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Chylomicronemia

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Introduction

The etiology of atherosclerosis may soon be unraveled by studies on the chylomicron. Chylomicrons, small, brilliant, dancing bodies of the blood, have again aroused the interest of research workers. Up to date, they have been useful as a means of studying postprandial fat digestion in health and disease. They have been thought in the past to serve as toxin adsorbers and have been suggested as the possible explanation of the non-specific immunological properties of the blood. But the role now being assigned to them as possible etiological agents in atherosclerosis far outshadows all past roles.

Our interest in the chylomicron was aroused by an article by Becker et al (1) in which that investigator states that there is a marked difference in postprandial chylomicronemia in the young and the aged, and that this fact is related to the incidence of atherosclerosis in the aged. Primarily because of this report, Herbert P. Jacobi thought it would be interesting to attempt to confirm Becker's work and to extend the work to include young diabetics. The aim was to see whether young diabetics have the same type of alimentary chylo-

micronemia as the aged of Becker's series and, if they do, then to see whether some chemical agent could reverse the chylomicronemia of the young diabetic to that of a young non-diabetic.

As work began, it was found that knowledge of the nature of chylomicrons was seemingly nowhere completely correlated; therefore it was thought advisable to include in this paper a complete history of the chylomicron and then to give a report of the results of our work.

History of Chylomicron Study

Early History

Aselius (2) in 1622 showed that the presence of food in the small intestine could cause the chyle and then the blood stream to become milky. But not until 1770 did experiments by Hewson (2) show that the milkiness was due to the fact that the fluids contained a lot of fat, and it was this investigator who first viewed microscopically the lipid particles. Seventy years later, Gulliver (2,3) described the Brownian movement of the lipid particles in chyle. He referred to them as "the molecular base of the chyle." Both of these men worked with the light field microscope. The first dark-field observations were made by Edmunds (4) in 1877. He thought the dancing particles of the blood appeared similar to "motes in a sunbeam", but did not venture to suggest their nature. In 1891 Munk and Rosenstein (3) observed the dancing particles , thought they consisted of fat, and named them fat dust. But in 1895 Kahne (5) concluded from his studies that the particles simply represented protein contaminants from the skin. The next year Mueller (3) in a report of his observations referred to them as hemaconia but did not add anything to the elucidation of their nature. Raehlmann (5) in 1905 and Newman (5) in 1907 confirmed the

presence of the particles, but could not definitely prove their composition. Newman reiterated the idea that the particles represented fat from ingested food. Raehlmann considered them to be granules of disintegrated leukocytes. Thus, at the turn of the century, the question of the nature of the dancing particles of the blood was still obviously not answered.

Biochemistry

The actual deciphering of the physical chemical nature of the chylomicron began with the work of Gage and Fish in 1924 (2). These two investigators considered the particles to be lipid in nature because of the following facts: 1) on prolonged standing in a slender cylinder the particles rise and form a cream on top, which can cause a grease spot; 2) the emulsion when dried and extracted with ether gives a fatty substance on evaporation of the solvent; 3) the particles have the iodine number and refractive index of fat; 4) their number increases in the blood stream after ingestion of fat, but not after protein or carbohydrate ingestion. Knudson and Grigg (6) in 1923 confirmed the view that the chylomicrons are associated with the fats in the blood by finding that the lipid concentration increases when the number of chylomicrons have increased after a fatty meal. But McDonagh (7) in 1927 and later Peters (8) thought that

the particles were protein because of the precipitability of the particles with complete saturation of ammonium sulfate.

Frazer et al (9), basing their conclusions on the light-reflecting power of the particle as observed in the dark field, divided the particles into three groups: brights, small brights, and dulls. After their work was published, some workers published papers suggesting that not all chylomicrons had the same chemical nature. Cunningham and Peters (10), for instance, by centrifuging pig serum at 15,000 rpm found the following facts: the bright particles would concentrate on top and the dull would precipitate out; pancreatin would decrease the number, but trypsin alone would not; ether could extract the large and the small bright particles, but not the dull. Based on the above results, these men concluded that the large and the small bright particles were neutral fat and the small dull particles were protein. Today, all three types of particles are considered to be lipid. The credit for proving this fact is given to Frazer (11) and Elkes (5), who performed carefully controlled fat absorption experiments and blood chemical analyses.

Though recent investigators all agree that the particles are lipid, they debate such questions as what lipids are present and in what proportion. Workers tried to solve these problems by attempting to correlate increases

in chylomicronemia with increases in various lipid fractions. No correlation has been found between cholesterol or lecithin increments (3,6). There is a rough correlation between increase in the chylomicronemia and increase in neutral fat (5, 9, 11, 12). But this method was not believed to be good enough to determine whether small amounts of cholesterol were present in the chylomicron. Moreton (13) and Becker(1) et al believe chylomicrons consist predominantly of neutral fat but also have about 3 % cholesterol. The presence of cholesterol is severely contested by Gofman and his group (14,15). He believes cholesterol is carried in the blood by lipo proteins which are not visible in the dark field. More investigation is necessary before this point can be decided.

Colloid Chemistry

The next important step in the unraveling of the nature of the chylomicron was the recognition of these lipid particles as colloid entities. Ludlum (16) was the first to recognize that chylomicrons formed a lipoid emulsion in the blood and to decide to study the nature of the chylomicron through colloid chemical means. Knowing that when an emulsion particle is coated with a film of protective agent its surface properties are those of the film, Ludlum et al (3, 16) reasoned that if the iso-

electric point of the chylomicrons could be determined, the isoelectric point of the surface film and thus its nature would be known. The isoelectric point was determined first by noting the pH at which maximum flocculation occurred. It was usually found to be 5.1, half-way between the isoelectric pH's of albumin and globulin. Desiring to obtain more accurate results, the experimenters used a cataphoretic method. The isoelectric point was again found to be 5.1 . Further evidence of the protein nature of the surface film was had from experiments in which such agents as 95% alcohol, ammonium sulfate, and 1% alkali were used. These agents precipitated the protein film, causing the particles to coalesce. These experiments clearly indicated that the surface film of the lipid particles was protein. Possibly because early workers failed to realize that the lipid particles could be coated by a protein film, they considered the entire particle as protein.

Ludlum (17) thought that the film was partly albumin and partly globulin because of the isoelectric pH found. But Elkes et al(5) and Frazer (18, 19) believe that the film consists only of globulin because the maximum flocculation was found by them to occur at pH of 5.3 and because half-saturation of ammonium sulfate can cause the lipid micelles to coalesce.

Ludlum (16) also was the first to suggest that lecithin might be present at the interface between the lipid micelle and the protein film. This concept is also mainatined by Elkes and Frazer (20), who showed that a phospholipid is an essential part of the stabilizing film of the chylomicron by forming colloid dispersions with and without the phospholipid. Because of the work of Sinclair (20), Elkes believes that the phospholipid is added in the intestinal cell. The significance of the presence of the phospholipid will be discussed later.

Physical Chemistry

Some of the physical chemical properties of chylomicrons have been determined. The charge on the particles has been determined by electrophoretic experiments and found to be negative (5,11). The size of the micelle varies. Gage (2) estimated the size to be between 1/2 micron and 1 micron with a few particles surpassing the latter size. Merling-Eisenberg (3), by a very special method, found the dimensions of Gage to be approximately accurate. The latter found that the dull particles were about 35 millimicrons, the small brights 1/3 to 1/2 micron, and the large bright 1 micron. Frazer (11) also believes the sizes to be in the above range.

Origin

Gage (2) in 1924 stated that the particles represented the neutral fat formed by the resynthesis of fatty acids and glycerine in the intestinal cells. This concept is essentially the same as the lipolytic hypothesis of Verzar and McDougall (21, 22). These writers state that after fat is completely hydrolyzed, the resulting fatty acids pass into the cells aided by bile salts. In the cell triglycerides are resynthesized after first being phosphorylated under the control of the adrenal cortex. Several years after, the lipolytic hypothesis was proposed. Frazer (23, 24) presented a new theory of fat digestion, which would give a new origin to the chylomicron. This author states that neutral fat is absorbed without being completely hydrolyzed to fatty acids and glycerin. He believes that the neutral fat ingested is only about 30% hydrolyzed, forming mono- and diglycerides. Then, in the presence of an emulsifying triad consisting of a monoglyceride, bile salt, and a fatty acid, the remaining ingested fat is spontaneously emulsified into particles 1/2 micron in diameter and having a negative charge. These emulsified particles pass directly into the intestinal cells through small canaliculi.

In summary, one theory is that the chylomicrons

represent resynthesized, ingested fat, whereas the other theory is that chylomicrons represent the ingested fat particles themselves.

Life

Once the lipid particles are in the intestinal cells it is thought by Sinclair (20) that lecithin is adsorbed at the oil water interface. Where protein is added is not stated, but it must be either in the lymph or blood stream. From the intestinal cells the coated particles enter the lymph, which carries them through the intestinal lymphatic ducts, cisterna chyli, and thoracic duct from which they pass into the blood stream. It has been shown by comparative simultaneous counts on the portal circulation and the systemic circulation that almost all of the particles follow this route in adult animals and about 50% in very young animals (25). The particles have been found to distribute themselves equally throughout the arterial tree and capillary bed and in less concentration in the venous tree (26). Gage (2) has estimated that there are 79,317,866 per cubic millimeter of blood at the height of digestion.

In the blood the particles form an oil in water emulsion, as already described. The emulsion within itself shows an interesting equilibrium. The equilibrium referred to is the one between the lipid in

solution and the chylomicron particles, the latter fraction representing 60% of the total lipid (2). The variation in sizes of the lipid micelles indicates the presence of an equilibrium, but whether the visible particles are directly related to lipid in solution has not been shown conclusively. It seems to us that Ahren's and Kunkel's work (27) work indicates this state to exist. These men were able to cause the appearance of chylomicrons by adding a lecithinase, Clostridium welchii lecithinase, to clear serum.

