Pancreatin with High Enzymatic Activity*,†

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A simple method of preparing pancreatin with high lipase content is outlined. An analysis, by 50 independent workers, of the pancreatin prepared by this method shows that it contains also over three times the amount of trypsin and of amylase required by the U. S. P. Thus it is an easily prepared "triple strength" pancreatin of high lipase content.

THE APPEARANCE on the market of "triple strength" pancreatin has aroused much interest in the method of its preparation. A study of several of these preparations shows that their amylase and trypsin content is three times that required for pancreatin, U. S. P. (1).

Since the literature does not give a method for preparing triple strength pancreatin in which the lipase content also is high, an investigation of this problem seemed timely. Furthermore, if such a preparation were to be used medicinally, it seemed that the effect of the stomach juices upon the lipase should be investigated.

There is given in this paper a method for making a pancreatin preparation which has a high lipase, trypsin, and amylase content. In addition, there is the evidence that shows that the acidity of the stomach irreversibly inactivates the lipase. As a result it appears that triple strength pancreatin must be enteric coated in order to get lipase activity in the intestines.

The lipase content of hog intestine was also investigated to see whether an intestinal extract that would contain more lipase than is found in pancreatin could be made from it. The results of this investigation were negative.

It appears that the pancreas is the principal source of lipase in the gastrointestinal tract and any dysfunction of the pancreas may hinder the secretion of pancreatic lipase, thus interfering with the proper digestion and absorption of fats.

HISTORICAL

In 1846, Claude Bernard (2) first observed the emulsifying action of pancreatic lipase on fat. In 1858, Marcet (3) found that lipolysis of neutral fat takes place in the stomach. Twenty-two years later, Cash (4) confirmed this finding, and also showed that extracts of the gastric mucosa could bring about fat digestion in the test tube. Ogata (5) confirmed these results. However, later authors rejected them. Volhard (6) showed that only emulsified fats are readily digested by gastric lipase.

The pancreas also contains esterases capable of splitting nonglyceryl esters, as shown by Balls and Matlack (7) in 1938 and Bauer and Masucci (8) in 1948.

In 1885, Pelouze (9) found lipases in flax seed and also in the seeds of colza, mustard, and poppy. Ricinus lipase was discovered in 1890 by Green (10) in the germinating seeds of the castor bean (*Ricinus communis*). Green found that this plant enzyme is a typical lipase, hydrolyzing true fats, similar to pancreatic lipase.

EXPERIMENTAL

Investigation of Hog Intestine for Lipase

It was suspected that the intestine might be a good source of lipase, since so much of the digestion takes place there. Although the literature states that the pancreas is the richest source of lipase, it was felt that perhaps there exists in the intestine, along with some active lipase, a lipasogen, or precursor of lipase, which could in some way be activated. A study of this problem might, it was felt, result in the finding of a new and rich source of lipase.

First, the intestine of a hog was assayed for lipase content. Any possible variation in lipase concentration throughout the length of the intestine was checked by the following method: a cleaned intestine was cut into seven pieces of approximately equal length. Each piece was numbered, beginning with number one for the lower end of the ileum, that leads into the caecum, and so on up to number seven, the proximal end of the duodenum. Each piece was also marked so that its upper and lower ends could be identified. Thus, if a concentration of lipase were to be discovered, its exact position in the intestine could be easily determined.

The method of assay of Willstätter, Waldschmidt-Leitz, and Memmen, as modified by Bauer and Wilson (11), was used.

Approximately equal-sized pieces of intestine were cut from the distal end of each of the seven portions, and each piece was weighed. They were then treated as follows:

Each piece was triturated in 50 cc. of water with some washed sand. Ten cubic centimeters of the resulting suspension was used in place of the 10 cc. of pancreatin suspension mentioned in the assay method. Four 10-cc. portions of each of the seven samples, labeled sample A, control A, sample B, and control B, were assayed. In the controls the enzyme activity was destroyed by heat (11). In all the following charts the average of sample A and sample B is given; the same is true of control A and control B. Since a preliminary assay had showed that the

* Received April 30, 1949, from the Massachusetts College of Pharmacy, Boston, Mass.
Presented to the Scientific Section, A. PH. A., Jacksonville meeting, April, 1949.
† This paper is based on a thesis presented to the Graduate Council of the Massachusetts College of Pharmacy by A. John Vazakas in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.
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active lipase content was disappointingly low, all samples were assayed at a constant temperature of 40° instead of 30°, and for a period of two hours instead of the one hour called for in the assay, so that any marked difference in the degree of concentration would be more obvious.

