.	53		43		1.6	
	53		41		1.6	
	7	38 \pm 20	0	28 \pm 19	4.5	2.6 \pm 1.3
$\frac{1}{4}$	32		27		2.1	
	32		23		1.9	
	18	27 \pm 6	13	21 \pm 5	3.4	2.5 \pm 0.6
$\frac{1}{2}$	50		33		1.9	
	32		19		2.4	
	18	33 \pm 11	8	20 \pm 9	3.5	2.6 \pm 0.6
$\frac{3}{4}$	66		49		1.7	
	67		51		1.7	
	28	54 \pm 17	19	40 \pm 14	3.0	2.1 \pm 0.6
1	70		60		1.6	
	78		63		1.5	
	59	69 \pm 7	44	56 \pm 8	1.7	1.6 \pm 0.1
$1\frac{1}{4}$	78		64		1.6	
	82		73		1.6	
	75	75 \pm 3	57	65 \pm 6	1.6	1.6 \pm 0
$1\frac{1}{2}$	79		64		1.6	
	79		62		1.6	
	61	73 \pm 8	44	57 \pm 8	1.6	1.6 \pm 0
$1\frac{3}{4}$	75		61		1.6	
	78		66		1.6	
	51	68 \pm 11	37	55 \pm 12	1.6	1.6 \pm 0

fractions with the calculated average deviation. This shows conclusively how large the margin of uncertainty actually is, if one assumes any single reading as representative of the total gastric contents at any particular moment.

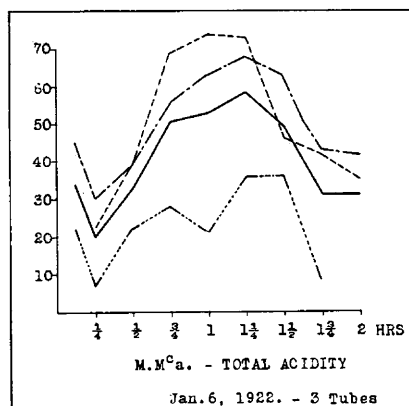


Fig. 8.—The ordinates 0-90 represent cubic centimeters of tenth normal sodium hydroxid per 100 c.c. of gastric contents; p_{H} from 1.0 to 3.0: the hydrogen ion concentration measured colorimetrically. The abscissae represent quarter hour intervals, and F. C.=fasting contents. The broken line represents the fraction taken from the tube at 50 cm.; the dash and dot alternately, represents the fraction taken from the tube at 45 cm.; the dash and several dots alternately, represents the fraction taken from the tube at 40 cm.; while the solid line represents the average of the three fractions taken.

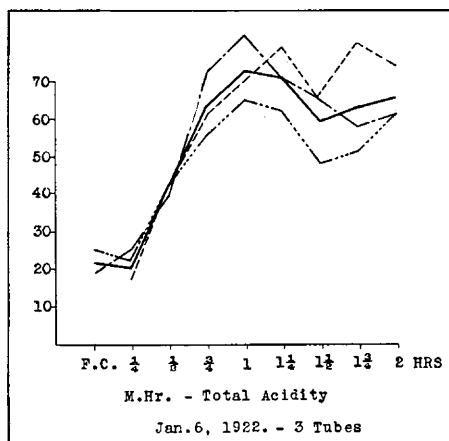


Fig. 9

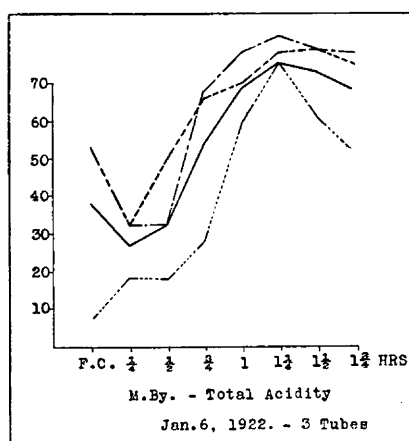


Fig. 10

Figure 11 brings this point out even more strikingly. Usually, the higher the acidity, the wider the margin of uncertainty—a generalization already referred to in Part I. It is apparent from this graph that curves of various kinds could be drawn within the shaded area, from one designated by Rehffuss isosecretory to one designated hyposecretory.

This substantiates my earlier contention that a single determination by the Rehfuß fractional method of gastric analysis is not sufficiently valid on which to base a conclusion.¹ It is clearly shown in the data here presented, that fractions taken from different parts of the stomach are not homogeneous in character, but differ widely in acidity. The roentgenograms indicate the relative position of the tubes during the analyses. One of these is shown in Figure 12.