Destination

The chylomicrons formed from the absorbed fat are next either metabolized immediately or stored in the fat depots (28, 29). Ludlum (3,16) believes both processes are possible because the particles are coated by a protein film, which enables the particles to become wetted and thus to be absorable through cellular membranes. Inside the cell the protein film could be destroyed by a proteolytic enzyme; then, either the fat could be metabolized or the particles could coalesce into fat droplets in the cells, which make up the fat depots (30). The fat from the depots is continually being given up to the general circulation. The particles from the depots are called by Frazer (9, 31) lipomicrons to distinguish them from the particles formed from ingested fat.

With the nature, origin, life, and destination of the fat particles of the blood and chyle in mind, attention can be turned to the various ways in which this knowledge was put to use.

Physiological and Pathological Chylomicronemia

Gage and Fish (2, 32, 33, 34) were the first to establish postabsorptive chylomicronemia as the usual phenomena that take place in healthy individuals. These authors stated that after an ordinary breakfast the chylomicrons increased in number, reaching a peak in 3-4 hours and returning to a base line in 8-10 hours. Frazer (9) confirmed this observation, but by using a more standardized technique found that the peak was in about 2 hours and the base level was reached in 4 hours. Observations by other authors are in agreement with Frazer's time. Gage (2),and later Frazer (9),noted that after fasting for twelve hours chylomicrons in the blood would reach an unreducible minimum. This minimum is what is referred to as the base line. Frazer (9) also noted that about every 2 hours in a fasting subject an increase above the base line could be noted. He referred to these increases of lipomicrons as fat crises and considered them to represent deliveries of fat from the fat depots. We have observed a fat crisis in a fasting subject. See record of subject 5 in Table 1.

Frazer (35) has also shown that if a person does not fast before a test, two peaks will be noted in the resulting chylomicrograph. The first occurs 15 minutes after the ingestion of food and ends in 1 hour. The second starts in 1 1/4 hours and ends in about 4 hours. The author interprets the initial rise as being due to the increase in bowel motility, a change which causes the absorption of fat remaining from the previous meal. The delayed rise is due to the fat just ingested. Bohm et al (36) studied the chylomicron curves in people who ingested 10 grams of fat every 2 hours. He was able to confirm Frazer's work and added the observation that at night the chylomicron levels were higher and persisted longer than during the daytime.

There are certain physiological states which can alter an individual's curve. It has been noted by Gage et al (2) and Frazer et al (9) that subjects who appear very anxious during a test have increased and prolonged curves. These investigators thought that this increase was due to decreased bowel motility and digestion. Exercise has been shown to increase the height of curves (2, 37, 38). Stature seems to make some difference. Stout people have prolonged and higher curves than those of normal individuals, and very thin people give lower curves. Frazer (23) said that this fact was due to the following finding: more fat goes directly to the fat

depots in stout individuals; more fat is hydrolyzed and taken by the portal vein to the liver in thin persons. Becker (1) has noted a marked difference between curves of the young and the aged. This point will be expanded later.

Cold exposure has been shown not to affect curves; whereas warmth gives slight increases (39). Radiation over pelvis, abdomen, and bifurcation of the carotid has been noted by Setala (40) to lower chylomicron counts. This author thought this radiation effect was due to the fact that lipid particles were leaving the blood stream through the altered membranes of the capillaries.

The action of drugs and chemicals upon the chylomicron curves has not been extensively studied. A few observations will be mentioned. Frazer (9) showed that pituitrin could decrease a count. He suggested that the decrease was due to a speed-up of the metabolism of the lipid particles. Ether has been shown by Day (41) to decrease a count , but this effect was not always obtained. Gage and Fish (42) have shown that glycerine, alcohol, and morphine can increase counts. Glycerine unites with fatty acids furnished by the body before it gives rise to chylomicrons. The action of the other drugs is not known. Lipase decreases number and reduces time necessary for the particles to return to the base

line. It is thought that the reason for this occurrence in the aged is that the lipase given overcomes the deficiency in lipase which is common in the aged (43,44). Sorbitan polyoxyethylene monoleate, "Tween 80", does the same thing in the aged as does lipase, but its action differs (1). It serves to help emulsify the fat ingested and thus facilitates the action of whatever lipase is present. In young people, under 50, the opposite effect is noted on the chylomicron curve. The explanation is not known. Becker (1) simply sta s that possibly a different mechanism of digestion may be present in the young. Another explanation to the action of "Tween 80" will be suggested.

The chylomicron curves have been found to be affected by certain pathological conditions. The base level has been found to be elevated in nephrosis, xanthamatosis, hypothyroidism, and i n uncontrolled diabetes mellitus (42, 46, 47). In controlled diabetes mellitus the base level has not been found to be elevated (42). In obstructive jaundice the count never increases above the fasting level (48). There is a decrease in the curve in vomiting of pregnancy (42) and in ulcerative colitis (48). Fourmann (49) has obtained t curves in diarrheas of different etiologies and in sprue. Cook et al (50) has found flat curves in conditions characterized by diarrhea. He calls these conditions anomalies

of fat digestion because no specific etiologies can be found. We have noted flat curves in non-tropical sprue and fibrocystic disease of the pancreas. Based on the work of Jones (51), we used sorbitan polyoxyethylene monoleate and found that this drug elevated the curves. In the sprue patient, because of her poor response to the usual therapy, adrenal corticotropic hormone was administered. A curve was determined after the patient had been on the latter therapy for two weeks. The results showed that this hormone can increase the chylomicron count. See Table V and graphs X, Y, Z.

Peters (8) has studied the changes in chylomicronemia in acute and chronic infectious processes. He states that in chronic infections the particles become large masses, brownian movement becomes sluggish, and a definite tendency to settle out is noted. In acute infections there is a rapid disappearance from the blood of the chylomicrons. The writer believes these changes are caused by toxins of the infectious agents which reduce the electric charge on the particles and, therefore, decrease the degree of dispersion of the particles with the result that they are flocculated out. A few workers, basing their investigations upon Peter's work, attempted to implicate the chylomicron as a non-specific, immunological agent and ventured so far as to give lipid infusions as treatment in certain illnesses characterized by symptoms

of toxicity (37, 38, 52, 53). During the past seven years no interest has been shown in this theory.

In non-infectious toxic states, such as chronic alcoholism, long continued narcosis,and phosphorus poisoning,chylomicronemia is observed (42). No reason for this was given, but the reason could be that damage to the liver resulted in a decrease in phospholipid production. The decrease in phospholipid would cause a shift in the lipid equilibrium, manifested by chylomicronemia.

The above paragraphs demonstrate the use of the chylomicron curve, but does not suggest how the curve is constructed and what it represents. In the following section, the construction and significance of the curve will be given in detail.

The Chylomicrograph

Construction of the Chylomicron Curve

In order for the chylomicron to be put to use as a tool in the study of fat digestion in physiological and pathological states, a method by which the chylomicron could be estimated was needed. To Gage and Fish (2) in 1921 goes the credit for developing the first method. Many methods have been developed since, but most are just modifications of that of Gage and Fish. Frazer (11, 35) in 1936 sought to standardize the technique, but almost all workers have instituted some modifications (54,55). Moreton (56) in 1948, completely disgusted with the apparent inaccuracy of the counting methods, developed a nephelometric method which he states is far more accurate. But it must be noted that this investigator uses venous blood, which has a different concentration of lipid particles than has arterial or capillary blood, for his count.

The method that we used is a modification of those of Gage (2), Frazer (11, 32, 35), and Becker (55). The most significant modification was the use of a paraboloid condenser instead of a cardioid and a 10X occular instead of a 20X. The entire procedure for securing the blood and making the count will be given in detail.

The subject is instructed not to ingest any food after 8 p.m. the evening before the test till receiving the test meal at 8 a.m. the following morning. Water is permissible. This interval allows enough time for the small intestine completely to empty itself of fat from previous meals.

A test meal is given, consisting of 2 slices of toast (60 gm.), 1 cup of tea without sugar, and an amount of oleomargarine equal to 1/2 gram per kilogram of body weight. The meal is eaten in 5-10 minutes (55). If insulin or "Tween 80" is to be used, it is given just before the meal.

The first sample of blood is obtained before the meal is given. Then a sample is obtained every hour thereafter for five hours. Before each puncture, the finger is cleaned with .2% mercuric chloride in 95% alcohol solution, then air dried (2). The puncture is made with a hemospast. This is kept sterile in 1:1000 zephiran, rinsed in 70% alcohol, and waved dry before use (2). Blood is drawn into capillary tubes. This procedure is facilitated by holding one end of the tube toward the floor. The blood is drawn only up to 9 cm. of 10 cm. tube, leaving a free end. The blood in the tubes is allowed to clot and retract, a process which takes about 10 min. The free end of the tube is sealed in a bunsen flame. The clot on remaining open end is

loosened. Now the tubes are centrifuged at a rate of 2000 rpm for 5 min. Then the end of the tube containing the clear serum is broken off from the end containing the packed cells. Porcelain chips or a diamond-point pen facilitates this part of the procedure. The serum is blown out of the capillary tubes by a modified eye-dropper which has a small nostril that just fits over the capillary tube. Space between tube and dropper nostril is sealed by finger. A small drop is placed in this way on a very clean, scratch-free glass slide, the thickness of which should correspond to slide thickness indicated by condenser used. Over the drop a No. 1 cover slip is placed. Excess serum is removed by pressing gently down on cover slip with forceps and dabbing up extruded serum at sides of cover slip with lens paper. Edges of cover slip are then sealed with castor oil. The preparation is now ready for examination.