The results are shown in Table I. In no case was the deviation in any duplicate set of assays greater than 0.5 cc.

TABLE I.—THE LIPOLYTIC ACTIVITY OF 8 GM. OF SAMPLE TAKEN FROM SEVEN SEGMENTS OF THE INTESTINE

Sample No.	Cc. of 0.1 *N* Alc. KOH Used by: Sample	Control	Activity in Cc. of 0.1 *N* Alc. KOH
1	18.6	18.0	0.6
2	17.8	17.2	0.6
3	17.4	16.45	0.95
4	18.3	18.0	0.3
5	19.5	18.45	1.05
6	20.1	18.25	1.85
7	19.8	18.9	0.9

An Attempt at Activation of Intestinal Lipasogen

It was thought that the pancreas might contain an activator for any lipase that was present as a lipasogen in the intestine. Consequently, a hog's pancreas and intestine were obtained, and 5 Gm. of each was assayed for lipase. The pancreas had a high degree of activity, whereas the intestine had a low degree. Then about 5 Gm. of each sample was taken. The two samples were triturated together according to the method of assay previously described, and the mixture was assayed. If any activation were to occur, the result of this assay would be higher than the sum of the results of the two separate assays. However, it was not higher, for the result of this assay was about the same as the total of the two results obtained when the pancreas and the intestine were assayed separately. Thus, even if the intestine did contain a lipasogen, it was not activated by fresh pancreas.

The results are shown in Table II. In no case was the deviation in any duplicate set of assays greater than 1.6 cc.

TABLE II.—THE AVERAGE LIPASE CONTENT OF 5 GM. OF INTESTINE, 5 GM. OF PANCREAS, AND OF A MIXTURE OF 5 GM. EACH OF INTESTINE AND PANCREAS

Sample	Cc. of 0.1 *N* Alc. KOH Used by: Sample	Control	Activity in Cc. of 0.1 *N* Alc. KOH
Pancreas	40.1	17.7	22.4
Intestine	14.9	13.9	1.0
Intestine and pancreas	39.0	18.8	20.2

Temperature and Time Suitable for Determination of Lipase Content of Hog's Pancreas

Since there was no high degree of lipase action from any portion of the intestine, and since the attempt to activate intestinal lipasogen failed, it was decided to abandon attempts to make a rich lipase preparation from the intestine; it was decided to attempt to make a pancreatin of high lipase content.

Assays were run on two pieces of fresh pancreas. One assay was run at 30° for one hour, and the other at 40° for two hours, in order to ascertain what effect an increase in temperature and in digestion time might have. The purpose of this assay was to determine at what temperature and for what period of time future assays should be run.

The average lipolytic activity, based on 10 Gm. of sample, was as follows:

At 30° for one hour..... 33.3 cc. of 0.1 *N* KOH
At 40° for two hours.... 65.0 cc. of 0.1 *N* KOH

The results above showed that there was no need to run the assays for longer periods of time than called for in the assay of Bauer and Wilson (11), or at a temperature of more than 30°, as was done in the assay of the intestines. A digestion period of one hour at 30° was sufficient to show the lipase activity of the pancreas.

The remaining portion of the assayed pancreas was then placed in toluene, for preservation. However, when it was assayed again three weeks later, a great drop in activity was noted. Evidently toluene does not preserve lipase. The result of this assay is seen in Table III.

TABLE III.—LIPOLYTIC ACTIVITY OF 10 GM. OF PANCREAS PLACED IN TOLUENE FOR THREE WEEKS, (ITS ACTIVITY WHEN FRESH HAD BEEN 33.3 CC. OF 0.1 *N* POTASSIUM HYDROXIDE)

	Cc. of 0.1 *N* Alc. KOH Required	Lipolytic Activity in Cc. of 0.1 *N* Alc. KOH	Av. Lip. Activity
Sample A	51.5	14.8	14.9
Control A	36.7		
Sample B	52.5	15.0	
Control B	37.5		

Search for a Lipase Activator

Various workers had been unable to agree whether alcohol activated or inactivated pancreatic lipase. It was decided to use various strengths of ethyl alcohol to see whether any lipase activation could be produced. Fifty per cent alcohol has been used to inhibit trypsin activity in the manufacture of insulin. Since trypsin destroys not only insulin, but also lipase, it was felt that storing the pancreas in alcohol would help obtain maximum lipolytic activity.