As might be expected, the free acidity values in Table 2, in general, run parallel to those obtained for total acidity. But it is noteworthy that this does not hold true for p_H . Here there is much closer agreement between the three fractions than when acidity is measured by titration. This means that the buffer salts are of paramount importance in the measurement of true gastric acidity. This lends further emphasis

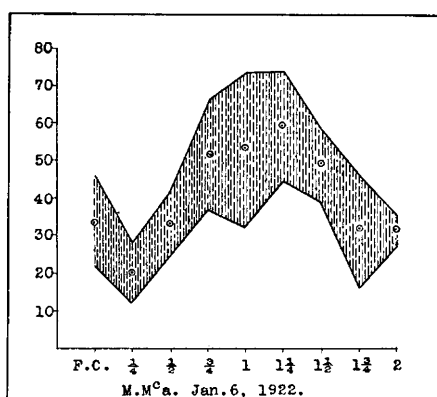


Fig. 11.—The points in circles in the shaded area represent the average of the three fractions at the time indicated on the abscissae, while the shaded area above and below each point indicates the possible range into which the reading at that moment might fall.

to the valuable work of Shohl⁵ and shows how misleading an index of gastric acidity a single titration may be.

The other tables bring out in varying degree the facts already referred to and do not require a repetition of the discussion. It is of interest, however, to note that the normal individual (M. McA.) exhibited even wider fluctuations in three fractions aspirated at the same time, than the two psychotic subjects (M. Hr. and M. By.), so that it cannot be said that the mental status has any particular bearing on the point at issue. Naturally, various individuals would be expected to differ with regard to the homogeneity of their gastric contents; nevertheless, this experiment indicates that it is erroneous to assume that any such homogeneity exists.

5. Shohl, A. T.: Determination of the Acidity of Gastric Contents, Johns Hopkins Hosp. Bull. **31**:152 (May) 1920.

In Tables 2, 3 and 4, a rather interesting phenomenon obtains. The fraction taken from the tube inserted at 40 cm. is almost invariably lower in total and free acidity than the fractions taken at a depth of 45 or 50 cm. Furthermore, these samples were totally different in physical character—being of a much lighter color often, water-white, and containing lesser amounts of starch. This is to be expected from the work of Prym⁶ and Sick,⁷ who have shown that the acidity of the pyloric gastric contents varies considerably from the fundal portion, but their methods are not as conclusive as the one herein described, since

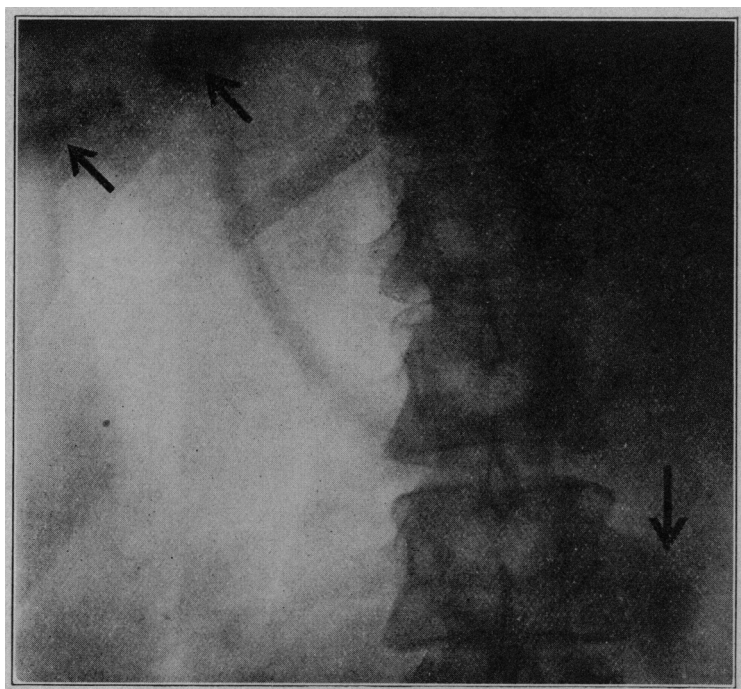


Fig. 12.—Relative position of Rehfuß tubes during fractional analysis with three tubes.

