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Lipase methods and lipase levels in the duodenal aspirate in the newborn, infant, and child

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(Appendix I)

LIPASE METHODS

AND

LIPASE LEVELS OF THE DUODENAL ASPIRATE IN THE NEWBORN, INFANT, AND CHILD

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I. INTRODUCTION

The importance of the digestive function of the pancreas has been known since the earliest days of clinical medicine and it has also been long realized that an estimation of the ferments in the duodenal juice would yield much more useful information on excretory function of the pancreas than their determination in the feces. There was no reliable way of collecting fluid from the duodenum until Einhorn (1) in 1910 devised his tube. It was then only a year until Hess (2) developed his tube for infants and published the first pediatric reports on enzyme activity. Since then many different methods have been devised for the estimation of the pancreatic enzymes.

Questions concerning the nature and origin of the lipidolytic enzymes are problems of major interest in physiology and clinical laboratory science. The earliest work of this kind was that of Hewlett (3) in 1904, who reported that after pancreatic injury there was in the urine an increase in the content of an enzyme which splits ethyl butyrate, or an esterase; also in one case there was observed in the urine an enzyme which splits the fats of olive oil, or a lipase. Later, Von Hess (4) in 1912 and Hiruma (5) 1923, using ethyl

butyrate as a substrate, obtained evidence indicating that there is an increase in the esterase content of blood after pancreatic duct ligation. In 1932 Cherry and Crandall (6) reported that after ligation of the pancreatic ducts in dogs, they could demonstrate in blood the presence of an olive oil-splitting enzyme (lipase), which normally did not appear in the blood stream. They also found that the activity of an enzyme normally present in blood, as measured by the hydrolysis of ethyl butyrate or tributyrin (esterase), did not show uniform increases after pancreatic duct ligation. They, therefore, concluded that the appearance in the blood of an enzyme which splits olive oil (lipase) is a specific indication of pancreatic injury. They also suggested that the term "lipase" be used to designate an enzyme which splits triglycerides of long chain fatty acids, as are in olive oil, and the term "esterases" be used for an enzyme which splits simple esters of short-chain fatty acids, such as ethyl butyrate. They found, however, that tributyrin is apparently split by the same enzyme that hydrolyses ethyl butyrate.

There is, however, a consistent difficulty in evaluating and comparing the findings of the different authors because only a few of them have used the same methods of estimation and every method yields different figures

for the same enzymic values. Moreover, the values various authors give to the same, the so-called normal ranges, or to the lowest limits of these, differ more widely than in any other field of medicine. Equally diverging are the opinions of the different authors as to the lipase enzymatic level of different age groups and the method best used to measure this.

It seemed, therefore, useful to report lipase levels in cases of newborn, infant, and child age groups; and to compare some of themore commonly used comtempory methods of determining lipase, giving some of the parallel observations already reported.

II. LIPASE DETERMINATIONS

A wide variety of esters have been utilized as substrates for the measurement of the esterolytic activity. From these studies it has become increasingly clear that there are at least two distinct esterolytic enzymes, esterase and lipase. Esterase is abundant in liver, kidney, blood serum, and pancreas, preferentially hydrolyzes esters of short chain fatty acids, and is inhibited partially by sodium taurocholate (7-9). Lipase is abundant almost exclusively in pancreas, preferentially hydrolyzes esters of long chain fatty acids and its activity isaccelerated by sodium taurocholate (7-9).

Although the measurement of serum esterase has not been demonstrated to be of clinical value, the measurement of serum and duodenal lipase has been found to be useful in the diagnosis of pancreatic disease (10-11).

The majority of the commonly used methods of lipase determination employ the same principle. In most of these methods the amount of acid liberated by the hydrolysis of substrate catalyzed by lipase has been measured by titration. The most widely used method is that of Cherry and Crandall (6) which titrates with N/20 sodium hydroxide the fatty acids liberated with an olive oil emulsion.

In Willstätter's method (12) a standard olive oil with a saponification number of 185 was used. A lipase unit, therefore, would equal 19.8 ml. of a N/10 solution of potassium hydroxide. The reaction is terminated by addition of a mixture of alcohol and ether 3:1. The indicator is Thymol blue.

Rona and Michaelis' (13) stalagnometric method has been used in its original form and in the modification of Willstatter and others. Ammoniacal buffers were used for stabilization and albumin, sodium oleate and calcium chloride for activation. It's results have yielded too wide variations to be of much value, however. It was felt that possible differences in the tributyrin solutions were responsible for this.

Freudenberg's method (14) indicates the tenth units, i.e., the amount of 0.1mL of N/10 solution of sodium hydroxide required for titration of 5 ml. of cows milk incubated with lipase solution. Neutral red was used as indicator.

Archibald (15) 1946 used commercially available esters of sorbitan and fatty acids which are completely soluble in water permitting one to study the effect of substrate concentration on enzyme activity and eliminate the complications which arise with the use of emulsions. Gorori (15) has employed esters with trade names Tween 40 and Tween 60 as substrates to detect, and to localize histologically, lipase activity in sections of tissue. He reported a method of measuring quantitatively lipase activity in which an aqueous solution of a polyoxyalkylene derivativative of sorbitan monolaurate is usedas substrate. This method holds much merit although it has not gained much popularity because of technical difficulties.