The preparations are observed under a microscope which has a paraboloid condenser and a built-in pointolite illumination. The oil immersion objective , containing a funnel stop which reduces aperature to less than .8, is used. A 10X ocular is used. The ocular is fitted with a net micrometer which has 100 squares. Each square in ours was determined to be .075 mm by .075 mm. A drop of immersion oil is placed between condenser and slide, and another between cover slip and objective. Microscope is then carefully focused. Three planes will be observed.

In two of the planes white specs, which are not moving, will be found. In the middle plane will be found particles which are dancing merrily. The latter are the chylomicrons. The non-moving particles are defects or dirt on the glass. We continually strived to reduce the latter. (After trying many methods, we finally decided that all glass ware to be used was first to be washed in alconox, rinsed with distilled water, then immersed for several minutes in boiling chromic acid, removed, and again rinsed very thoroughly in distilled water. While still wet, the glass ware is put into 95% alcohol, from which it is removed in a few minutes and put into storing jars containing 95% alcohol. When ready to use, slides and cover slips are removed and wiped dry. They are polished with silicon paper.)

The condenser is again adjusted; a final focus is made,and without further manipulation a count of ten squares is done. This procedure is repeated for five fields and an average of the five is taken as the count for that preparation. (We counted all moving articles, dull or bright, because all particles are considered lipid. Some workers have counted just the bright.) The count for the preparation is multiplied by 10; the resulting figure is used in the construction of the graphs. The count obtained by the above method is graphed with the abscissa representing time and the ordinate the number

of chylomicrons counted. The experimental error is about 5 percent for the low counts and 15-20 per cent for the highest counts.

Interpretation of the Chylomicrograph

There are a multitude of factors that must be considered in the interpretation of the chylomicrograph. The factors can be divided into two groups: physiological and experimental. Gage and Fish(2) state that there are five physiological processes that must be considered in the interpretation of the graph: digestion, absorption, the movement of the absorbed fat from the intestinal mucosa to the blood stream, assimilation of the fat after it reaches the blood, and the metabolism of the fat. These processes have been described in a previous section. Another factor, emptying time of the stomach, should be considered. Anneger et al (59) states that excess fat ingestion decreases emptying time of the stomach to different degrees in different individuals. This fact would mean that graphs obtained from individuals who were fed excess amounts of fat would not be comparable. Frazer and Stewart (60) agree that the above physiological points must be considered, and these authorities place particular emphasis on the belief of Gage and Fish (2) that each point on the chylomicrograph represents the

resultant between the amount of fat added to the blood minus the amount removed. This latter point is most important in the interpretation of a graph.

Experimentally, many factors are brought in because of the possibilities of error. First, there are possibilities of error in the technique itself, mainly because the constantly moving particles are difficult to count. Irwin et al (39) found that all the particles are not removed from the packed erythrocytes by centrifuging. Different depths of focus bring different numbers of chylomicrons into view. But by experience, by using a standardized method, and by having one person make all counts, many of the difficulties stated are minimized and qualitative comparative results can be obtained. Also, there are some definite unique advantages to be found in using the chylomicrograph. The fraction of the lipids that change most rapidly after ingestion of fats can be directly studied (40); the method does away with the rather inaccurate time-consuming lipid concentration determining methods (39,57); and capillary blood can be used instead of venous (58).

Following the above presentation of the method of construction and the factors involved in chylomicrograph interpretation, the experimental work will be discussed.

Experimental Work

Chylomicrographs of Normals

The purpose of the first experiment was to obtain graphs of young and old normals and then to compare the results with those of Becker et al (1). In graph A is represented the difference found between young and old normals. Essentially, it amounts to a delay in the peak by one hour and a slight tendency to chylomicronemia in the aged. This result presents somewhat of a contrast to Becker's work. Becker's graph (graph B) shows that the peak in the aged is delayed seven hours longer than the peak in the young and that chylomicronemia is prolonged nineteen hours over that of the young. The curves of the young compare fairly well. The difference lies in the curves of the aged. Possibly, the reason for the difference is that the subjects were not followed for a long enough period. Becker et al followed their subjects for 24 hours.

Gofman (66) has informed us that White and Ralston of the Birmingham Veterans Hospital have found that bed-ridden elderly patients give the type of curve reported by Becker et al. There are still other points which tend to depreciate the results of Becker et al. The psychological state and the stature of a subject may

affect the curve (2,9). The influence of these factors is illustrated in graphs D and E. These are graphs of young subjects and show prolonged curves. Setala (40) states that each individual has a characteristic curve, which may vary far from what is accepted as a normal curve. In graph F is seen what Becker et al consider a typical young curve; but in graph G, which is also of a young subject, the curve would have to be interpreted as prolonged. In graph H is found what Becker et al consider a typical curve of an old normal; but in graph I, which is also of an old normal, is found a typical young curve. The most important evidence against the validity of the results of Becker et al is that nowhere in the literature can post-prandial curves similar to Becker's be found. Setala (40) determined curves in young and old normal subjects in order to see later whether radiation could affect the curves. In graph C it is seen that the curves of Setala are similar to the ones in graph A but not in graph B. Gage and Fish (2) and Frazer (9) have graphs of young and old normals which are similar to graph A,but not to graph B.

Eye Ground Correlation

Eye ground findings have been frequently used as a rough index of the degree of the atherosclerotic

process in the body; if the assumption of Becker et al were true, that is, that postpranidal chylomicronemia is an index of atherosclerosis in the body, then possibly eye ground findings might correlate with postpranidal chylomicronemia. Some evidence of prolongation occurred in the graphs of subjects having arteriosclerotic retinopathy from grades I-III, graphs K, L, M. The young normal curves in graph A and graph J represent subjects that have grade 0 to 1/2 retinopathy. An interesting observation was that 8 out of 10 young diabetics had some degree of retinopathy; whereas none of the 12 young normals had any degree of retinopathy.

This work would tend to substantiate observations of Becker et al; but before it could be positively stated that the eye ground findings in atherosclerosis can be correlated with postpranidal chylomicronemia, the number of subjects would have to be increased. (Grade 0 represents no evidence of retinopathy, and grade 4 represents severe arteriosclerotic retinopathy. The dividing of the grades into halves was done on the basis of clinical judgment by Dr. Filkins and Dr. Ellis.)

Chylomicrographs of Diabetics

The next step in the investigation was to obtain curves of young and old diabetics. It was reasoned that

since old normals had markedly elevated and pr onged curves, possibly young diabetics, and most certainly old diabetics, would have markedly elevated and prolonged curves if the contention of Becker et al is true. The curves were not found to be markedly elevated and prolonged. In graph N it is seen that young diabetics, both with and without insulin administered before the test meal, do give a slightly more prolonged curve than do young normals, but not as marked as the aged normal curve of Becker et al, graph B. In old diabetics the curve, graph O, is still more elevated and prolonged, but yet not as much as the curve secured by Becker et al. All of the diabetics were under control at the time of the experiment; however, they still would be expected to give markedly elevated and prolonged curves, inasmuch as it is well known that even controlled diabetics are markedly prone to developing atherosclerosis.

Effect of Insulin

Since it was known that insulin can reduce pathological lipemia in diabetics, it was thought important to see whether insulin might not affect postprandial chylomicronemia. Graphs N and O are composite graphs which illustrate that insulin tends to lower the height of the curve. This point was more critically studied by doing

curves with and without insulin in the same subject. In graphs P and S insulin seems to have definitely lowered the height of the curve; but in graph Q it has increased the height of the curve. In graph R insulin seems to have caused the peak to come earlier. It can only be concluded from these results that there seems to be a tendency for postpranidal chylomicronemia to be reduced by insulin. Possibly if the patients had not already been under control, a more marked decrease would have been noted with insulin.

Effect of "Tween 80"

In the case of four young diabetic subjects, the curves of whom had been established with insulin, it was decided to see what effect "Tween 80" would have if given along with the insulin before the test meal. In normal young the curves are elevated; in normal old the curves are decreased (1). In graphs T and W, where "Tween 80" was used, definitely elevated and prolonged curves are noted. In graph V the detergent caused the peak to occur earlier but did not prolong the curve. In graph U the peaks were not changed but the curves were prolonged. Thus, seemingly, no benefit, but actual harm, could come from the use of "Tween 80" if the theories of Moreton (56, 61, 62) and Becker et al (1, 55) are true. These authorities hold that atherosclerosis is the result of a life-time bombardment of the arterial wall by chylomicrons.

Recently, Zinn and Griffith (63) have published some work in which they noted that diabetics have a larger number of the bright type of chylomicrons, in relation to the dull, than have non-diabetics. In the dark-field, brighter and coarser particles are more abundant in the diabetic than in the non-diabetic sera. In the subjects that received "Tween 80", the bright and coarse particles were decreased in number. Possibly, an explanation for this point can be found in the fact that "Tween 80" has been shown to be able to increase the cholesterol phospholipid ratio in rabbits with atherosclerosis (64, 65). This observation is significant because the cholesterol phospholipid ratio has been shown to be decreased in diabetes, nephrosis, hypothyroidism, and xanthamatosis, all conditions which predispose to atherosclerosis (27). It can reasonably be postulated that a deficiency of phospholipid could cause the increase in the bright particles. As has been already brought out, there exists a phospholipid at the interface between the lipid of the chylomicron and the protein protective layer. The phospholipid is essential for proper emulsification of the fat in the blood (20). So, when phospholipid is deficient, the emulsion state breaks down and large particles appear. This fact implies that an equilibrium exists within the lipid fraction of the blood; that is, there exists lipid particles of all sizes from the large bright

chylomicrons to very small, invisible particles. There is some evidence to support this concept. In any dark-field, as Frazer (31) has noted, are seen three groups of particles which vary in size. Gofman (14, 15) by ultra-centrifuge methods has shown there are various lipid fractions which can be separated because they have different sizes. Gofman's fractions include not only chylomicrons, but also particles that are invisible in the dark-field. Ahrens and Kunkel (27) have shown that particles which look like chylomicrons appear when clostridium lecithinase is allowed to act on clear sera, destroying the phospholipid of the sera. This work seems clearly to indicate the importance of phospholipids in the lipid emulsifying system and suggests that a lipid equilibrium exists. By the use of the "Tween 80" observations, a new concept as to how lipids exist in the blood has been presented. Only circumstantial evidence has been offered in proof.

