

THE EXAMINATION FOR DIAGNOSTIC PURPOSES OF THE ENZYME ACTIVITY OF DUODENAL CONTENTS *

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Since the introduction of the duodenal tube by Einhorn¹ and by Gross,² this method of reaching the duodenum has been employed for a variety of purposes. It has been used to maintain nutrition in diseases of the stomach and other conditions,³ to treat a variety of constitutional diseases by irrigation⁴ and to secure fluid for a study of the bacterial flora of the upper intestine, and likewise the activity of the pancreatic enzymes and the detection of bile. Despite the many possibilities which have been opened by this method of entrance into the intestines, it would appear to have received attention only in a few circles. As a method of ascertaining the activity of the pancreas for diagnostic purposes, it is obviously more logical than the various indirect methods entailing an examination of the feces or urine.

The results which have thus far been obtained in the examination of duodenal contents deserve brief review. It has been shown by Hess⁵ in infants, and more recently by MacNeal and Chace⁶ in adults, that this method may be employed to excellent advantage in the study of the bacterial flora of this part of the intestines. MacNeal and Chace observed that, normally, when free hydrochloric acid was present in the stomach, the contents of the duodenum were comparatively sterile, but when free acid was absent, numerous organisms were present. In a case of typhoid which they had the opportunity to examine, typhoid bacilli were present in large numbers, while in an infant, aged 22 months, Hess made a similar observation.

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1. Einhorn: *Med. Rec.*, New York, 1910, lxxvii, 98.

2. Gross: *New York Med. Jour.*, 1910, xci, 77.

3. Einhorn and Rosenbloom: *Am. Jour. Med. Sc.*, 1911, cxlii, 7; Einhorn: *Post-Graduate*, 1913, xxviii, 550; Hess, A. F.: *The Use of a Simple Duodenal Catheter in the Diagnosis and Treatment of Certain Cases of Vomiting in Infants*, *Am. Jour. Dis. Child.*, 1912, iii, 133; Morgan: *Am. Jour. Med. Sc.*, 1912, cxliii, 670.

4. Gross: *New York Med. Jour.*, 1911, xciii, 171; *Med. Rec.*, 1912, lxxxi, 706; Jutte: *New York Med. Jour.*, 1912, xcv, 543.

5. Hess, A. F.: *Jour. Infect. Dis.*, 1912, xi, 71.

6. MacNeal, Ward J., and Chace, A. F.: *A Contribution to the Bacteriology of the Duodenum*, *THE ARCHIVES INT. MED.*, 1913, xii, 178.

The first paper of importance on the enzyme activity of the duodenal contents was that of Einhorn and Rosenbloom,⁷ who studied the influence of various agents on the secretion and activity of the juice in a series of persons. In general, they were able to confirm the physiologic observations on animals. They noted that the secretion was active during fasting, while the administration of secretin, hydrochloric acid, pilocarpin, etc., caused an increased secretion of fluid, and generally a more active one. The activity of the juice in diseases possibly involving the pancreas was not considered.

Several very instructive papers on the examination of the juice in infants have appeared by Hess,⁸ who conducted much of his work in the baby wards of this hospital. He observed that bile was rarely excreted during the first twelve hours of life, while in the subsequent twenty-four hours it was variable. In cases of marked jaundice it was observed to be profuse, the jaundice preceding the excretion of bile into the duodenum. The three pancreatic ferments were found in the infants before they had been put to the breast, and thus without the stimulus of food to incite the secretion. In older infants, a month or more of age, with the increase in the secretion of the juice, a decided increase in the amylolytic power was observed. The development of this starch-splitting enzyme so early in life is particularly interesting. In certain atrophic infants it was noted that, although they secreted little gastric juice to act as a stimulus, a very large amount of thin, watery juice containing all the pancreatic ferments, though weak in lipase, could be aspirated from the duodenum.

In a series of cases of adults without pancreatic disease, Frank⁹ found active trypsin in all cases.

While the present work was in progress an interesting paper appeared by Crohn¹⁰ on the diagnosis of the functional activity of the pancreatic gland by means of ferment analyses of the duodenal contents. His series included a number of cases of interest, namely one case of acute pancreatitis, three cases of obstructive jaundice, five cases of cholelithiasis and six of diabetes. In the case of pancreatitis, amylase and trypsin were found absent from the duodenal juice and likewise from the feces. The cases of obstructive jaundice showed an absence of bile, but a normal enzyme activity, except in one case, in which the amylase and trypsin

7. Einhorn, Max, and Rosenbloom, Jacob: A Study of the Duodenal Contents in Man, *THE ARCHIVES INT. MED.*, 1910, vi, 666.

