PANCREATIC JUICE IN NORMAL INDIVIDUALS AND IN SPRUE

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Recently there has been a wide discussion on the character of the gastric and duodenal contents in sprue. In reviewing the literature, several investigators are found to have studied this problem. Bahr(1), in his Ceylon cases, found either diminution in the amount, or entire absence of the pancreatic ferments. T. R. Brown(2) found entire absence, of pancreatic ferments or these existed in minimal amounts falling within the limits of experimental error in his two cases from Porto Rico and three from the Philippines. Silverman and Denis(3) found the pancreatic enzymes to be present to a considerable extent in the duodenal contents. Pratt and Spooner(4) at first reported pancreatic insufficiency in sprue; later, in another case, they failed to corroborate their first observations. Sokhey and Malandkar(5) came to the conclusion that there is no insufficiency of the pancreas. Ashford(6) and myself have found a reduction in pancreatic ferments.

These discordant findings in many cases are due to the differences in the methods of analysis. Referring to Sokhey and Malandkar's work let it be said that while they have used the same methods that we have used, they introduce a modification which would seem to explain the difference in results, as will be described later. These authors seem not to have taken into sufficient consideration the physiology of the gastro-intestinal tract and we should like to comment on this phase of the subject.

Dolinsky (1895) discovered that acids brought into contact with the mucous membrane of the duodenum promptly set up a secretion of pancreatic juice. Since this discovery it has been believed that the acid gastric juice is what serves to initiate the flow from the pancreas. As soon as the acid contents of the stomach begin to pass through the pylorus this action commences. Assuming that the pancreatic gland possesses secretory nerve fibers, it was thought at first that the acid stimulates these fibers reflexly, that is, the acid in the duodenum, acting upon sensory endings, causes a reflex stimulation of the efferent secretory fibers. It has been shown that the

same effect takes place after section of the vagus and splanchnic nerves. But Bayliss and Starling have called attention to another and more probable explanation. These authors find that if the mucous membrane of the duodenum is scraped off and treated with 0.4 per cent hydrochloric acid, the extract thus made, when injected into the blood, sets up an active secretion of pancreatic juice. They have shown that this effect is due to a special substance, secretin, which is formed by the action of the acid upon some substance (prosecretine) present in the mucous membrane. Secretin is not an enzyme, since its activity is not destroyed by boiling nor by the action of alcohol. The experimental evidence at present favors the view that the normal sequence of events is as follows: The acid of the gastric juice upon reaching the duodenum brings about the production of secretin; this in turn is absorbed by the blood, carried to the pancreas, and stimulates this organ to activity. The pancreatic secretion furnishes, therefore, another example of the group of substances designated by Starling as *hormones*. According to the evidence at present, we must conclude that the pancreatic secretion, like the gastric juice, consists of two parts: First, a nervous secretion caused by the stimulation of the secretory fibers in the filaments of the vagus and splanchnic; second, a chemical secretion due to the action of secretin. These two secretions are said to present quite different characters. The former is thick, opalescent, rich in ferments and proteins, but poor in alkalies. The trypsin contained in it may be secreted in active form, and the secretion is suspended by the action of atropine. Administration of pilocarpine, on the contrary, excites this secretion. The chemical secretion, on the other hand is thin and watery, contains relatively little ferment or proteins and is rich in alkali. The trypsin in it is secreted in an inactive form, and the secretion is not affected by the administration of atropine. The normal relation of these two forms of secretion in an ordinary meal is not so apparent as in the case of the gastric secretion. Nothing is known of its nature or the point of departure of the afferent stimuli responsible for it, but assuming its existence as a part of the normal machinery we may infer that the nervous reflex is preparatory and provides for a flow of pancreatic juice in the earlier stages of intestinal digestion, while the action of the secretin serves to maintain this flow until the stomach is completely emptied.