they did not use more than one tube. Very little difference was found between fractions obtained at 45 and 50 cm., respectively. However, the fact that these two fractions are usually in close agreement indicates that in that region the stomach contents were, perhaps, of a more homogeneous character. In plotting the curves of the fractions taken at each level, a certain general similarity may be noted, despite the recrossing at numerous points. This might seem to indicate that if the level of the tube were kept constant, single determinations might yield a characteristic curve. However, the errors are too fundamental

6. Prym: *Deutsch. Arch. f. klin. Med.* **90**:310 (June) 1907.

7. Sick: *Deutsch. Arch. f. klin. Med.* **88**:169 (Oct.) 1906.

to be thus lightly disregarded. For despite the similarity in curves, each curve has its peculiar margin of uncertainty, and thus the possibility of obtaining the characteristic curve would be seriously diminished. In other words, an average deviation of ± 12 for every sample, or practically 25 c.c., in the first subject, implies a wide margin of uncertainty.

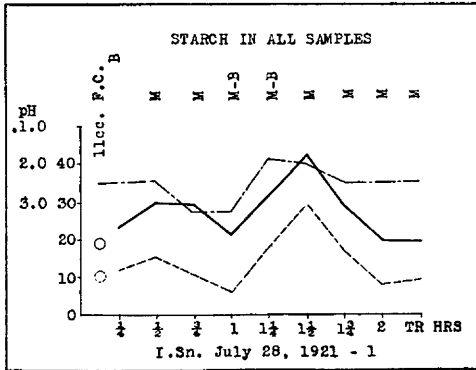


Fig. 13

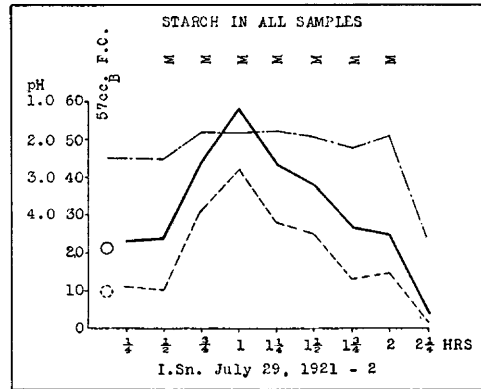


Fig. 14

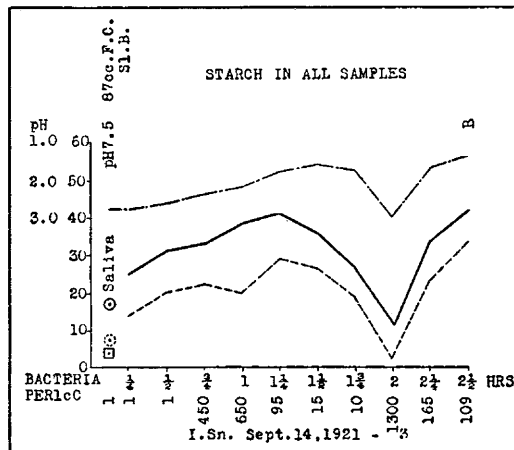


Fig. 15

Figs. 13, 14 and 15.—Fractional analysis of stomach contents of a natural regurgitator.

PART III

The problem of determining whether or not a small aliquot is truly representative of the total gastric contents was approached from still another aspect. Through the kindness of Dr. William C. Garvin, superintendent of Kings Park State Hospital, there was transferred to Ward's Island a psychotic patient who could be called "a natural regurgitator," i. e., one who could deliver his total gastric contents

at will, or on very slight stimulation of the palate. This patient gave a history of frequent vomiting after meals for which no adequate explanation could be given other than that it was an hysterical symptom. Since admission to Manhattan State Hospital, the vomiting has become relatively infrequent and generally occurs only after the patient has been visited. Several fractional gastric analyses were performed on him in the usual way (Figs. 13, 14 and 15). Other than the relatively low acidity, there is little that is worthy of comment in Figures 13 and 15; while in Figure 14, the curve is typically isosecretory.

The power to regurgitate at will was utilized to study the homogeneity of the gastric contents. The subject was given the standard Ewald meal on several different days and instead of removing the contents by the fractional method, the total gastric contents were regurgitated on the first day after one-quarter hour, the next day after one-half hour, and so on, in such a way as to make it possible to plot a

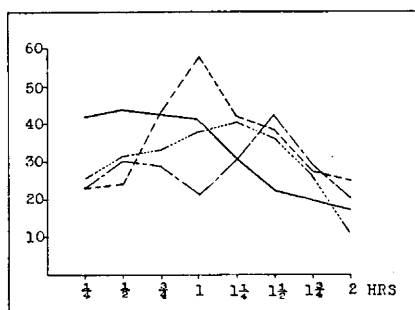


Fig. 16.—Homogeneity of gastric contents. Only the total acidity is shown. The solid line represents the total contents. The broken lines represent the fractional contents.

curve over the two-hour period, similar in scope to the usual fifteen minute fractions taken on the same day. A comparison of this curve with those obtained by the usual method is presented in Figure 16.