8. Hess, A. F.: A Study of Icterus Neonatorum by Means of the Duodenal Catheter, *Am. Jour. Dis. Child.*, 1912, iii, 304; The Pancreatic Ferments in Infants, *Am. Jour. Dis. Child.*, Oct., 1912, iv, 205. A Consideration of the Pancreas and Its Ducts in Congenital Obliteration of the Bile-Ducts, *THE ARCHIVES INT. MED.*, 1912, x, 37.

9. Frank: *Arch. Verdauungskr.*, 1912, xviii, 387.

10. Crohn: *Am. Jour. Med. Sc.*, 1913, cxlv, 393.

were absent on the first examination. The duodenal juice in the cases of cholelithiasis and of diabetes was found normal. This observation in diabetes would appear to emphasize the point, sometimes misunderstood, that there is no disturbance of the external secretion of the pancreas in this condition.

A study of the enzyme activity of duodenal juice was begun more than a year ago. The objects of the investigation were (1) to establish the normal limits of variation in the activity of the pancreatic enzymes, (2) to ascertain the chemical composition and enzyme activity of the duodenal juice in a variety of pathologic conditions, (3) to determine what influence the gastric acidity might exert on the composition of the duodenal juice, and (4) to perfect methods of determining the admixture of bile by the estimation of the bile pigments and cholesterol. In view of the scant attention which the problem has attracted, we have thought it desirable to report the results which have been obtained in the examination of duodenal juice in thirty cases. It is planned to continue the study on interesting cases as they may arise.

METHOD OF OBTAINING DUODENAL JUICE

The technic employed in obtaining the duodenal juice has already been described by MacNeal and Chace.¹¹ A small soft rubber tube (external diameter of 3.5 mm.) capped with a perforated gold tip, is placed in the patient's mouth with instructions to swallow. With the patient lying on his right side, the tube is usually carried by the peristaltic waves of the stomach into the duodenum in about twenty minutes. Its position can be demonstrated either fluoroscopically or by examination of the aspirated contents. The tube is usually allowed to pass to about 80 cm. It was generally given at 10:30 p. m. and the duodenal contents aspirated with a glass syringe at about 9:00 a. m.; then with the tube still in place an Ewald test-breakfast was given and the duodenal contents aspirated one hour later. Occasionally the tube was passed at 7 a. m. and the duodenal contents aspirated at 9 a. m. This method is generally as accurate, and is more acceptable to the patient.

Duodenal Juice.—The duodenal juice obtained in this way is normally a clear or nearly clear, golden yellow, slightly viscid fluid. It is faintly acid, neutral or faintly alkaline in reaction to litmus and has a specific gravity of about 1.005. The juice contains bile as its color would indicate, and active amylolytic, lipolytic and proteolytic enzymes.

Methods of Examination.—The methods which have been employed in the examination of the duodenal juice are briefly described below. They are slight modifications of well-known methods which have been simplified in so far as possible for use in this connection. It was apparent in this work that the determinations of the enzyme activities were only relatively quantitative. On this account, and because of the small amounts of fluid often obtainable, a relatively simple technic has been employed.¹² With the technic described, complete data can be secured with 6 to 7 c.c. of juice.

11. MacNeal, Ward J., and Chace, A. F.: A Contribution to the Bacteriology of the Duodenum, *THE ARCH. INT. MED.*, 1913, xii, 178.

12. These methods have been described by Myers and Fine: *Essentials of Pathological Chemistry*, New York, 1913, p. 25.

After securing the juice, it was taken to the laboratory without delay and placed in a refrigerator at 0 C., unless the examination was to be made at once. In the latter half of the present series this was the case. It is not believed, however, that refrigeration, at least for a short period, produces any appreciable change in the enzyme activity of the juice. It was very soon observed that little or no amylolytic activity could be demonstrated in a juice which was slightly acid to litmus, even though the juice had been at once neutralized when received. The proteolytic activity was affected in the same way, though to a much less extent. In the case of the lipase it was observed that the juice, which was faintly alkaline to litmus, always showed a weak lipolytic activity. It should be noted here, however, that the juice was not previously acidified. The initial reaction of the juice is obviously an important factor in the observed enzyme activity.

(a) *Reaction and Total Acidity.*—The reaction was ascertained with strips of red and blue litmus, while the total acidity was titrated with N/20 NaOH, using phenolphthalein as indicator, and employing as large amounts of material as might be available.