Determination of Lipase Content in Different Parts of the Pancreas.—In the assay just mentioned it was assumed that lipase is present uniformly throughout the pancreas. Before any further work was done, it was decided to assay a pancreas to determine whether or not it is.

A fresh pancreas of a hog was obtained and was cut transversely into four approximately equal parts. These parts were then assayed in the usual manner. The results, on a basis of a 10-Gm. sample, are shown in Table IV.

In no case was the deviation in any duplicate set of assays greater than 2.6 cc.

Table IV shows that a fairly uniform distribution of lipase existed throughout the pancreas, and that

TABLE IV.—THE LIPOLYTIC ACTIVITY OF A PANCREAS ASSAYED IN FOUR 10-GM. SEGMENTS

Segment No.	Cc. of 0.1 N Alc.-KOH Used by: Sample	Control	Activity in Cc. of 0.1 N Alc. KOH
1	45.2	17.1	28.1
2	51.4	20.7	30.7
3	48.2	20.7	27.5
4	57.1	26.6	30.5

under that assumption, this investigation might proceed.

Some Experiments to Discover the Effect of Various Strengths of Alcohol on Lipase.—It was decided to assay some fresh pancreases of hogs; to tabulate the lipase content; and then to place these pancreases in various strengths of alcohol to see whether any particular strength of alcohol would cause activation of lipase to any marked degree. The results of the assays done to determine the lipase content of the fresh pancreases are shown in Table V.

TABLE V.—THE LIPOLYTIC ACTIVITY OF FOUR 5-GM. SAMPLES TAKEN FROM FOUR FRESH PANCREASES

Pancreas No.	Cc. of 0.1 N Alc.-KOH Used by: Sample	Control	Activity in Cc. of 0.1 N Alc. KOH
1	22.1	15.9	6.2
2	42.0	14.9	27.1
3	36.4	15.0	21.4
4	22.1	15.2	6.9

In no case was the deviation in any duplicate set of assays greater than 4.4 cc.

When the activity of each pancreas had been recorded, the pancreases were placed in alcohol solutions of various strengths, as follows:

Pancreas 1 . . . One-half placed in 20% alcohol
One-half placed in 40% alcohol
Pancreas 2 . . . One-half placed in 60% alcohol
One-half placed in 80% alcohol
Pancreas 3 . . . Placed in undiluted alcohol
Pancreas 4 . . . Placed in 50% alcohol

Five days after the first assay the samples were assayed again for lipolytic activity. The results are shown in Table VI. In no case was the deviation in any duplicate set of assays greater than 3.8 cc.

The results in Table VI show that there was a slight increase in the lipolytic activity of the pancreas which had been placed in the 20% alcohol for the five-day period. The lipolytic activity of all the others had become less.

TABLE VI.—THE LIPOLYTIC ACTIVITY OF 5 GM. OF EACH SAMPLE FIVE DAYS AFTER IT HAD BEEN PLACED IN ALCOHOL

Sample No.	Cc. of 0.1 N Alc.-KOH Used by: Sample	Control	Activity in Cc. of 0.1 N Alc. KOH
1—20%	23.8	15.0	8.8
1—40%	18.1	14.5	3.6
2—60%	38.2	18.8	19.4
2—80%	41.7	18.2	23.5
3—Alc.	32.8	16.4	16.4
4—50%	19.7	15.4	4.3

Eleven days after the pancreases had been placed in alcohol, assays were run again to determine the lipolytic activity of each. The results are shown in Table VII. In no case was the deviation in any duplicate set of assays greater than 3.2 cc.