Summary

Although the chylomicron has been discussed from widely different points of view, the point wished to be most emphasized in this thesis is the relation of the chylomicron to the atherosclerotic process. Moreton (56) feels very strongly that atherosclerosis is directly

related to chylomicronemia. Becker et al (1) not only feel that the chylomicron is the eticlogical agent in atherosclerosis , but also believe that the chylomicrograph may be taken as an index of atherosclerosis in the body. The experimental work done does not add confirmation to the latter belief. It has been suggested that atherosclerosis may be due to an overabundance of the large and coarse chylomicrons, a condition resulting from a disruption of the lipid equilibrium caused by a deficiency of phospholipid. In order to see whether the latter assumption is true, the effects of phospholipid precursors on chylomicronemia in diabetics is being studied.

Conclusions

1. The chylomicron is a complex consisting of a central nucleus , which is either entirely neutral fat or neutral fat with 2-3 per cent cholesterol, surrounded by first a phospholipid and then a protein.

2. The chylomicron represents ingested lipid; whereas lipomicrons represent the fat from the fat depots.

3. The chylomicrograph can be used to study many physiological and pathological disease processes.

4. The chylomicrograph is not as markedly different in the young and the aged as has been suggested by Becker et al (1).

5. Postprandial curves in young and old diabetics have been determined.

6. Insulin has been shown to decrease slightly chylomicronemia in controlled diabetics.

7. The chylomicron, per se, may represent the etiological cause of atherosclerosis.

TABLE I

Young Normals

No	A	Wt	F	Count						BP		P	R	T	Bld	Eye	Mis.
				0	1	2	3	4	5	S	D						
1	20	55	27	6	15	67	12	30	34	98	60	84	20	98	N	0	
2	22	71	36	7	6	23	18	10	12	126	66	68	16	98.2	N	0	
3	22	60	30	11	17	76	34	11	21	114	72	92	16	98	N	0	
4	23	84	42	8	60	66	61	53	28	110	80	72	16	97.4	N	0	
5	23	72	36	1	2	1	13	5	-	120	80	72	16	98.6	N	0	Fasting
5a	23	72	36	24	23	56	-	37	41	128	80	96	16	98.6	N	0	Nervous
6	24	71	36	20	35	120	30	7	-	110	72	96	16	98.6	Sl	0	
7	26	59	30	1	9	24	13	14	5	110	90	72	14	98.2	N	0	
8	27	71	36	3	11	37	28	40	20	128	78	88	16	98	N	0	
9	29	78	39	4	25	32	31	22	40	130	88	72	25	97.6	St	0	
10	30	77	39	2	37	16	30	10	36	124	82	96	16	98.6	N	0	
11	38	49	25	10	24	86	41	23	12	98	48	92	12	98.4	Sl	0	
12	40	104	52	23	38	39	74	64	41	146	102	72	25	98.6	St	0	

Key:

No - Number

A - Age

Wt - Weight in kilograms

F - Oleomargerine in grams

BP - Blood pressure

P - Pulse

R - Respiration

T - Temperature

Bld- Stature
N - Normal
Sl - Slender
St - Stout

TABLE II

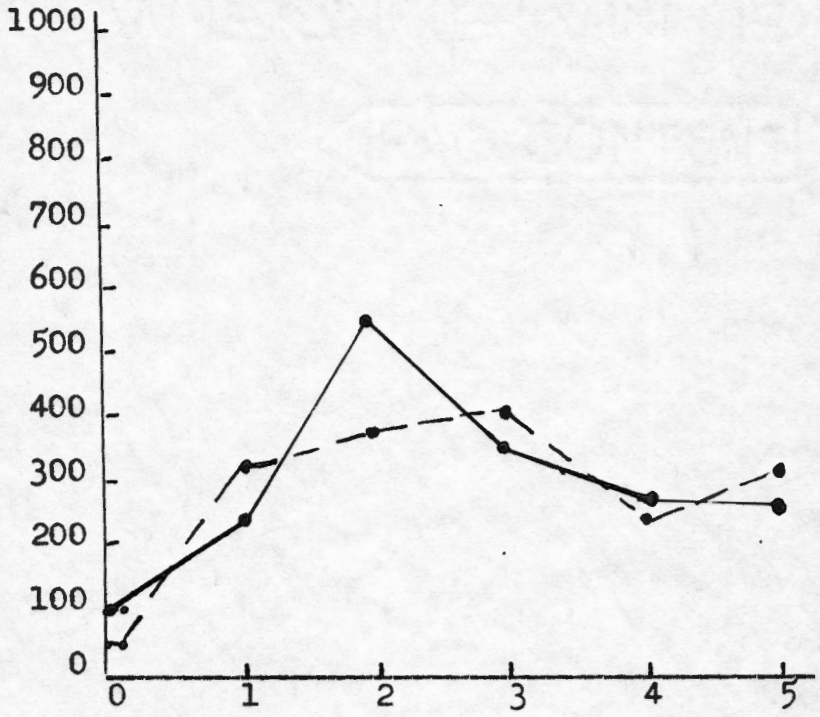
Old Normals

No	A	Wt	F	Count						BP		P	R	T	Bld	Eye
				0	1	2	3	4	5	S	D					
13	50	54	27	1	1	11	14	10	76	230	100	76	16	97	Sl	3
14	64	64	38	5	32	38	32	23	44	120	80	96	16	98	N	2
15	68	68	34	1	68	76	36	3	9	140	98	76	12	98.4	N	1.5
16	69	83	42	5	38	21	30	15	17	170	80	72	16	98.2	St	1/2
17	69	45	23	6	13	52	36	20	6	128	64	84	16	97.6	Sl	1/2
17a	69	45	23	9	29	46	57	49	20	128	64	84	16	97.6	Sl	1/2
18	69	56	28	5	10	47	92	34	28	140	76	60	14	97.4	N	1.5
19	71	80	40	16	23	28	21	13	9	170	60	72	14	97	St	-
20	75	70	35	2	2	35	50	51	53	200	100	92	20	97.6	N	1
21	75	90	45	4	2	14	35	48	41	138	90	90	12	98.2	N	-

Key:

No - Number
A - Age
Wt - Weight in kilograms
F - Oleomargerine in grams
BP - Blood pressure
P - Pulse
R - Respiration
T - Temperature
Bld- Stature
N - Normal
Sl- Slender
St- Stout

Composite Graphs of Old and Young Normals

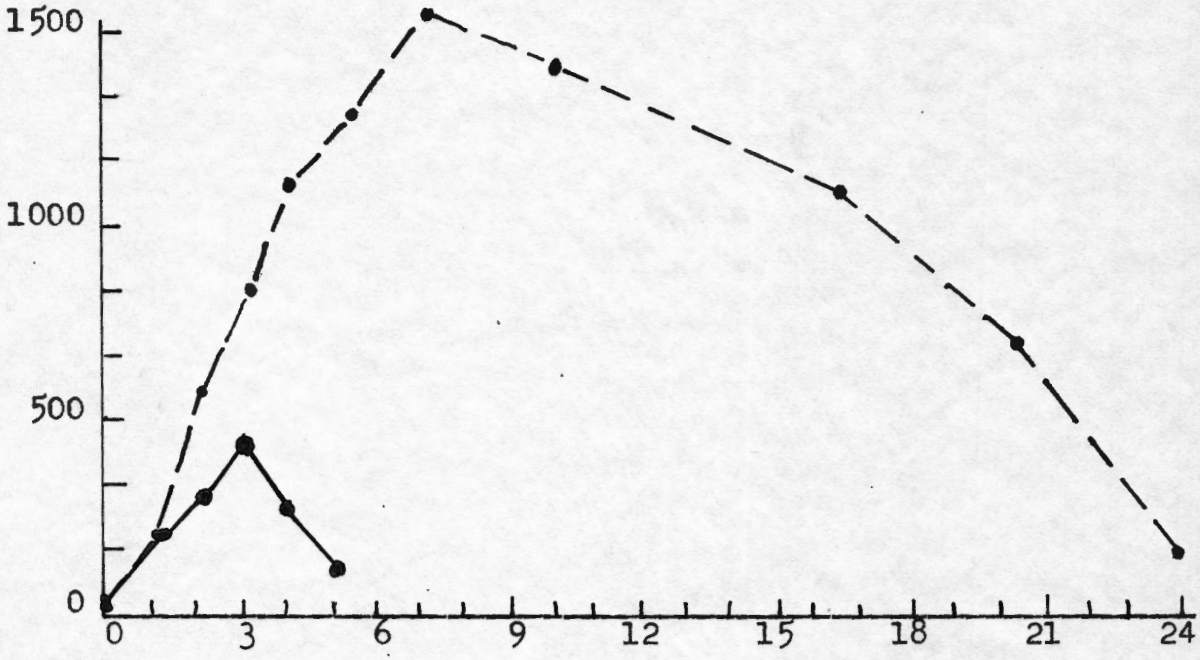


Graph A:

(———) - Young normals (20-40). Av. 25 years. Subjects 1-12 inclusive.

(— — —) - Old normals (50-75). Av. 68 years. Subjects 13-21 inclusive.