(b) *Amylase (Amylopsin).*—The Wohlgemuth method is simple and fairly satisfactory. Into each of six small test-tubes are introduced 5 c.c. of 1 per cent. soluble starch solution. Tube one serves as a control and to the remaining five tubes are added 0.05, 0.1, 0.25, 0.5, and 1.0 c.c. of the juice diluted one-half with distilled water. The tubes are then incubated at 38 C. for thirty minutes, immediately nearly filled with cold water, two drops of N/10 iodine added, and the tubes shaken. The tube is selected as positive which shows an entire disappearance of all blue color. The enzyme activity is expressed in the number of c.c. of starch solution 1 c.c. of undiluted juice is capable of digesting. If it takes 1 c.c. of juice to digest 5 c.c. of starch, the activity is five; if digestion is accomplished by 0.25 c.c., it is twenty, etc. For the five tubes as diluted above, the activity figures are 200, 100, 40, 20 and 10.

(c) *Lipase (Steapsin).*—Into each of two test-tubes are introduced 1 c.c. portions of the juice, one of which is boiled to serve as control. To each of these tubes are added 1 c.c. of neutral ethylbutyrate, 10 c.c. of distilled water and 1 c.c. of toluene. The tubes are shaken and placed in the incubator at 38 C. for twenty-four hours, shaking several times during the interval. At the end of this time they are removed to porcelain dishes and titrated with N/20 NaOH, using phenolphthalein as indicator. The titration result of the boiled tube is subtracted from the unboiled to obtain the figure for the lipolytic action.

(d) *Protease (Trypsin).*—Two methods have been employed, the O. Gross casein method, and a modification of the Fermi gelatin method. Casein has the disadvantage that it is also attacked by erepsin, though this is probably a negligible factor here, while the gelatin digestions must be carried on at room temperature.

Casein Method.—Into each of six small test-tubes, as in the amylase method, are introduced 5 c.c. of 0.1 per cent. pure casein in 0.1 per cent. sodium carbonate,¹³ and the same amounts of duodenal juices added as in the case of the amylase. The tubes are incubated for fifteen minutes at 38 C. and then acidified with two drops of dilute acetic acid. The tube which remains perfectly clear, that is, in which digestion has been complete, is recorded. The tryptic activity is calculated in the same way as the amylolytic activity, except that the tryptic activity, according to the Gross formula, is an expression of the power of 1 c.c. of the juice on 10 c.c. of the casein solution, instead of 1 c.c., as in the Wohlgemuth method. The five tubes, according to the Gross scheme, represent activities of 20, 10, 4, 2 and 1, that is, one-tenth of the values obtained in the case of the amylase.

13. The soluble starch and casein solution were made up a liter at a time, a little chloroform and toluene added, and kept in a refrigerator at 0 C.

ENZYME ACTIVITY OF DUODENAL CONTENTS TABULATED ACCORDING TO GASTRIC ACIDITY
GROUP I. CASES SHOWING AN ABSENCE OF FREE HYDROCHLORIC ACID

Case	Diagnosis	Gastric Acidity		Total Duodenal Acidity	Volume of Fluid c.c.	Con- dition §	Reaction to Litmus	Amy- lase	Trypsin	
		Free HCl	Total Acidity						Casein	Gelatin cm.
1—B. A.	Myxedema	0	6	5	14	F	Neutral	5	2	3.9
2—M. McG.	Chr. gastric ulcer.	0	8	8	19	F	Neutral	200	4	3.7
3—J. K.	Asthenic gastritis.	0	10	10	5	F	Neutral	20	4	3.8
4—J. L.	Pernicious anemia	0	20	9	20	F	Neutral	10	2	5.6
		15	F	100	4	4.2

GROUP II. CASES SHOWING AN HYPERACIDITY, THE FREE HCl BEING ABOVE 25

5—I. S.	Gastric ulcer	50	77	12	20	F	Neutral	100	4	3.2
		10	6	E	Faintly alk...	100	10	2.1
		10	7	F	Faintly alk...	10	10	4.2
6—W. L.	Gastric ulcer	50	70	5	6	E	Neutral	100	4	4.3
7—C. L.	Gastric ulcer	50	75	..	5	F	Faintly alk...	100	4	3.1
		23	15	E	Faintly acid..	5	0	3.2
		25	6	E	Faintly acid..	0	0	5.3
8—M. P.	Hyperchlorhydria	30	70	10	10	F	Neutral	200	4	3.9
		2	E	Neutral	3.9
9—R. L.	Alcoholic gastritis..	28	58	55	15	F	Acid	0	0	0.4
		75	13	E	Acid	0	0	..
		85	10	F	Acid	0	0	..
		65	27	E	Acid
		35	10	F	Acid
		80	24	E	Acid	4.2
		80	25	E	Acid	1.0
		E	Acid	0.7
10—S. W.	Neurosis	30	60	15	9	F	Faintly acid..	10	4	4.9
		10	27	F	Neutral	20	10	5.2
		4	E	Neutral	10	4	4.2
		10	23	F	Faintly acid..	0	2	3.5
11—T. B.	Duodenal ulcer ...	73	89	5	5	F	Faintly alk...	10	2	2.6
		15	15	E	Neutral	5	..	3.4
12—A. B.	Gastric ulcer	90	105	0	40	F	Alkaline	2	10	4.8
	Gastro-enterostomy	0	20	E	Alkaline	40	10	4.5