The Free and Myers (16) method of lipase determination is popular using phosphate buffer, bile salts, and tributyrin as a substrate forming a semiemulsion which is required for the reaction. The reaction is stopped with alcohol and titrated with N/10 sodium hydroxide with phenophthalein as an indicator.

Other methods used are Chase and Myers (17) using

ethyl butyrate as a substrate; McClure, Wetmore, and Reynolds (18) employed cotton seed oil; and Wadsworth and Aaron (29) utilized tricacetin. In addition each author appears to have his own modification of the standard methods.

In 1934 a committee appointed by the American Gastroenterological Association (20) conducted a study of the methods used to determine the enzyme activities of duodenal contents. A report of this committee stated: "It is obvious that this survey denoted the existence of unsolved technical difficulties in pancreatic enzyme analysis, otherwise there would be more unanimity of opinion favoring one system of tests which is sufficiently accurate and simple to be routinely adopted for clinical purposes." Since that time there appears to have been little progress, as there appears yet to be little agreement as to the ideal method that will employ optimal environmental conditions, applicable to use in a routine clinical biochemical laboratory, that could be readily evaluated by the clinician.

Many different criticisms have been offered in regard to other methods of lipase determinations by various authors while defending their own method. Archibald (15) states that heretofore mostmethods for the determination of lipase activity have employed emulsions of fatty acid esters in water. These emulsions

have been stabilized by use of such agents as gum acacia. The disadvantages ofsuch methods are that 1. the emulsions break up to a considerable and variable extent during the course of the incubation, and 2. the enzyme is presumed to be in the aqueous phase, whereas the substrate is only dispersed (not in solution) in aqueous phase. Attempts to study the relation of substrate concentration to the rate of hydrolysis are complicated by the fact that the substrate and enzyme are in different phases. The rate of hydrolysis in these cases is dependentpartly on the degree of dispersion of the substrate rather than on its concentration: Confirming this observation Madock, Farber, and Shwachman (49) who reported on parallel determinations using different olive oils as substrates different values were found because of the different state of emulsion in each specific product.

Therehave been several objections raised to the use of tributyrin (24) as a substrate for lipase estimation, since it has been maintained that the digestion of tributyrin is defended by Free and Myers (16) by running 500 determinations using tributyrin and olive oil emulsion as substrates. In every one of the sample studies, the amountsof acid liberated from the tributyrin and from olive oil emulsion were practically identical. In no

instance would the clinical interpretation of the findings be any different regardless of the substrate used.

Seligman and Nachlas (21) states that because "Tween" and tributyrin are extensively hydrolyzed by esterase as well as by lipase, the mostwidely used method is that of Cherry and Crandall (6) using olive oil emulsion. Although this method may be reliable in experienced hands, technical difficulties, such as the unsatisfactory end-point in the presence of protein, instability of the oil emulsion, and occasional spontaneous hydrolysis of the oil, limit its usefulness as a routine laboratory procedure.

Wilburt Davison (22) states that hegives discouraging comments being recorded to save others the same disappointments, and also to warn them not to place too much faith in quantitative enzymic titrations. He states the following as some of the factors which often are responsible for the inaccuracies in the reports of the enzymic activity of duodenal contents. 1. Enzymes are measured by the rate of change they produce rather than by a given end-point as in other chemical determinations, and therefore errors are more likelyto occur, even when all determinations are made by the same individual. Parallel determinations rarely give the same result, so

averages of several titrations are used. But even averages of parallel titrations were far from accurate.

The reliability of enzymic titrations may be guaged more precisely by calculating the Standard Deviation and Standard Error. The Standard Deviation was calculated as the square root of the sum of the squares of the difference of the individual determinations from the mean, divided by the total number of parallel determinations. The Standard Error is the Standard Deviation divided by the square root of the total number of parallel titrations. For results to be significant, the titrations must be 3 X S.E.

2. The amount and dilution of the enzyme used are important factors in the reliability of the results. Usually a preliminary "trial and error" titrations is necessary in order to avoid too fast or too slow a reaction.

3. Variations of 1 or 2 degrees in temperature at which the enzymes are titrated change the rateof enzymic reactions. An accurately controlled water bath is necessary for comparable results.

4. Substrates greatly affect the results as has already been discussed.

5. The end-products of the reaction may interfere with the activity of the enzyme by combining with it or

rendering it inactive.

6. The position of the collecting tube in the duodenum isan important factor as specimens obtained just beyond the bend in the second portion of the duodenum may be four times as active as those collected just below the pylorus orin the terminal portion of the duodenum.

7. Changes in enzymic activity occur if duodenal contents are kept one or more days, even when on ice, with or without thymol. Usually the potency fell but occasionally it increased, probably through the action of the enzymes of infecting bacteria. The time between dilution of the enzyme and its assay also is a factor in accuracy. Titrations of fresh dilutions and of those allowed to stand for an hour or more usually are different.

In addition to theabove difficulties which affect the enzymic duodenal contents, the amount of duodenal secretion and the enzymic activity of the specimens are subject to several variables.