Composite Graphs of Old and Young Normals

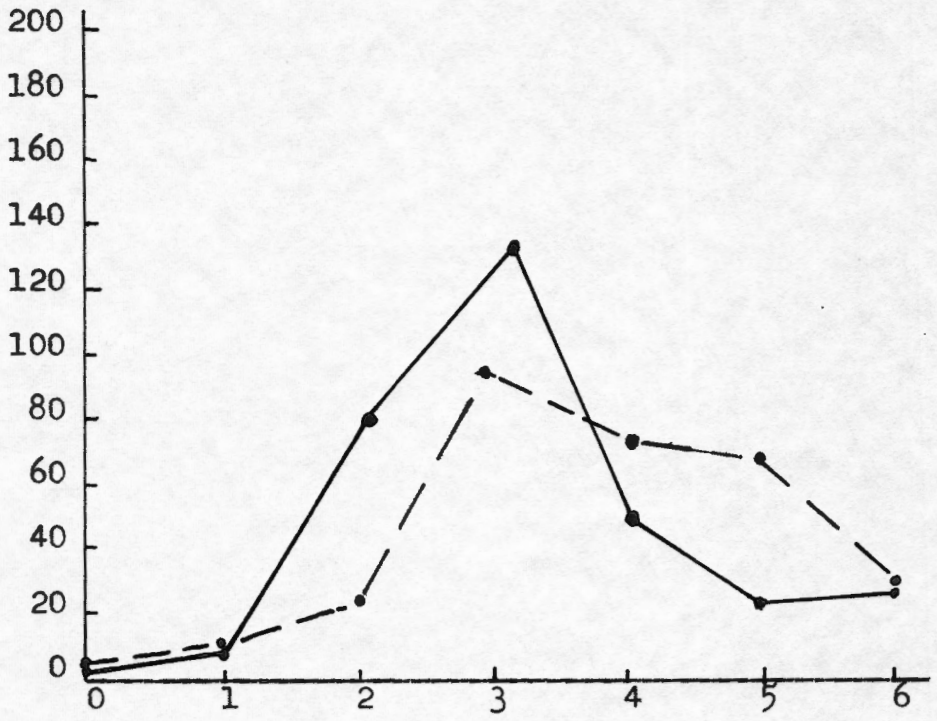


Graph B: After Becker et al (1).

(———) - Young normals (10-34). Av. 18 years.

(_ _ _) - Old normals (50-87). Av. 76 years.

Composite Graphs of Old and Young Normals

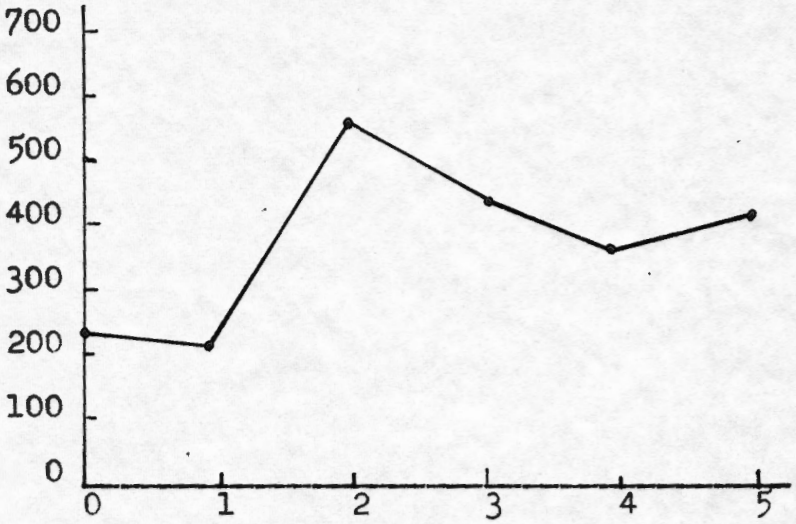


Graph C: After Setala (40).

(———) - Young normals (19-49). Av. 27 years.

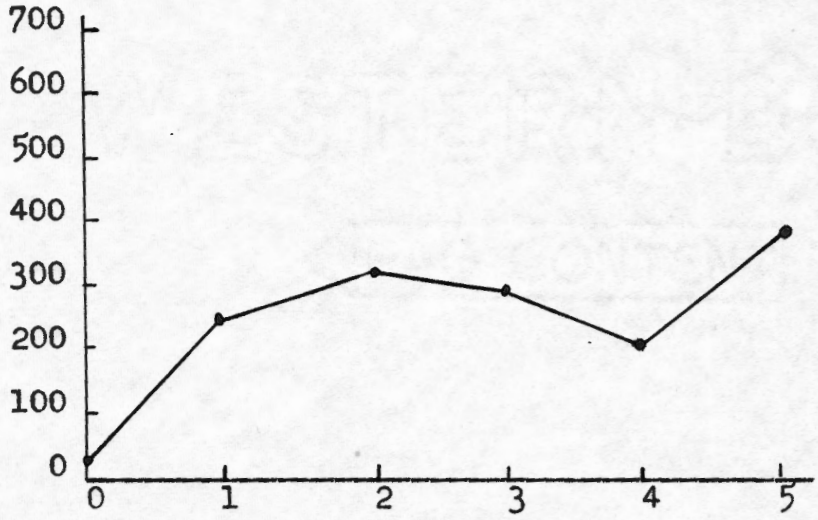
(— — —) - Old normals (64-77). Av. age 68 years.

Effect of Extrinsic Factors on Curves



Graph D: Subject No. 5a.

Illustrates effect of nervousness .

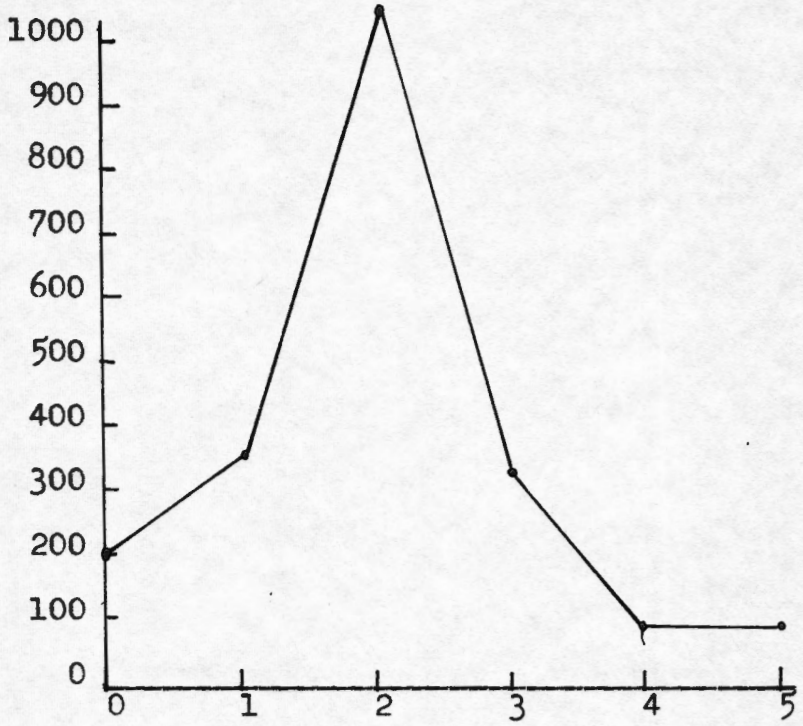


Graph E: Subject No. 9.

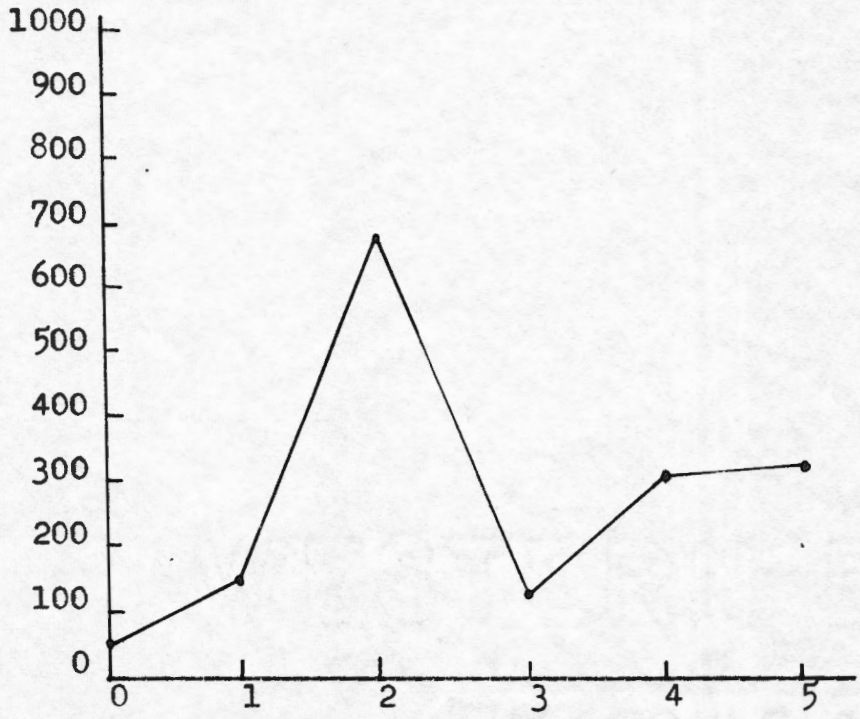
Illustrates relation of stature to curve.

Stout individual.