GROUP III. CASES SHOWING NORMAL ACIDITY

13—B. B.	10	35	10	17	F	Neutral	40	0.3	10	2.8
14—A. P.	21	60	35	6	E	Acid	0	...	2	0.6
15—G. H.	10	37	8	15	F	Faintly acid...	0	1.7	0.5	1.7
16—B. D.	15	50	13	10	F	Faintly alk...	40	2.3	10	5.8
17—F. S.	20	35	15	6	F	Faintly alk...	100	2.1	10	4.7
18—C. E.	30	54	13	15	F	Faintly acid...	5	1.9	4	3.3
19—E. H.	24	56	10	12	E	Faintly acid...	2	3.1	4	3.7
20—R. E.	13	27	5	10	F	Neutral	10	3.8	2	3.0
21—A. O'C....	5	8	F	Neutral	5	1.7	4	3.4
	5	8	F	Neutral	2.0	2	3.7
	E	Neutral	0.9	..	3.6
	0.9	..	3.1
22—S. Z.†....	8	16	..	3	F	Faintly alk...	40	...	4	3.6
	2	F	Faintly alk...	100	...	2	..
	5	19	F	Faintly alk...	100	0.6	4	4.4
	8	7	F	Faintly alk...	200	0.4	4	3.6
	8	9	F	Faintly alk...	40	2.2	4	4.5
	45	8	*	Neutral	100	2.0	4	5.4
	17	53	..	5	F	Faintly acid...	0	...	4	3.1
	20	11	F	Faintly acid...	0	1.8	0.5	2.6
	15	9	F	Faintly acid...	0	1.6	2	3.2
	5	F	Faintly acid...	0	1.3	0.5	1.4
23—S. S.	26	46	..	1	F	Faintly alk...	0	...	0	..
	4	26	F	Faintly alk...	0	0.6	0	0.0

GROUP IV. MISCELLANEOUS CASES

24—J. F.	20	12	F	Faintly alk...	40	2.9	10	3.0
	15	15	E	Faintly alk...	100	1.2	10	3.4
25—W. D.	3	10	F	Neutral	40	0.3	3	3.0
	3	3	E	Neutral	100	...	2	3.5
26—M. G.	12	30	5	10	F	Neutral	10	1.9	10	5.3
	0	12	5	8	F	Neutral	20	1.8	10	4.5
27—L. L.	10	20	E	Neutral	40	0.8	20	5.4
	10	10	E	Neutral	2	1.3	4	4.3
28—A. N.	5	10	E	Neutral	40	0.4	4	2.8
	2	E	2.6
29—S. G.†....	10	10	F	Neutral	100	0.1	1	4.3
30—J. O.	7	32	7	16	F	Faintly alk...	100	1.8	10	4.5

* Test meal of milk, cream and boiled starch. † Bile absent from duodenal juice on each examination. ‡ Bile present in duodenal

Gelatin Method.—Into a small test-tube are pipetted 1 c.c. of undiluted duodenal juice, 3 c.c. of water, 1 c.c. of 0.5 per cent. sodium carbonate and 1 c.c. of toluene. Into the test-tube is then inserted two 3 cm. gelatin tubes,¹⁴ and the test-tube allowed to incubate for forty-eight hours at room temperature with occasional gentle mixing. At the end of this time the amount of digestion is measured with a millimeter scale and the measurements added together.

(e) *Bile.*—The test employed for bile has been the simple Gmelin nitric acid test, though ordinarily sufficient bile is present to make a qualitative test unnecessary. In future analyses we plan to employ modifications of the Huppert test for bile pigments, and the Liebermann-Burchard test for cholesterol as the basis of colorimetric methods for estimating these substances.

The subjects of the present study were adults of an average age of about 35 years, sixteen being males and thirteen females.