TABLE VII.—THE LIPOLYTIC ACTIVITY OF 5 GM. OF EACH SAMPLE ELEVEN DAYS AFTER IT HAD BEEN PLACED IN ALCOHOL

Sample No.	Cc. of 0.1 N Alc.-KOH Used by: Sample	Control	Activity in Cc. of 0.1 N Alc. KOH
1—20%	19.1	14.7	4.4
1—40%	18.2	15.0	3.3
2—60%	37.8	16.9	20.9
2—80%	45.2	18.5	26.7
3—Alc.	29.4	15.6	13.8
4—50%	21.8	16.1	5.7

The assays in Table VII show that after eleven days none of the samples showed any increase in lipolytic activity. In fact, all the samples showed a decrease in activity.

It is interesting to note from the figures of Tables V, VI, and VII that, although no great difference in lipase content exists in different parts of the pancreas of any one animal, a great difference does exist among the pancreases of different animals.

Preparation of a Concentrated Form of Lipase

Since all efforts to activate lipase by alcohol had failed, it was decided to proceed with the attempt to make a pancreatic preparation containing lipase in concentrated form.

The Willstätter and Waldschmidt-Leitz method (12) of making a pancreatic lipase preparation consists essentially of de-fatting the fresh pancreas by repeated washings with acetone and then of drying it with ether. The resulting dry preparation is then powdered as thoroughly as possible and is sifted. Willstätter and Waldschmidt-Leitz used this powder in their experimental work as a source of lipase. It retains its activity "for a long period of time."

This method, devised by Willstätter and Waldschmidt-Leitz, is an improvement on the method of Pottevin (13), in which alcohol and ether were used. The use of alcohol reduced the lipase content of the resulting preparation, as Willstätter and Waldschmidt-Leitz asserted, and as this investigation confirmed. Willstätter and Waldschmidt-Leitz proved this sensitivity of lipase to alcohol by preparing a sample of dried pancreas by their acetone-ether method; by then placing it in absolute alcohol for thirty minutes; by filtering with suction and drying with ether; and by then assaying it. The loss in lipolytic activity in this period of time was 14%.

The Preparation of a Rich Lipase Product by the Method of Willstätter and Waldschmidt-Leitz.—Fresh pancreases of hogs were scraped as free of fat and clinging tissue as possible. They were then put through a meat grinder four or five times, until further grinding was ineffectual. Then the glandular mass was stirred in a large mortar, 3 Kg. at a time, with a convenient amount of acetone, 1 L. at a time, until most of the fat was removed. It was then washed into large flasks with more acetone and was left to stand for several hours. The mass was then strained, and the whole process was

repeated. After the second straining, a mixture of equal parts of acetone and ether was used to wash the glandular mass. After this was done, it was washed with ether alone. By then the material was fairly gritty. It was spread upon drying paper and was dried in air. After the material was dry, it was powdered and sifted. A quantity of fibrous material remained in the sieve at the conclusion of the sifting. This material could not be pulverized. The lipolytic activity of this fibrous material was as high as that of the powdered material.

Determination of Amylase and Trypsin in the Lipase Preparation

Willstätter and Waldschmidt-Leitz had devised this method of preparing a concentrated lipase preparation so that they might have a supply of lipase constantly on hand for their experimental work. Since this method was superior to other methods of preparing pancreatin of high lipase content investigated by us, it was also of interest to determine whether this method in any way reduced the amylase and trypsin content of the pancreas. Although the method of preparation was designed to preserve as much of the lipase as possible, there is no reason why the amylase and trypsin should not be present also.

Two well-known brands of pancreatin and this new lipase preparation were assayed by the method of assay for amylase and trypsin found under "Pancreatin" in the U. S. P. XIII. It was found that the lipase preparation contained three times the amount of trypsin required by the U. S. P. and twice the U. S. P. requirement for amylase. Thus, pancreatin prepared by this method appears to be satisfactory since its amylase and trypsin content is greater than that of well-known preparations on the market today. The results of these assays are shown in Tables VIII and IX.

Although this method of making the lipase preparation is fairly easy and gives a pancreatin preparation superior to pancreatin, U. S. P., it does not seem to be in common use. The reason for this is undoubtedly that no mention has been made of its trypsin and amylase content. No reference to a commercial preparation made by this method was to be found in the literature. Although there are several triple strength pancreatin preparations on the market, the literature does not tell how they are made. Companies to whom inquiries were sent either refused to tell their method, or else referred inquiries to the United States Dispensatory (14). In the methods in the Dispensatory, alcohol, which materially reduces the lipase content of the finished product, is used.