In sprue, it has been demonstrated that the entire nervous system, particularly the autonomic nervous system, is functionally altered, and therefore the nervous reflexes that control the flow of pancreatic

juice are also probably altered. If we have a diminution in quantity of pancreatic juice do we also have a diminution of the enzymes? Let us consider first the enzyme action of the pancreatic ferments. Sherman and Schlesinger(7) have demonstrated in their work on amylases, that 1:100,000,000 in a one per cent starch solution converted 100,000 times its weight of starch to the erythrodextrin stage in thirty hours and within ninety-six hours had completely digested the starch and intermediate dextrins to products giving no reaction with iodine and had formed over 500,000 times its weight of reducing sugar calculated as maltose. So when the relationship between the extent of transformation and the time which may chance to obtain in a given instance of an enzymic hydrolysis, one quantitative relationship remains invariably true, namely, that *the time required to attain a given amount of transformation of the substrate is inversely proportional to the concentration of the enzyme.* There appears to be no deviation from this rule which is not immediately explicable by decomposition of the enzyme or such adventitious factors as fluctuation of temperature, reaction, etc.

As the digestion of the chyme in the duodenum persists for approximately one half hour, that is why when testing the pancreatic enzymes in vitro, the periods of digestion must conform to the period of digestion in the human body. For this reason the Gross method for determining the proteolytic enzymes, as well as the Wohlgemuth method for determining the diastase activity have been used. The Gross method reads as follows:

Prepare a series of tubes containing 10 cc of a 0.1 per-cent solution of pure, fat-free casein which has been heated to a temperature of 40° C. Add to the contents of the series of tubes increasing amounts of pancreatic juice under examination and leave them at 40° C for 15 minutes. At the end of this time remove the tubes and acidify the contents of each with a few drops of diluted acetic acid (1 per-cent). The tubes in which the casein is completely digested will remain clear when acidified, while those tubes which contain undigested casein will become more or less turbid under these conditions. Select the last tube in the series which exhibits no turbidity upon acidification, thus indicating complete digestion of casein, and calculate activity of the enzyme solution under examination.

Calculation: The unit of tryptic activity is an expression of the power of 1 cc of the fluid under examination, exerted for a period of 15 minutes, to digest the casein in 10 cc. of 0.1 per-cent casein solution. For example, if 0.5 cc of the pancreatic juice completely digests 10 cc of 0.1 per-cent casein solution in 15 minutes, the activity of the solution would be expressed as follows:

$$\text{Tryptic activity} = 1 \div .5 = 2$$

Such a trypsin solution or pancreatic juice would be said to possess an activity of 2. etc., according to the dilution.

Sokhey and Malandkar have modified this method, using 5 cc. of 0.1 per-cent casein solution and an incubation period of *two hours*. Of course, having one half the quantity of casein solution and an incubation period of two hours, any small quantity of the trypsin present will digest all the casein solution in any reasonable proportion, as has been already demonstrated.

The Wohlgemuth method for diastase activity reads as follows:

Arrange a series of test tubes with diminishing quantities of the enzyme solution under examination. Introduce into each tube 5 cc of 1 per-cent solution of soluble starch and place each tube at once in a bath of ice-water. When all the tubes have been prepared in this way and placed in the ice-water bath, they are transferred to a water-bath or incubator and kept at 38° C for 30 minutes. At the end of this digestion period the tubes are again removed to the bath of ice-water in order that the action of the enzyme may be stopped.

Dilute the contents of each tube with water to within a half inch of the top, add a drop of N/10 solution of iodine and shake the tube and contents thoroughly. A series of colors ranging from dark blue through bluish-violet and reddish-yellow will be formed. The dark blue color shows the presence of unchanged starch, the bluish-violet indicates a mixture of starch and erythrodextrin; whereas the reddish yellow signifies that erythrodextrin and maltose are present, and the yellow solution denotes the complete transformation of starch to maltose. Examine the tubes carefully before a white background and select the last tube in the series which shows the entire absence of all blue color, thus indicating that the starch has been completely transformed into dextrin and sugar.

Calculation: The amylolytic activity of a given solution is expressed in terms of the activity of 1 cc of such a solution. For example, if it is found that 0.02 cc of an amylolytic solution, acting at 38° C, completely transformed the starch in 5 cc of a 1 per-cent solution in thirty minutes, the amylolytic activity of such a solution would be expressed as follows:

$$\frac{D \times 38^{\circ}}{30} = 250$$

This indicates that 1 cc of the solution under examination possesses the power of completely digesting 250 cc of 1 per-cent solution in thirty minutes at 38°.