DISCUSSION OF RESULTS

As may be observed from the tabulated data, the activities of the pancreatic enzymes show some little variation from day to day under normal conditions. Similar observations were made by Einhorn and Rosenbloom and by Crohn. The greatest variation was noted in the amylase and lipase, and was apparently dependent in part on the initial reaction of the juice. The favorable reaction for these two enzymes is quite opposite, neutral or faintly alkaline for amylase and faintly acid for lipase. The activity of the trypsin was more constant. In Patient 21 — A. O'C. — the tryptic activity was quite uniformly 4 over a period of several days, whereas the activity of the amylase varied from 40 to 200, and that of the lipase from 0.6 to 2.2. In the entire series, the amylolytic activity ranged from 5 to 200, the lipolytic activity from 0.3 to 3.8 and the proteolytic from 0.5 to 10 (casein method) and 1.4 to 5.6 (gelatin method).

It may be noted in Case 4 — J. L. — with pernicious anemia, that though there was an almost complete absence of gastric juice, the duodenal juice was very active, especially the trypsin (gelatin test). In Case 29 — S. G. — with infective jaundice, the lipolytic activity was very low, 0.1. The activity of the pancreatic enzymes was normal in the case of hypertrophic cirrhosis of the liver, 25 — W. D. In Group II, Case 9 — R. L. — with alcoholic gastritis, the juice was found to be strongly acid. This was believed at first to have been due to an error in technic, but the active lipase and the good digestion of gelatin on the one occasion when the acidity was comparatively low, would indicate otherwise. The results suggest an inability on the part of the pancreatic juice to neutralize the acid of the gastric juice. The results in Case 12 — A. B. — with gastric ulcer (gastro-enterostomy), are in striking contrast. Here the free gastric

14. The gelatin tubes were prepared by dissolving 10 gm. of gelatin and 1 gm. of sodium fluorid in boiling distilled water, deeply coloring with a clear solution of cochineal, making up to 100 c.c. and then filling glass tubing of 2 mm. inside diameter. The tubes 3 cm. in length are cut just previous to use.

acidity was 90 and the total acidity 105. The duodenal juice in this case was decidedly alkaline to phenolphthalein. The lipolytic activity was weak, but the proteolytic activity very strong. When this is considered, together with the large volume of juice obtained, it would appear to indicate that the pancreas had responded to the stimulus of the high gastric acidity. From the tabulated data it seems evident, however, that the acidity or lack of acidity of the gastric juice is without definite influence on the enzyme activity of the pancreatic juice, although a strongly acid gastric juice is apparently able in most cases to stimulate the production of a sufficiently alkaline duodenal secretion to neutralize it.

The normal variation in the activity of the pancreatic enzymes is so great, as evidenced by the data tabulated above, that only an absence of the enzymes would appear to render the juice of diagnostic value. The absence of pancreatic enzymes in the duodenal juice, would, however, be positive evidence of either pancreatitis or of non-potency of the pancreatic ducts, while the lack of bile in the juice would be pathognomonic of the occlusion of the common bile duct. By means of an analysis of the duodenal juice, the surgeon should in certain cases be able to determine the involvement or non-involvement of the pancreas prior to operating on the gall-bladder or gall-ducts.

In the case of chronic pancreatitis, 23 — S. S. — the enzyme activity of the duodenal juice was negative, except for 0.6 of lipase. The reaction of the juice was favorable for the detection of amylase and trypsin — faintly alkaline — and sufficient material, 26 c.c., was available for very careful examination. The stools were typical of pancreatitis. This coincides with the results obtained by Crohn in his case of pancreatitis, though it should perhaps be noted that there the fluid was acid.

In the case of carcinoma of the gall-ducts and the pylorus, 22 — S. Z. (diagnosis confirmed by operation) — the pancreatic ducts were not involved as shown by the active enzymes. (The absence of amylase was probably due to the reaction of the juice, faintly acid.) Bile could not be detected in any of the four specimens obtained.

CONCLUSIONS

Active amylolytic, lipolytic and proteolytic enzymes are present in duodenal juice, though the activity of these enzymes is apparently subject to considerable variation under normal conditions.

The acidity of the gastric juice appears to be without influence on the activity of the enzymes present in the duodenal juice.

In a case of carcinoma of the gall-ducts and pylorus with biliary obstruction, there was an entire absence of bile from the duodenal juice. In a case of chronic pancreatitis, the amylolytic and proteolytic activity was entirely negative, while the lipolytic activity was comparatively weak.

The absence of pancreatic enzymes from the duodenal juice would appear to be positive evidence of either pancreatitis or non-potency of the pancreatic ducts, while the lack of bile would appear to afford similar evidence of the occlusion of the common bile duct. Further observations on these conditions are desirable.

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