To judge the reliability of enzymatic titrations with ordinary laboratory technique this author, using a modification of the Cherry-Crandall method with olive oil substrate, ran 9 parallel runs using simultaneous incubation with the same duodenal drainage enzyme. Phenophthalein was used as the indicator and N/10 NaOH

as titrant. The results were as follows:

Tube No.	cc.of N/10 NaOH with enzyme		cc.of N/10 NaOH in blank		Result
1.	5.1cc.	-	3.6cc.		1.5
2.	5.1cc.	-	3.4cc.		1.7
3.	5.1cc.	-	3.4cc.		1.7
4.	4.9cc.	-	3.3cc.		1.6
5.	4.7cc.	-	2.8cc.		1.9
6.	4.7cc.	-	2.7cc.		2.0
7.	4.7cc.	-	2.6cc.		2.1
8.	4.8cc.	-	2.4cc.		2.4
9.	4.4cc.	-	1.9cc.		2.5
Averages	4.8 ± .4	-	2.9 ± 1.0	=	1.93 ± 5.7

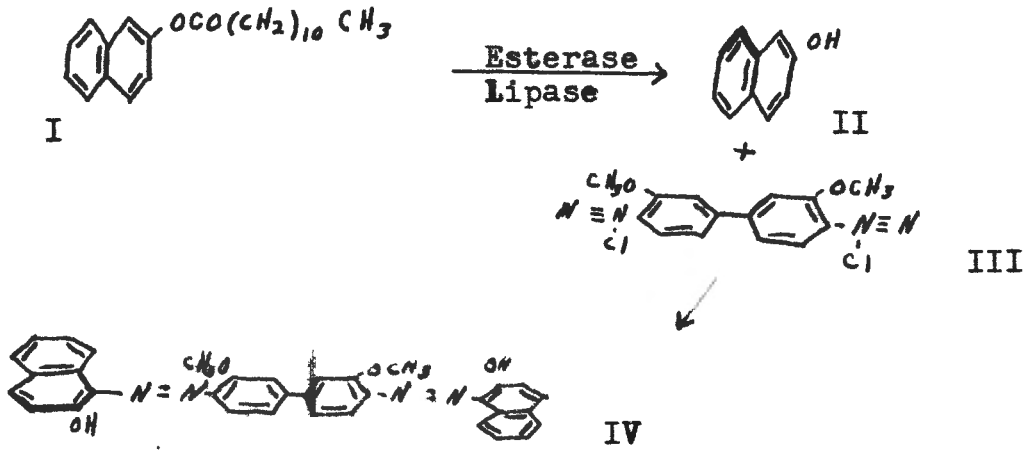
Meq. / 2 hours / cc. duodenal juice

The variations found in these parallel studies are great enough to give unreliable results to the clinician and further supports the undesirability of quantitative enzymatic titrations. The greatest source of error in these studies was thought to be caused by the difficulty in measuring accurately the exact amount of olive oil substrate and the indefinite and variable endpoint of the titration using phenophthalein as an indicator in olive oil.

A new method of lipase determination was presented by Seligman and Nachlas in 1949-50 (21,24) which is specific, sensitive, and convenient to perform. Because it ishighly recommended, gaining favor, and was used in our lipase determination the method will be gone into with some detail.

Following the development of a histochemical method for demonstrating esterase (23) with the substrate beta naphthyl acetate, long chain fatty acid esters of beta naphthol were prepared (9) and their hydrolysis by the esterolytic enzymes of several tissues in several species was explored, together with the effects of various known inhibitors and accelerators of esterase and lipase activity (9). It was then found that the lipase content could be measured accurately by these newer substrates. Increases in serum lipase after injection of mecholyl and eserine could also be readily demonstrated in dogs, particularly when lipase activity was stimulated by sodium taurocholate (24). The latter effect was so constant and striking that the substrate, beta naphthyl laurate (I) which is hydrolyzed by both esterase and lipase, can be used to measure each of the enzymes accurately by a convenient colorimetric method. The beta naphthol (II), produced by enzymatic hydrolysis, is converted to a purple azo dye (IV) by the coupling of two molecules with tetrazotized diorthoanisidine (III). This pigment is then extracted colorimetrically.

Figure I



I II III IV

A description of the method and its use in determining lipase in normal and abnormal pancreatic juice follows. The dilution of the pancreatic juice is variable by necessity because ofthe great sensitivity of the Beckman photometer. Ordinary runs use 1:2000 dilution if values under 1000 are anticipated. Exceptionally high values will require 1:4000 dilution, and abnormal ly low values dilutions of 1:1000 will give greater accuracy to results. The other variable is the amount of diluted pancreatic juice to be added during each run. In routine runs 0.1cc. is recommended, but if low values are expected 0.2cc. may be added giving higher concentrations and more accurate results. Determinations are made in duplicates if possible. With a 0.1cc pipette, diluted pancreatic juice is transferred into each of four glass stoppered centrifuge tubes, two of which contain 1.1cc of water each, and two of which contain 1cc. of sodium taurocholate solution (890 mgms. in 100cc. of water and