The literature also states that glycerin may be used in the preparation of pancreatic lipase, but nowhere do the authors give the trypsin and amylase content of such a preparation. No effort was made to determine the value of glycerin in the preparing of a pancreatin of high lipase content.

TABLE IX.—AMYLASE ACTIVITY OF DIFFERENT SAMPLES OF PANCREATIN[a]

Digestion Time, Min.	No. of Assays	Color of Special Iodine Soln. — Sample I	Sample II	Sample III
1	2	Blue	Blue	Blue
2	2	Blue	Light blue	Blue
3	2	Blue	Colorless	Light blue
4	2	Blue	Colorless	Trace of blue
5	2	Blue	Colorless	Trace of reddish violet

[a] Sample I does not meet U. S. P. requirements for amylase content.
Sample II meets U. S. P. requirements and may be looked upon as double strength in amylase content.
Sample III barely meets U. S. P. requirements for amylase content.
These data are based upon the results of 10 independent workers.

Determination of Lipase Content of Various Pancreatins

The three samples of pancreatin having been compared for amylase and trypsin content, an assay for the lipase content was done to make the information about the three pancreatins complete. The assay was done by the usual method, and the results showed that the new pancreatin preparation had about twice as much lipolytic activity as did sample III, and about three times as much lipolytic activity as did sample I. The results are shown in Table X.

TABLE VIII.—TRYPSIN ACTIVITY OF DIFFERENT SAMPLES OF PANCREATIN[a]

Digestion Time, Min.	No. of Assays	Amount of Precipitate — Sample I	Sample II[b]	Sample III
10	5	+++++	+++	+++++++
15	5	+++	++	++++++
20	5	++	−	+++
25	5	+	−	++
30	5	−	−	+
35	5	−	−	−
40	5	−	−	−
45	5	−	−	−
50	5	−	−	−
55	5	−	−	−
60	5	−	−	−
65	5	−	−	−
70	2	−	−	−

[a] Key: + means precipitation. − means absence of precipitate.
Samples I, II, and III meet U. S. P. minimum requirements for trypsin content.
Sample II is a triple strength pancreatin in trypsin content.
These results are based upon the work of 62 independent workers.
[b] Sample II is the lipase preparation made by the method of Willstätter and Waldschmidt-Leitz.

TABLE X.—THE LIPOLYTIC ACTIVITY OF 300 MG. OF PANCREATIN FROM 3 DIFFERENT PANCREATINS

Sample No.	Cc. of 0.1 *N* Alc. KOH Used by: Sample	Control	Activity in Cc. of 0.1 *N* Alc. KOH
1	27.5	17.4	10.1
2	48.8	17.4	31.4
3	37.4	20.1	17.3

The Necessity for Enteric Coatings on Lipase Preparations

A method for making a pancreatin rich in lipase having been found, the next step was to determine whether the digestive juices of the stomach would inactivate pancreatic lipase, and thus make necessary the enteric coating of lipase preparations. For this assay, a *p*H of about 1.1, which is similar to that of the stomach, was created as directed under the monograph for pepsin in the N. F. VIII. For this purpose, 35 cc. of normal hydrochloric acid was mixed with 385 cc. of distilled water.

The Method of Assay.—Three hundred milligrams of pancreatin was put into 10 cc. of the prepared hydrochloric acid, and was left standing for two hours. The acid was then exactly neutralized with 0.1 *N* sodium hydroxide and the pancreatin was assayed for lipase activity. The question was whether acidity destroys the lipase irreversibly or merely inactivates it. If the lipase is not destroyed, the neutralization of the acid should cause the lipase to be active at the *p*H of its optimum activity. If destroyed, the lipase would not be brought back upon the neutralization of the acid.

A second aim of the assay was the determination of the effect of pepsin, in the presence of hydrochloric acid, on the lipase. Since pepsin is active only in an acid medium, the two-hour digestion period at room temperature would give the pepsin a chance to destroy the lipase, if it had the power to do so.