Sokhey and Malandkar have used these methods with the exception that they incubate for a period of two hours, thus giving ample time for digestion in vitro of any reasonable quantity of starch, as has already been demonstrated.

For lipase we have used the ethylbutyrate method. This method reads as follows:

Place 1 cc of alkalinized duodenal contents in each of two large test tubes. Boil the contents of one, and cool. To each tube now add 1 cc of ethylbutyrate,

10 cc of water and 1 cc of toluene. Shake well, and incubate at 37° C for 24 hours; shaking several times in the interval. Then titrate acidity, using N/10 NaOH and phenolphthalein. The difference (in cubic centimeters) between the results of the two titrations represents the lipase. The normal being considered to be 0.2 — 2.0.

Sokhey and Malandkar employ the Kanitz method of using olive oil. This method reads as follows:

Five cc of olive oil (Heinz) with 2 cc of duodenal contents were incubated for 2 hours. Fatty acids produced were titrated with N/10 NaOH using phenolphthalein as indicator.

In order to illustrate our thesis we shall give the analyses of normal duodenal contents as well as of duodenal contents of sprue patients which have been worked out by the two methods.

HEALTHY INDIVIDUALS

DUODENAL ANALYSES—TRYPSIN ACTIVITY

GROSS METHOD

Case	Name	1 cc	0.5 cc	0.25 cc	0.1 cc	0.05 cc	0.005 cc
1	J. A. L.	clear....	clear....	clear....	clear....	clear....	clear
2	H. D.	clear....	clear....	clear....	clear....	clear....	clear
3	A. F.	clear....	clear....	clear....	clear....	clear....	clear
4	R. L. I.	clear....	clear....	clear....	clear....	clear....	clear
5	A. B.	clear....	clear....	clear....	clear....	clear....	clear

SOKHEY AND MALANDKAR'S MODIFICATION

Case	Name	1 cc	0.5 cc	0.25 cc	0.1 cc	0 05 cc	0 005 cc
1	J. A. L.	clear....	clear....	clear....	clear....	clear....	clear
2	H. D.	clear....	clear....	clear....	clear....	clear....	clear
3	A. F.	clear....	clear....	clear....	clear....	clear....	clear
4	R. L. I.	clear....	clear....	clear....	clear....	clear....	clear
5	A. B.	clear....	clear....	clear....	clear....	clear....	clear

SPRUE PATIENTS

DUODENAL ANALYSIS—TRYPSIN ACTIVITY

GROSS METHOD

Case	Name	Quantities of Duodenal Contents					
		1 cc	0.5 cc	0.25 cc	0.1 cc	0.05 cc	0.005 cc
1	A. M.	clear....	clear....	clear....	clear....	opaque..	opaque
2	D. G.	clear....	clear....	clear....	opaque..	opaque..	opaque
3	J. H. P.	clear....	clear....	clear....	clear....	opaque..	opaque
4	B. H.	clear....	clear....	clear....	clear....	opaque..	opaque
5	J. O.	clear....	clear....	opaque .	opaque..	opaque..	opaque
6	M. C.	clear....	clear....	clear....	opaque..	opaque..	opaque
7	P. V.	clear....	clear....	clear....	opaque..	opaque..	opaque

DUODENAL ANALYSIS

SOKHEY AND MALANDKAR'S MODIFICATION

Case	Name	Quantities of Duodenal Contents					
		1 cc	0.5 cc	0.25 cc	0.1 cc	0 05 cc	0.005 cc
1	A. M.	clear	clear	clear	clear	clear	clear
2	D. G.	clear	clear	clear	clear	clear	clear
3	J. H. P.	clear	clear	clear	clear	clear	clear
4	B. H.	clear	clear	clear	clear	clear	clear
5	J. O.	clear	clear	clear	clear	clear	clear
6	M. C.	clear	clear	clear	clear	clear	clear
7	P. V.	clear	clear	clear	clear	clear	clear

NORMAL INDIVIDUALS

DIASTASE ACTIVITY—WOHLGEMUTH'S METHOD

Case	Name	1 cc	0.5 cc	0.25 cc	0 1 cc	0.05 cc	0 005 cc
1	J. A. L.	clear	clear	clear	clear	clear	clear
2	H. D.	clear	clear	clear	clear	clear	clear
3	A. F.	clear	clear	clear	clear	clear	clear
4	R. L. I.	clear	clear	clear	clear	clear	clear
5	A. B.	clear	clear	clear	clear	clear	clear