stored at 4°C. for as long as a month). A fifth tube containing 1.1cc of water is used asa control. The substrate solution is prepared by adding 5cc. of the stock solution of beta-naphthyl laurate (200 mgms. per 100cc. of acetone in the refrigerator stored in tightly stoppered bottle) through a submerged pipette into an agitated aqueous mixture composed of 35cc of water and 10cc of veronal buffer (0.1M, pH 7.4, made by mixing 58cc. of a solution containing 10.3 gms. of sodium diethyl-barbiturate in 500cc. of water, with 42cc. of 0.1 hydrochloric acid. Stored at 4°C., it could be used for a month, but if stored longer, or at room temperature, non-enzymatic hydrolysis of the substrate was observed). A colloidal suspension is produced which contains 0.2mgm. per cc. Five cc. of substrate is then added to each of the five tubes above, and incubation at 37°C. isallowed to proceed for one hour. To each test tube is then added 1cc. of tetrazotized diorthoanisidine, prepared immediately before use from 40 mgms. of the powder in 10cc. water. This was then followed in one minute by 1cc. of 40% trichloracetic acid solution added to each tube with agitation. The acid must be 40% concentration and its addition timed accurately following the addition of the dye as there is a progressive color formation which is stopped

by the addition of acid, 15 second differences in its addition give significant alterations in final results. The purple pigment is then extracted by vigorously shaking each tube with 10cc. of ethyl acetate. The tubes are then centrifuged for 5 minutes, and 5cc. ofeach clear red organic layer was transferred with a pipette to a colorimeter tube. The color density is then determined with a photoelectric colorimeter thru a 540Mu filter.

When lipase is present in the pancreatic juice the color density of the tubes containing taurocholate is greater than that of the tubes without it, because of stimulation of lipase activity by this substance. Thedifference in the color density between the tubes is related to the amount of lipase irrespective of the esterase content. Color density is converted to milligrams of naphthol from a calibration curve (Figure II), prepared by substituting various quantities of beta naphthol for the substrate solution, measuring them on the Beckman photometer, and carrying out the procedure outlined above.

One unit of lipase is defined as the mgms. of beta naphthol laurate liberated by 1cc. of pancreatic juice, in the presence of sodium taurocholate, from beta naphthyl laurate at 37°C. in one hour incubation, in

excess of that liberated by the esterase present in the same quantity of pancreatic juice.

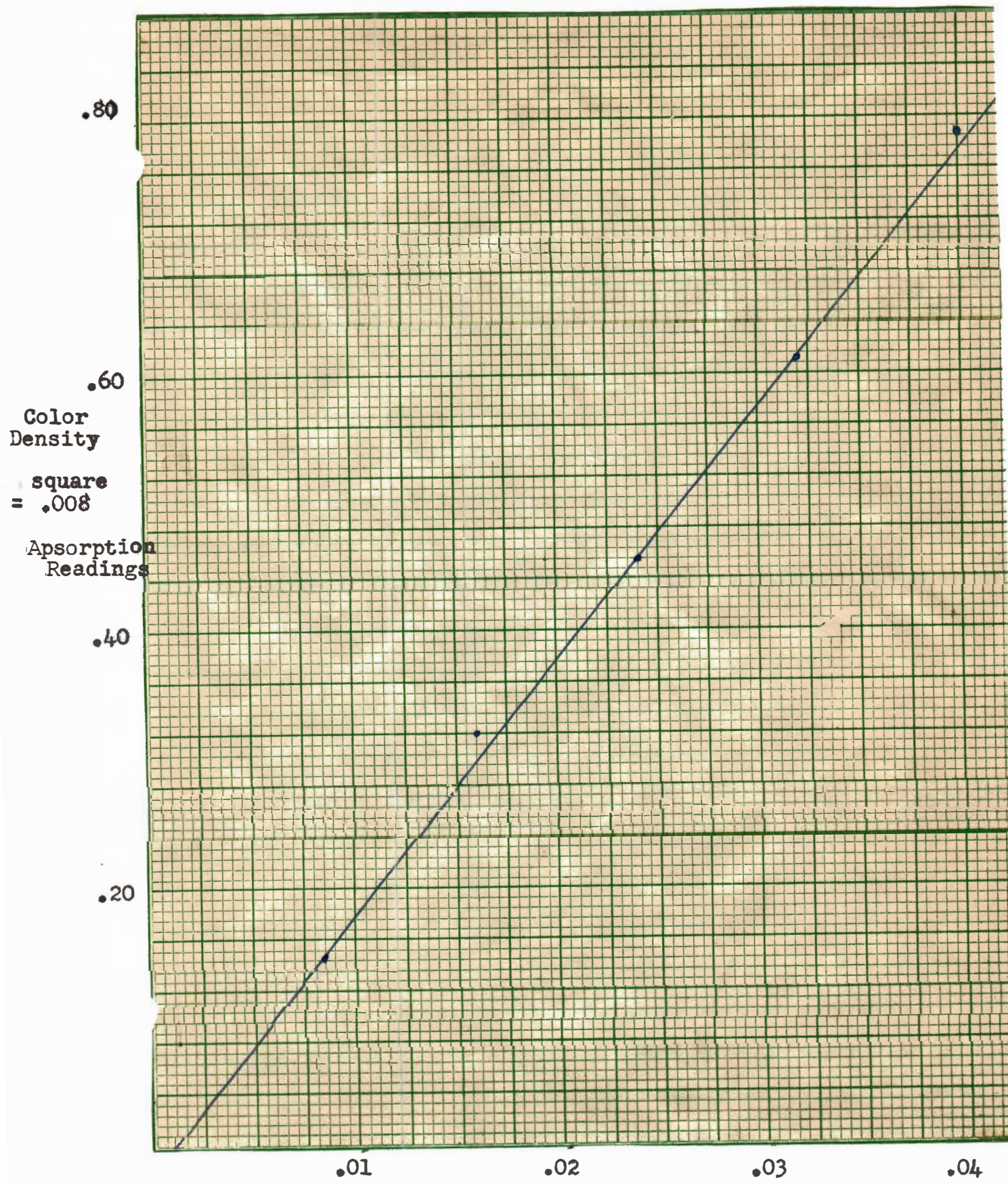
Sample calculation for a routine run is then as follows:

Mgm. beta naphthol liberated X 10 X 2000 =
mgm. beta naphthol/cc. juice / hr.

Lipase - Esterase - Blank = Lipase units.

Gibbs (62) states some confusion may possibly arise from the presence of variable amounts of bile in the specimens having effect on the lipase and esterase. A large amount of bile probably does not influence the total lipase and esterase sum but could possibly make the esterase value sum low. Esterase is a relatively small componant of the duodinal aspirate in comparison to lipase so the significance of this point is not great.

FIGURE II CALIBRATION CURVE



Fixed increments of beta naphthol were added and the prodedure carried out as described. The values are an average of four experiments using the Beckman DU spectro-photometer.

III. PANCREATIC LIPASE LEVELS IN NEWBORN, INFANT, AND CHILD

The embryologist, Needham (25) who has given the best of the role and development of enzymes in prenatal life is not very happy over the accuracy of available knowledge about these matters. Having tabulated the results of researches, up to 1931, he states that "the main bulk of the large literature on this subject enzymes in mammalian embryos has been the work of medical investigators in obstretrical clinics, using questionable methods, giving little value to the tables." In an introduction to the same chapter states, "a good deal of the work which must be mentioned in this section seems to have been inspired by the idea that, if one could get hold of the original egg-cell, no enzymes would be found to be present at all, and that they all arise, in a kind of ontogenetic procession from an ovum completely innocent of any." This idea was stated for its absurbidity to be recognized for it is more probable that no living cell can exist without an assortment of fundamental enzymes.

This conception removes much of the mystery (though not the fundamental one) as to the origin of these active principles in the infant's body, and makes it necessary only to survey what is known as to the fetal age at which digestive enzymes can first be demonstrated

with certainty. Enzymes have been demonstrated in the duodenum and intestine in the fourth fetal month (26-27) and although the sequence of their specific appearance has not been firmly established their presence at birth is generally accepted. Pancreatic lipase and and tryp - sinogen are probably present in the fetus of four months (28) and after the fifth there seems to be no doubt about any pancreatic fermentexcept amylase. This sub- stance may appear at birth, but Keene and Rewer (28) did not find it consistently then. In few prematures, infants 3-4 pounds, no amylase was found in duodenal contentsat three weeks after birth although the other pancreatic ferments appeared with almost unimpaired strength (28). Whatever be the time-table of their fetal appearance, no substantiated evidence has been brought forward indicating any critical lack of protein, sugar, or fat-splitting ferments in the intestinal juices at birth, even when the infant is born a month or more prematurely (29). As to the part played by the pancreatic enzymes, duodenal analyses have shown that lipase is not very actively formed. Ibraham (30) and Klumpp and Neale (31) report that the impotency of lipolytic activity of infants under 1 year of age is notable, as was that of amylolytic enzymes. A few cases show values so low as to be almost negligible. In children

over one year of age there is a moderate increase in activity, which maintaines a surprising uniform level throughout childhood. With the exception of the first group of children, 1-3 months, in which there isa feeble liplolytic activity, there was a uniform increase in the averages following the test meal, suggesting the secretion of a lipolytic enzymes. Smith (28) states that there is a moderate deficiency of lipase for about three months (31). Gschwind (32) studied the pancrease of 38 premature infants and 18 full term babies dying of a variety of causes and found the amylase and lipase values in most of the prematures were very low, with some proteolytic activity present. Venuti (33) found no significant change in enzyme assays of duodenal juice during the first year. Droese (34) investigated the amount of lipase recovered from the duodenum that could be a clinically useful gauge for the recognition of fat intolerance in 321 premature, new-born, and older babies. His normal lipase values ranged between 2 - 14 cc.NaOH using a tributryin method, the majority of values falling between 5 - 9cc. with no significant difference between any of the age groups. Carnevale, Cocozza, andAngelis (35) recently reported a study of 33 sucklings (7 prematures, 8 fullterm infants, and 18 suckling of different ages) concerning duodenal enzyme concentrations in relation to human milk. The

immature group of lipase levels varied between 3-6cc. NaOH, neonatal levels between 5-9cc. and the variable age group (up to 12 months) between 8-12 cc. NaOH.