Extreme Variations in Old and Young Graphs

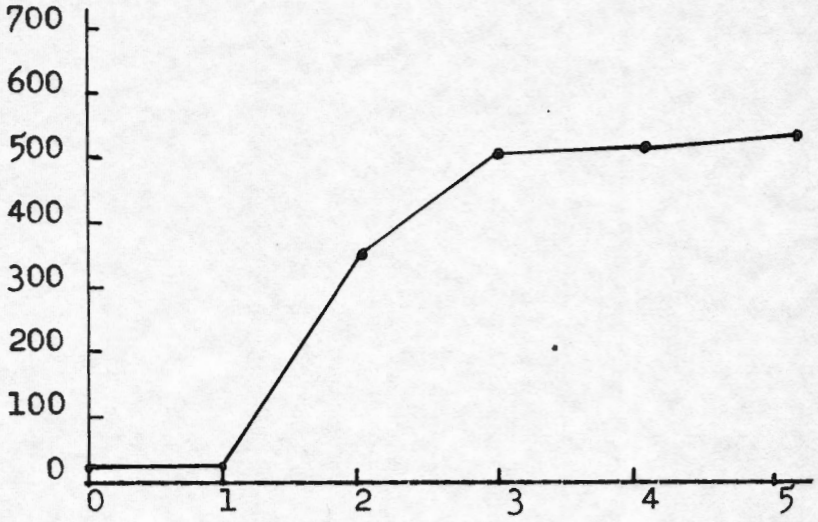


Graph F: Young normal. Subject No. 6.

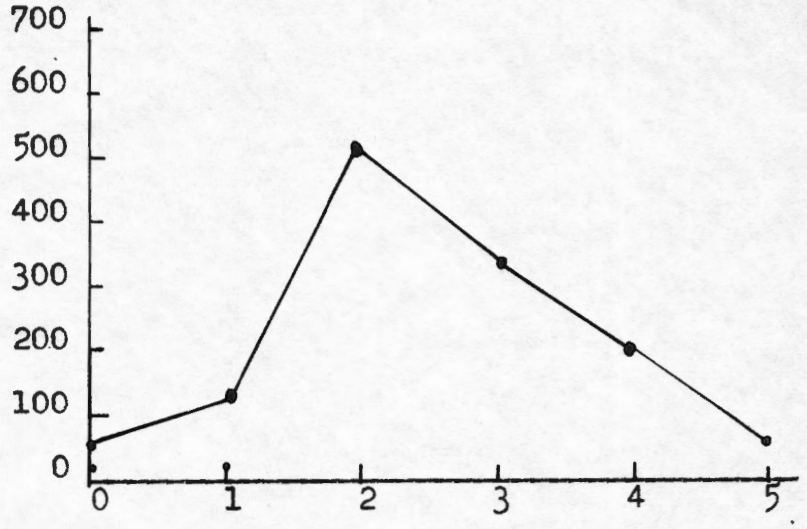


Graph G: Young normal. Subject 1.

Extreme Variations in Old and Young Graphs

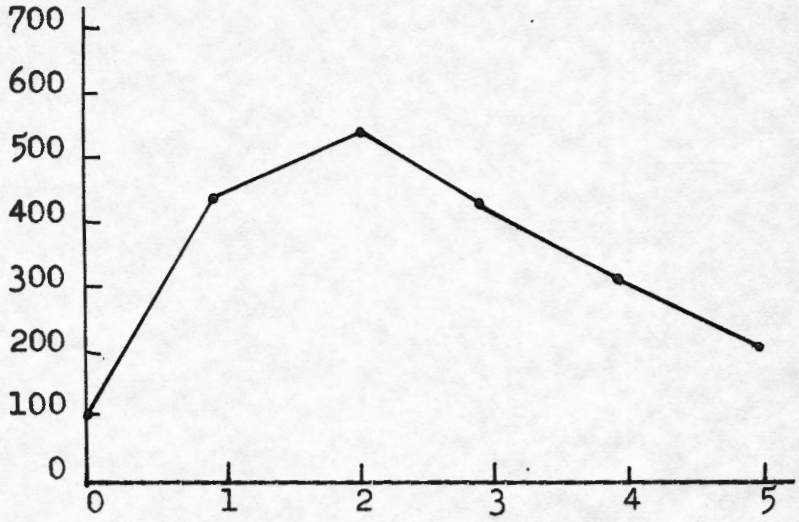


Graph H: Old normal. Subject No. 20.
In bed throughout experiment.

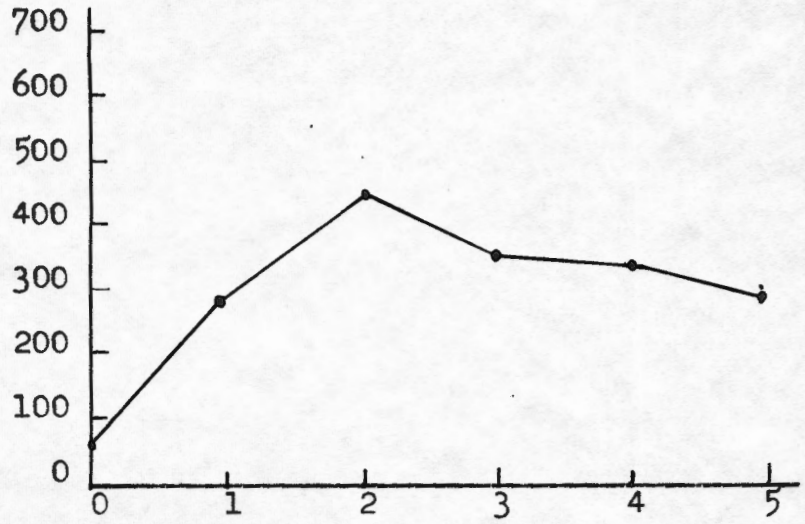


Graph I: Old normal. Subject No. 17.
Active during experiment.

Composite Graphs Relating Eye Grounds to Chylomicrograph

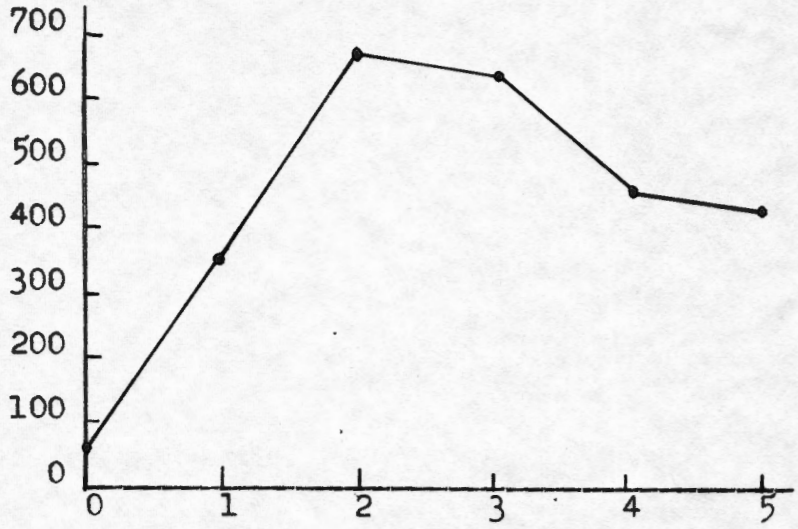


Graph J: Subjects No. 17, 18, 34, 36, 45.
Grade 1/2 retinopathy.



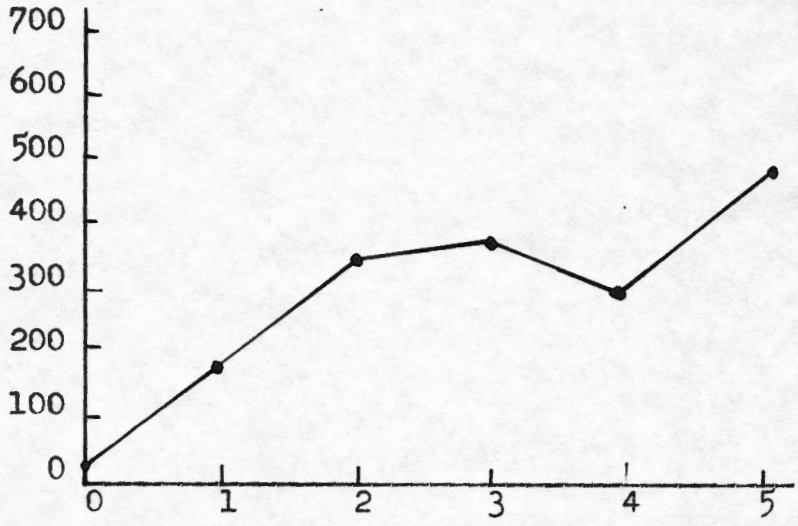
Graph K: Subjects No. 22, 24, 37, 38, 39, 40, 50, 51.
Grade 1 retinopathy.

Composite Graphs Relating Eye Grounds to Chylomicrograph



Graph L: Subjects 16, 20, 42, 43, 49.

Grade 1 1/2 retinopathy.



Graph M: Subjects 14, 15, 46, 47, 48.

Grade 2 and 3 retinopathy.