SOKHEY AND MALANDKAR'S MODIFICATION

Case	Name	1 cc	0.5 cc	0 25 cc	0.1 cc	0 05 cc	0.005 cc
1	J. A. L.	clear	clear	clear	clear	clear	clear
2	H. D.	clear	clear	clear	clear	clear	clear
3	A. F.	clear	clear	clear	clear	clear	clear
4	R. L. I.	clear	clear	clear	clear	clear	clear
5	A. B.	clear	clear	clear	clear	clear	clear

SPRUE PATIENTS

DIASTASE ACTIVITY—WOHLGEMUTH'S METHOD

Case	Name	Quantities of Duodenal Contents					
		1 cc	0.5 cc	0.25 cc	0.1 cc	0.05 cc	0 0005 cc
1	M. A.	clear	clear	clear	bluish-violet	bluish-violet	blue
2	D. G.	clear	clear	clear	clear	clear	bluish-violet
3	J. H. P.	clear	clear	clear	clear	clear	clear
4	B. H.	clear	clear	clear	blue	blue	blue
5	J. O.	clear	clear	clear	violet-blue	violet-blue	blue
6	M. C.	clear	clear	clear	violet-blue	violet-blue	violet-blue
7	P. V.	clear	clear	clear	clear	clear	clear

SOKHEY AND MALANDKAR'S MODIFICATION

Case	Name	Quantities of Duodenal Contents					
		1 cc	0.5 cc	0.25 cc	0.1 cc	0.05 cc	0.005 cc
1	A. M.	clear	clear	clear	clear	clear	clear
2	D. G.	clear	clear	clear	clear	clear	clear
3	J. H. P.	clear	clear	clear	clear	clear	clear
4	B. H.	clear	clear	clear	clear	clear	clear
5	J. O.	clear	clear	clear	clear	clear	clear
6	M. C.	clear	clear	clear	clear	clear	clear
7	P. V.	clear	clear	clear	clear	clear	clear

NORMAL PERSONS

LIPASE

ELTHYLBUTYRATE METHOD

Case	Name	Quantity
1	J. A. L.	2.2
2	H. D.	1.9
3	A. F.	2.7
4	R. L. I.	1.8
5	A. B.	0.9

/SPRUE PATIENTS

LIPASE

ETHYLBUTYRATE METHOD

Case	Name	Quantity
1	M. A.	0.11
2	D. M.	0.48
3	J. H. P.	0.24
4	B. H.	1.5
5	J. O.	0.1
6	M. C.	1.2
7	P. V.	0 2

SOKNEY AND MALANDKAR'S

OLIVE OIL METHOD.

Case	Name	Quantity of cc of N/10 NaOH to neutralize
1	M. A.	27.3
2	D. M.	19.6
3	J. H. P.	0.7
4	B. H.	2.5
5	J. O.	4.7
6	M. C.	6.7
7	P. V.	6.2

Sprue is said by Ashford to be an exhaustive process and he has produced evidence of physiologic exhaustion of the bone marrow, corroborated by pathologic sections. He believes that this physiologic exhaustion involves the pancreas and that the function of enzyme production is reduced and intermittent. In this case, the presence of even a normal amount of the enzymes at any given time might be followed by much longer periods of lessened amounts or even total absence. Such abrogation of function is profoundly affected by psychic conditions working through a disordered autonomic nervous system and the same statement may be applied to other organs of digestion. For these reasons one analysis of duodenal contents would not seem to be enough to determine the integrity of the pancreatic function, and only a large number of cases will ever establish the average value of pancreatic secretions in sprue.

SUMMARY AND CONCLUSIONS

A review of the pancreatic digestion is made. The mechanics of enzyme action is taken into consideration. Comparison is made of the digestion, in vivo, and in vitro. The methods used are described. Comparison is made of the Gross and Wohlegemuth's Methods with modification made by Sokhey and Malandkar in which they do not take into consideration the enzyme activity and periods of digestion. Sokhey and Malandkar prove the enzyme activity, and not the amount of enzyme present.

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