Thus there remains some disagreement as to the exact lipase levels of the newborn by various authors. However, most recent investigation appears to reveal that although the lipase level of the newborn and during the first 3 months of life is decreased from normal values found after that period there is an average level present only slightly below normal which is adequate for normal digestion and lipolytic activity and which is far above any pathologically low values found in pancreatic pathology. This is confirmed by our studies which will be presented later.

Several previous studies on the pancreatic secretion in normal infants are recorded. The pioneer work was done in 1912 by Hess (36) who showed by qualitative tests that all three enzymes are present in early infancy. Davison (37) in 1925 studied the trypsin and amylase in normal infants and in infants convalescing from diarrhea. Andersen 1942 (38) concluded in her study of pancreatic enzymes in duodenal juice that lipase is present in normal infants of all ages, and the concentration definitely is reduced in pancreatic deficiency. She reported 3 patients with severe marasmus

with depressed lipase levels which later returned to normal with clinical improvement. This depression of pancreatic function probably represented merely a specific example of the depression of function of the organism as a whole, but it may well contribute a component to the clinical picture because of consequent difficulties in digestion. The lipase levels found using the Willstätter method from ages of birth to 5 years varied using mean lipase units from 18.8 - 34.6, lowest value found was 10.4.

Waldschmidt (39) using a modification of the Willstatter method found a enzyme activity in normal children 13.92 Lipase units with Average percent from 46-145%.

McDougall (40) presented a paper showing relationships between symptomalology and duodenal enzyme findings in 165 children who had or were suspected of having chronic intestinal disease. Using a modification of the Cherry and Crandall method, the normal value being 10 or more, 76 children showed normal potency of pancreatic enzymes, and 25 cases were found to have reduced lipase in the presence of normal trypsin and amylase. At the time the determinations were repeated only one child continued to be sick, this one continued to run suppressed lipase activity. Twenty three of the children

had pancreatic deficiency with fibrocystic disease of the pancreas. The rest of the children evidenced normal lipase enzyme activity.

Veghelyi in 1949 (41) made parallel observations using different methods of assay on a group of healthy children, studying normal conditions and to determine a common denominator of methods of estimation used which would indicate the normal or pathological character of the findings. In 47 normal and healthy children from 11 months to 10½ years of age the parallel lipase results with Willstätter's method of 182 estimations showed a mean value of 26 units, with a high of 53 and low of 13; with 143 estimations of Leubner's method revealed a mean value of 35% with high of 72% and low of 8%1 and with 113 estimations with Freudenberg's method values a mean of 5.3 units with high of 8.5 units and low of 3.0 units. He proposed that the lowest normal values yielded by the different methods of assay to expressed as 100%, thus expressing the results of pathologically low findings as fractions of these. He found no difference in the mean values found in different age groups although there was no subjects under 11 months ofage. Taking serial observations he proved that fasting secretion continuously occurs in healthy children and the amounts of enzymes put out is constant per units of time. This is in

accordance with Lagerlof's (42) findings but disagrees with Boldyreff (42) who felt there was a periodicity in the output of enzymes. With practically no exception, he found that the curves of the three enzymes,-lipase, trypsin, and amylase- ran parallel in all cases of healthy children, which confirms many authors investigations. This phenomen occurred so regularly in all cases that fluctuations of a single enzyme which does not correspond within the limits of experimental error, must be considered pathological. This strict parallism exists almost exclusively in healthy subjects but conditions become entirely different as soon as external secretion of the pancreas is affected by some pathologic process. This further emphasizes the need for more elaborate diagnostic laboratory procedures than the simple test for trypsin in the study of patients suspected of having cystic fibrosis of the pancreas, which has been shown to have an occasion dissociation of enzyme activity (43). He finally repudiated the common belief that the production of enzymes is adapted to the food given. The changes found during the administration of different diets were irregular and not substantial and could by no means be connected with the type of food eaten.

Gibbs and associates (44), have to report 50

duodenal drainages and lipase determinations from 28 normal children varying between 1 day and 16 years of age, and 7 children with fibrocystic disease of the pancrease, using Seligman's method of lipase determination. One lipase unit is defined as the mgms. of beta-naphthol liberated per cc. of pancreatic juice per hour incubation at 37 degrees C. in presence of sodium taurcholate and in excess of esterase present in the same quantity of pancreatic juice. The pancreatic juice was collected continuously for 20 minute intervals with a preliminary period followed by various pancreatic stimulants such as secretin, hydrochloric acid, cream, and amino acids. The results are presented in chronological order as follows using units of lipase / esterase:

TABLE NO. 1

LIPASE DETERMINATIONS IN CHILDREN AND INFANTS
WITHOUT FIBROCYSTIC DISEASE

Patient	Age	Preliminary	Secretin	HCl	Cream	Amino Acids
1.	1 day	QNS	225/0	95/0		
2.	1 day	QNS	355/30			
3.	1 day	16/9*	131/27	27/2		
4.	3 days	QNS	160/40	15/0		
5.	3 days	125/0	55/0			
6.	4 days	340/5		120/5		
7.	14 days	900/60	1210/0			
8.	"	670/0		410/0		
9.	1½ months	105/0	315/0			
10.	"	QNS		1140/105		
11.	"				235/45	
12.	"	QNS	745/30			710/70
13.	"	290/0		900/45		
14.	"				670/105	
15.	3½ months	435/15	265/0			
16.	4 months	1350/110	550/30	410/30		
17.	4 months	310/20	740/80	55/0		
18.	5½ months	820/35	1520/50			
19.	"	395/45			485/30	930/40
20.	6 months	390/55	155/55			
21.	7 months	1040/60	585/25			