TABLE III

Young Diabetics

No	A	Wt	F	Count						B P		P	R	T	Bld	Eye	Med.
				0	1	2	3	4	5	S	D						
22	19	54	27	2	62	21	32	57	38	118	70	114	18	98.4	Sl	1	NI
23	20	60	30	3	84	65	85	70	61	130	92	72	16	97.4	St	1/2	TI
23a	20	60	30	1	33	23	63	31	44	130	94	72	16	97.5	St	1/2	I
24	20	67	33	4	32	54	52	6	2	120	78	84	16	98	N	0	TI
24a	20	67	33	13	22	38	4	10	39	130	84	84	14	98	N	0	I
25	24	50	25	2	62	39	38	20	12	110	84	72	14	98.4	N	1/2	TI
25a	24	50	25	7	28	52	38	28	26	120	82	66	16	98.4	N	1/2	I
26	25	74	37	1	35	7	10	62	1	120	80	96	16	99	N	0	NI
27	23	64	32	47	40	78	63	48	57	122	86	95	16	98.4	N	1/2	TI
27a	23	64	32	20	37	96	61	46	28	122	86	95	16	98.4	N	1/2	I
27b	23	64	32	3	71	107	81	57	23	126	86	88	14	98	N	1/2	NI
28	31	60	30	5	35	22	19	19	27	128	82	96	16	98.8	N	1/2	I
29	31	55	28	14	48	35	31	33	48	126	80	96	14	99	N	1/2	NI
30	48	59	30	13	37	26	15	47	77	170	88	96	16	98.4	N	1	NI
31	45	74	37	17	56	47	26	47	27	160	112	90	16	98.6	St	1	NI

Key:

I - Insulin taken before test.

NI - No insulin taken before test.

T - "Tween 80".

For others see Table I.

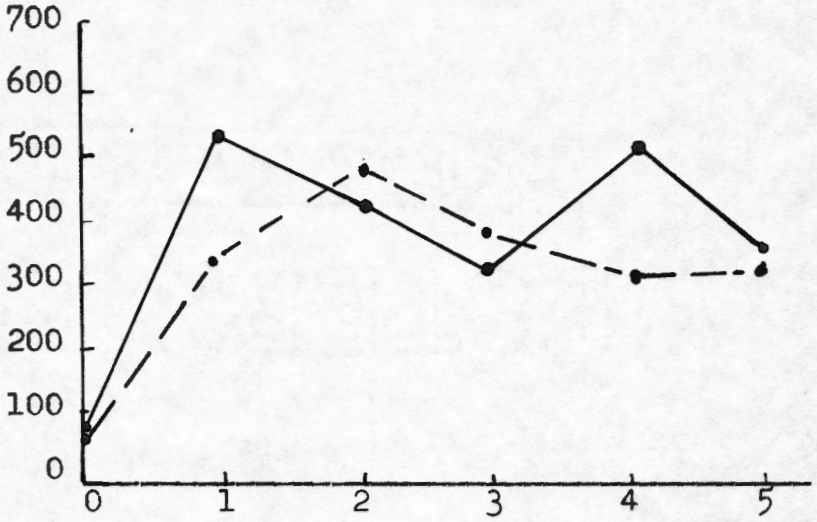
TABLE IV

Old Diabetics

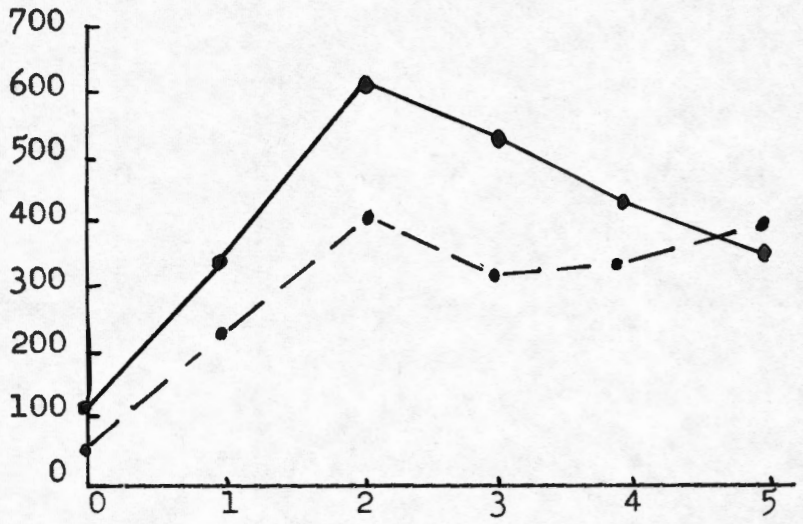
No	A	Wt	F	Count						B P		P	R	T	Bld	Eye	Med.
				0	1	2	3	4	5	S	D						
32	55	61	30	3	11	39	34	13	8	126	86	108	14	97.6	N	1	I
32a	55	59	30	6	3	29	22	7	3	110	66	78	14	97.4	N	1	NI
33	57	66	33	6	15	24	10	43	78	190	94	72	16	97.6	N		I
34	58	54	27	8	19	59	29	34	53	140	90	84	12	97.6	N	1.5	I
34a	58	56	28	2	24	46	62	32	24	150	82	102	16	98.6	N	1.5	NI
35	60	84	42	21	75	60	52	54	55	156	92	81	14	98.6	St	1.5	NI
36	61	54	27	24	50	-	42	45	22	126	64	72	14	98	N	1/2	NI
37	65	63	32	5	13	52	45	31	42	210	104	90	16	97.4	N	2	NI
38	68	64	32	1	1	44	54	47	60	128	70	96	14	98.2	N	2	NI
38a	68	64	32	1	44	34	48	36	21	142	80	96	16	97	N	2	I
39	73	68	34	19	68	112	112	112	92	178	94	72	16	98.6	St	1.5	NI
40	73	64	32	1	34	86	49	17	12	216	92	66	14	98.2	St	1	NI
41	79	70	35	11	29	64	61	40	35	164	82	78	14	98.2	St	1	NI

See tables I, II, and III for symbols.

Young and Old Diabetic Composite Curves With and Without Insulin

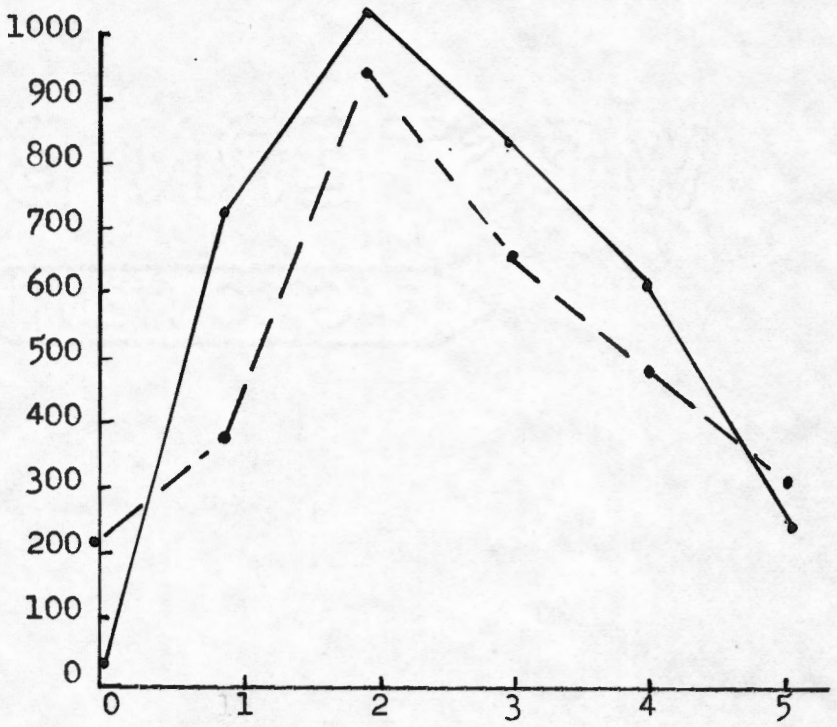


Graph N: Young diabetics (19-48). Av. age 32 years.
Subjects No. 22-31 inclusive.
(———) - No insulin before test.
(– – –) - With insulin before test.



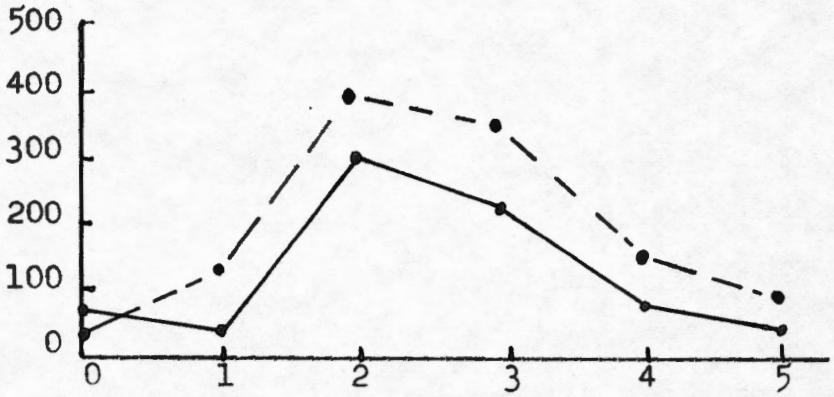
Graph O: Old diabetics (55-79). Av. age 66 years.
Subjects No. 32-41 inclusive.
(———) - No insulin before test.
(– – –) - With insulin before test.

Autocomparative Graphs With and Without Insulin



Graph P: Subject No. 33

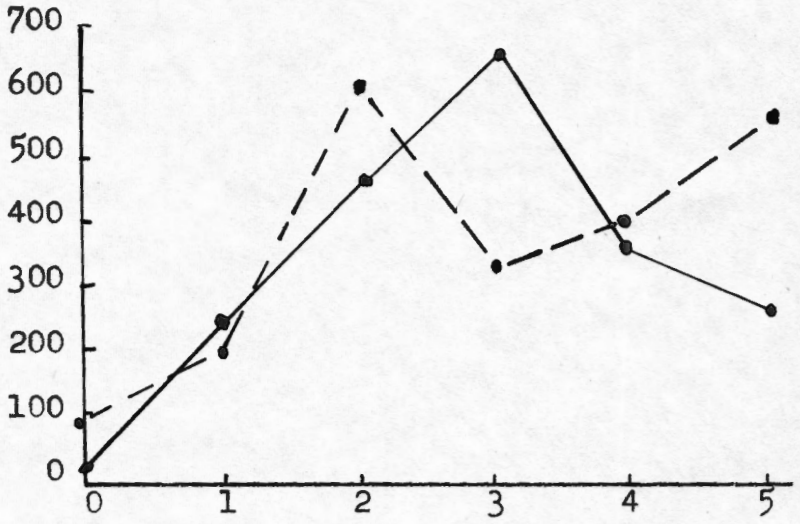
(———) - No insulin before test.
(– – –) - Insulin before test.