This curve representing the acidity of the total gastric contents has a higher initial acidity and a lower final acidity than that ordinarily found. Furthermore, instead of showing a gradual rise and decline, it is decidedly atypical in starting high and declining steadily. These peculiarities can scarcely be ascribed to the lapse of time between analyses, since the usual fractional method conducted at about the same intervals showed no marked changes, and the patient remained in about the same physical and mental condition so far as could be ascertained. While this method of conducting a gastric analysis is unusual, it gives a more adequate picture of the subject's gastric cycle of digestion than the routine method in vogue. Gorham² states that "the true fractional analysis necessitates the giving of successive test meals and extracting them at different periods."

A feature of some importance was added to the procedure described above, namely, an aliquot sample of 10 c.c. was taken by means of the Rehfuß tube immediately prior to the regurgitation of the total gastric contents; in fact, the removal of the Rehfuß tube in these instances stimulated "natural regurgitation." The comparison of each aliquot with the corresponding total contents is given in Table 5.

In a subject having a rather low gastric acidity, as has already been pointed out, large differences could not be expected. However, it is significant that there are noticeable differences. This fact, together with the others mentioned in Parts 1 and 2, serves to complete the evidence against the usual technic of the Rehfuß method in which it is assumed that the aliquot withdrawn is representative of the total gastric contents, showing that such an assumption has little basis in fact.

While these experiments may appear to be entirely destructive in character, nevertheless, certain constructive points have been estab-

TABLE 5.—COMPARISON OF ALIQUOT WITH CORRESPONDING TOTAL GASTRIC CONTENTS OBTAINED BY "NATURAL REGURGITATION"

Time	Total Acidity in C.c. N/10 NaOH	
	Aliquot	Total Contents
1 hour.....	49	41
1½ hours.....	37	22*
2 hours.....	22	17

* Some bile present.

lished. First and foremost, in using the Rehfuß method, it is essential to repeat the analysis until a satisfactory agreement in curves is obtained. Second, the tube should be kept at a constant level. This can easily be accomplished by fastening the tube to the cheek by means of a strip of surgeon's adhesive tape. Third, the aliquot fractions should be as large as possible. Fourth, the subject should expectorate freely, swallowing as little saliva as possible. Finally, the acidity should be measured in terms of hydrogen ion and buffer salts, and not by the inaccurate titration method in common usage.

SUMMARY

1. A wide variation was found in the acidity of 10 c.c. fractions withdrawn in rapid succession by means of the Rehfuß tube three-quarters of an hour after the test meal was ingested. This was more noticeable in subjects having high rather than low gastric acidity.

2. By inserting three Rehfuß tubes in one individual and aspirating the fractions simultaneously at fifteen minute intervals, it was found that there was considerable variation of acidity in different parts of the stomach at the same moment. Roentgenograms established the relative position of the tubes.

3. From these data it is shown that widely divergent curves of acidity may be plotted which depend entirely on the experimental error of the method and not on the subject's gastric condition.

4. The inadequacy of the titration method and the importance of hydrogen ions and buffer salts in measuring gastric acidity is indicated.

5. By comparing the periodic "natural regurgitation" of total gastric contents with the fractions ordinarily obtained by the Rehfuß method, discrepancies are noted.

6. Results obtained by the Rehfuß method may be more validly interpreted if: (a) the analysis is repeated until a satisfactory agreement in curves is obtained; (b) the tube is kept at a constant level; (c) aliquot fractions are large; (d) little saliva is swallowed; (e) acidity is measured in terms of hydrogen ion and buffer salts.

CONCLUSION

Aliquot fractions obtained by the Rehfuß method of gastric analysis cannot be assumed to represent accurately the total gastric contents.

I am indebted to the individuals who made these experiments possible and to the members of the staff for their generous assistance. Particular thanks are due Dr. C. O. Cheney for his many valuable suggestions and help on the wards, and to Mr. E. J. Kennedy for his efficient and painstaking work in the laboratory.