Patient	age	Preliminary	Secretin	HCl	Cream	Amino Acids
22.	8 months	200/50	65/35	110/30	375/35	
23.	8 months	750/30	940/30			
24.	8 months	450/45		300/0		440 /20
25.	9 months	295/30	415/20			
26.	"			450/25		
27.	"				140/0	
28.	12 months	355/205	95/0	5/0*	1190-/0	
29.	12 months	460/15		150/15	255/15	280 /5
30.	14 months	400/65	410/55			
31.	14 months		115/30	670/60		
32.	" months	675/50	205/25			
33.	15 months	450/35	1600/35	475/28	425/15	
34.	18 months	410/85	135/65	350/45	245/35	
35.	"		95/0			
36.	18 months	802/35	1200/85	620/60		
37.	2 years	840/45	460/65	620/60		
38.	2 years	460/15	400/85	540/26		
39.	3 years	410/85	350/60	620/35		
40.	3½ years	490/55	970/50			
41.	6 years	435/50	45/30	10/15*		
42.	7 years	1600/85	215/10	1180/45	1500/90	
43.	16 years	620/0	115/0			
MEAN Lipase/Esterase		540/52	502/43	403/32	552/46	590/34
MINIMUM Lipase		105	45	55	140	280
MAXIMUM Lipase		1600	1600	1180	1500	930

TABLE NO. 2

LIPASE DETERMINATIONS IN PATIENTS
WITH FIBROCYSTIC DISEASE

Case	Age	Preliminary	Secretin
1.	4 weeks	0/50	15/30
2.	2½ months	25/40	5/45
3.	9 months	0/220	0/220
4.	10 months	45/15	65/10
5.	12 months	44/3	22/3
6.	4 years	10/15	10/15
7.	9½ years	45/15	65/10
MEAN Lipase/esterase		24/51	26/48
MINIMUM Lipase		0	0
MAXIMUM Lipase		45	65