Also, in addition to the usual controls, a control would be run in which the pancreatin would be placed, not in water, but in a solution in which 10 cc. of the acid had been neutralized by the calculated amount of alkali. This control was used to insure that no interference could be caused by the sodium chloride caused by the neutralization, which might lead to a false interpretation of the result.

The Assay.—Eight samples were prepared for analysis. The first two samples consisted of 300 mg. of pancreatin in 10 cc. of the hydrochloric acid solution. After two hours at room temperature the acid was neutralized and the preparation was assayed for lipase activity.

The next two samples consisted of 300 mg. each of pancreatin and of pepsin in 10 cc. of the hydrochloric acid solution. This mixture was left standing two hours at room temperature; then the acid was neutralized and the preparation was assayed for lipase content.

The fifth sample consisted of 300 mg. of pancreatin that was left standing in distilled water for two hours, and then assayed by the method used above. This assay was done in order to ascertain whether immersion in water for two hours would materially reduce the lipolytic activity of pancreatin.

The sixth sample consisted of 300 mg. of pancreatin assayed by the usual method for lipase content to show maximum lipolytic activity.

TABLE XI.—THE EFFECT OF HYDROCHLORIC ACID UPON THE ACTIVITY OF PANCREATIC LIPASE

Sample No.	Cc. of 0.1 *N* Alc. KOH Required
1	18.2
2	18.6
3	22.0
4	22.0
5	51.7
6	23.3
7	52.7
8	50.8

In the seventh asssay, the lipase of 300 mg. of pancreatin was destroyed by heat before the pancreatin was assayed for lipase content. Thus this assay was a control.

In the final assay, 300 mg. of pancreatin was placed in a neutralized solution of the acid to show whether the sodium chloride had any effect upon this method of determination. After the two-hour digestion period at room temperature the pancreatin was assayed in the usual manner for lipolytic activity.

Discussion.—The results in Table XI show that lipase is destroyed and not reversibly inactivated by an acidity equal to that of the stomach. Whether pepsin acts upon the lipase cannot be determined by this method, since the acid must be present in order for the pepsin to act.

SUMMARY

1. Hog's intestine contains very little, if any, lipase. If any lipase is present in the form of a precursor, it is not activated by fresh pancreas.

2. Solutions of 20, 40, 50, 60, and 80 per cent of alcohol, as well as undiluted alcohol, slowly inactivate pancreatic lipase.

3. The lipase content of a hog's pancreas is fairly uniform throughout the gland, but there is a marked difference in lipase content among pancreases of different hogs.

4. A method for the manufacture of a pancreatin of high lipase, trypsin, and amylase content is outlined.

5. Lipase is irreversibly inactivated by hydrochloric acid. It must be protected from the action of stomach juices to be therapeutically effective.

REFERENCES

(1) Bauer, C. W., and White, A. K., THIS JOURNAL, **36**, 119(1947).
(2) Bernard, C., quoted from Gillespie, A. L., "The Natural History of Digestion," Charles Scribner's Sons, New York, 1898, p. 16.
(3) Marcet, *Med. Times & Gazette*, **18**, 210(1858).
(4) Cash, *DuBois' Archiv.*, **1880**, p. 323.
(5) Ogata, *DuBois' Archiv.*, **1881**, p. 115.
(6) Volhard, quoted from Stade, W., *Beitr. chem. physiol.*, **3**, 291(1902).
(7) Balls, A. K., and Matlack, M. B., *J. Biol. Chem.*, **123**, 679(1938).
(8) Bauer, C. W., and Masucci, P. E., THIS JOURNAL, **37**, 124(1948).
(9) Pelouze, J., *Compt. rend. soc. biol.*, **40**, 605(1855).
(10) Green, J. R., *Proc. Roy. Soc.*, **48**, 370(1890).
(11) Bauer, C. W., and Wilson, E. E., THIS JOURNAL, **36**, 109(1947).
(12) Willstätter, R., and Waldschmidt-Leitz, E., *Z. physiol Chem.*, **125**, 132(1923).
(13) Pottevin, H., *Bull. soc. chim.*, **35**, 693(1906).
(14) "The Dispensatory of the United States of America," 24th ed., J. B. Lippincott Co., Philadelphia, 1947, p. 816.