* Inadequate volumn made determinations inaccurate

QNS- Insufficient quantity of duodenal juice for determination

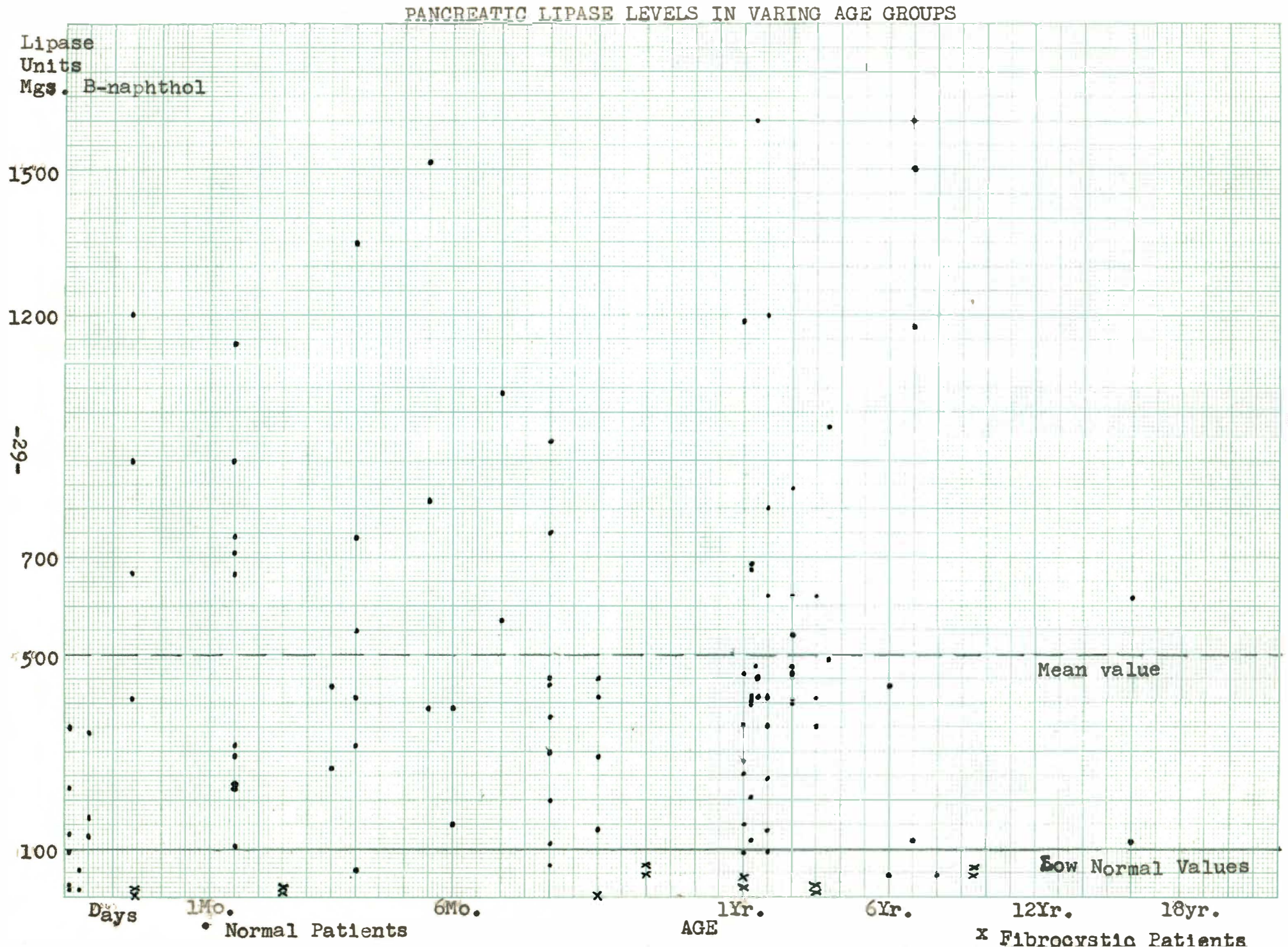


Figure III

Using mean values, there would obviously be little difficulty in telling abnormally low lipase levels. Using 500 Lipase Units as normal mean fasting levels for all ages, and 100 Lipase Units as low normal values; it will be noted that, on occasion, normal subjects went below the latter value. In each of these cases; however, either the preliminary 20 minute sample, or the sample of stimulated pancreatic juice produced values well within the normal range. This reveals the necessity for routine use of pancreatic stimulants for all duodenal enzyme determinations. Lipase deficiency should only be diagnosed when there is uniformly low values of all determinations and not on any one value alone.

Six cases, 4 days and younger, had a mean lipase value of 137 units, which is far below that of the mean found for all ages, 500 units. We may conclude from this evidence that there is a relative lipase deficiency found in the newborn which within 14 days returns to normal childhood levels.

The patients with fibrocystic disease uniformly gave subnormal values of preliminary and secretin stimulated runs. In none of the normal cases could lipase deficiency be considered and in none of the fibrocystic patients were normal lipase values found.

IV CONCLUSIONS

The most commonly used methods of lipase determinations at the present time use olive oil, tributyrin, and triolein as substrates. From the number of modifications of these methods and the contraversial literature concerning the accuracy of each method, it is obvious that none of them are ideal. The lack of lipase specificity of the substrate, difficulty in determining exact endpoints with titrametric methods, unstability of formed emulsions, and technical difficulties, have limited their usefulness as routine laboratory procedures.

Seligman and Nachlas's presentation of a method of lipase determination using beta-naphthol laurate as the substrate in the presence of sodium taurcholate using a photometer for determinations, gives the first method which gives us exact lipase levels independant of the amount of esterase present, using a colorimetric determination. We have found the method consistant and accurate in all parallel runs, reasonably technically simple, possessing a short incubation period, and applicable to serum or pancreatic lipase determinations which may be used and relied upon for clinical use.

Although there has been and is at this time a great diversity of opinion as to the lipase levels of the

newborn more recent authors appear to have come to some agreement. Early investigations, using rather inadequate methods of determination, were unanimous in the opinion that the lipase level at birth was low to absent. More careful studies have shown, particularily in the last 5 years, that premature and full term babies have only relative low values of lipase in the neonatal period which is safely above the pathologically low values found in pancreatic deficiency, but below the mean values found in childhood. Some authors have found the lipase values to return to normal at the age of one year, some at 3 months, and some feel that there is no decrease in lipase following birth. We found normal values following the 14th day and will conclude that mean values of lipase may be found in the normal children following the first month of life. In a normal infant following birth there is at no time a lipase deficiency present that is of sufficient magnitude to cause symptoms refereable to its deficiency.

In making duodenal enzyme determinations it is a necessity to use pancreatic stimulants, particularily when small volumnes of pancreatic juice is expected as are found in newborns, for abnormally low values have been found in normal children on separate runs, but never on all runs simultaneously.

The following are pancreatic lipase levels of children giving mean, maximum, and minimum values of different methods:

	Willstätter's Units Olive oil	Leubner's Units Triolein	Seligman's Units B-naphthol Laurate	Meyer's Units Tributyrin
MEAN	26	35	500	7
MAXIMUM	53	72	1600	11
MINIMUM	16	10	100	2

V. SUMMARY

1. A brief review of the different methods of lipase determination has been presented with evaluation of results, reliability, and undesirability of the various methods.
2. Seligman and Nachlas's colorimetric method of lipase determination is presented in detail and adapted for use of lipase determinations in duodenal drainages using the Beckman DU spectro-photometer.
3. A report is made of the pancreatic lipase levels in the newborn, infant, and child.
4. A series of 50 duodenal drainages from 28 normal children and 7 children with fibrocystic disease of ages varying from 1 day to 16 years is reported using

Seligman's method of lipase determination. Mean, and low normal values are reported using this method.

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