Graph Q: Subject No. 39.

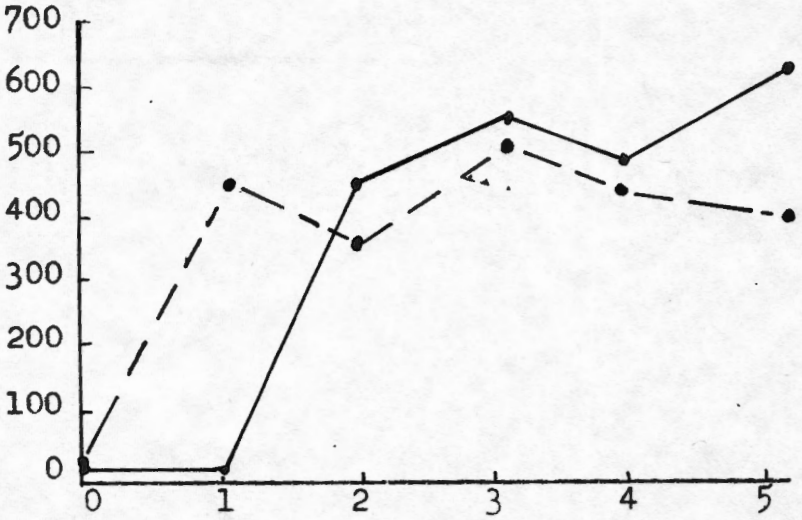
(———) - No insulin before test.
(– – –) - Insulin before test.

Autocomparative Graphs With and Without Insulin



Graph R: Subject No. 41.

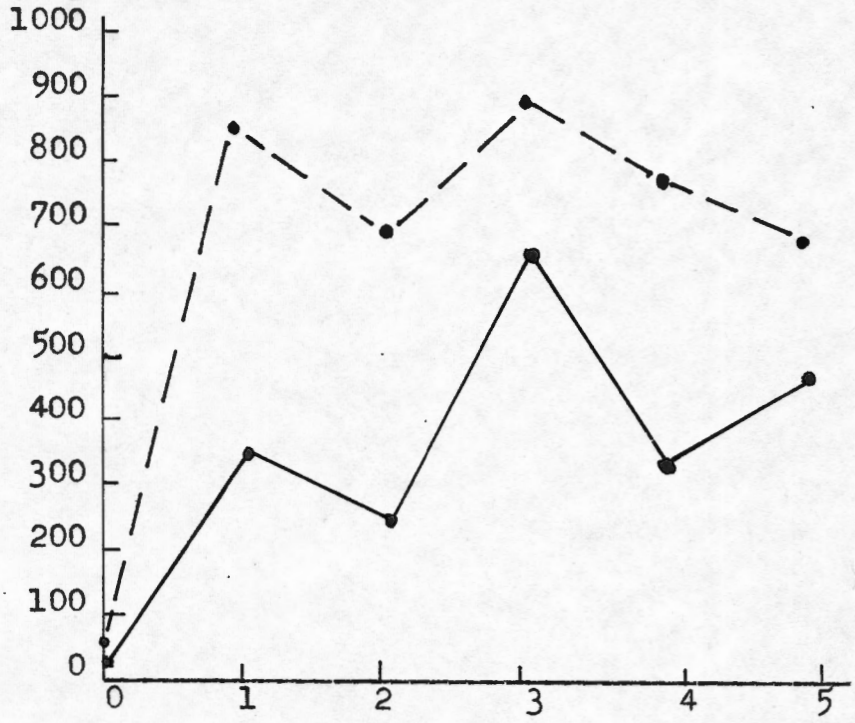
(———) - No insulin before test.
(– – –) - Insulin before test.



Graph S: Subject No. 45.

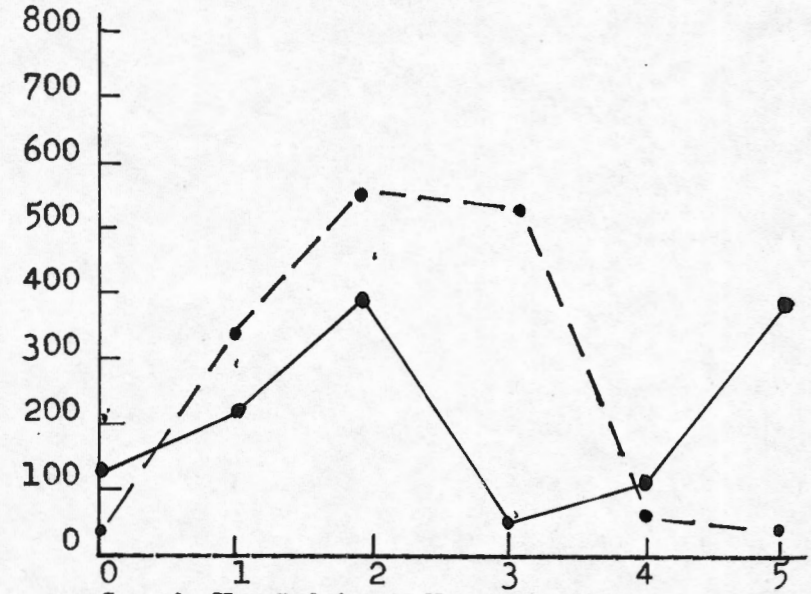
(———) - No insulin before test.
(– – –) - Insulin before test.

Autocomparative Graphs Using "Tween 80" and Insulin and Insulin Alone



Graph T: Subject No. 23, 23a.

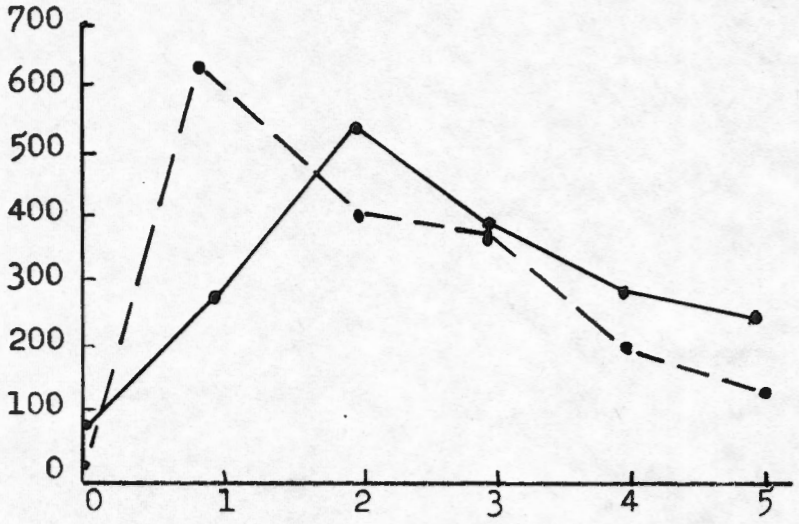
(———) - Insulin only.
(– – –) - "Tween 80" (1.5 gm) and insulin.



Graph U: Subject No. 24, 24a.

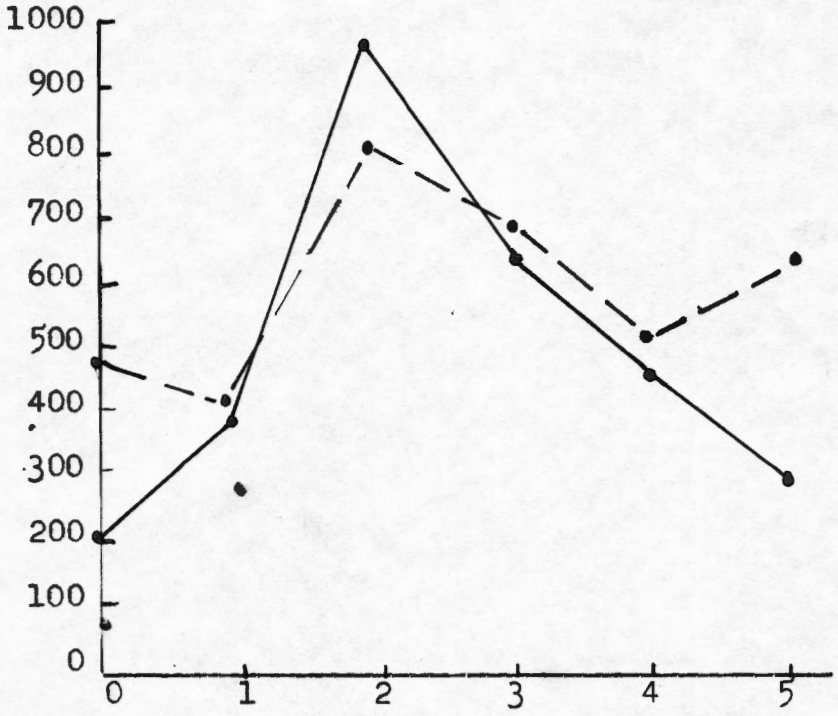
(———) - Insulin only.
(– – –) - "Tween 80" (1.5 gm) and insulin.

Autocomparative Graphs Using "Tween 80" and Insulin and Insulin Alone



Graph V: Subject No. 25, 25a.

(———) - Insulin only.
(— — —) - "Tween 80" (20 gm) and insulin.



Graph W: Subject No. 27, 27 a.

(———) - Insulin only.
(— — —) - "Tween 80" (1.6 gm) and insulin.

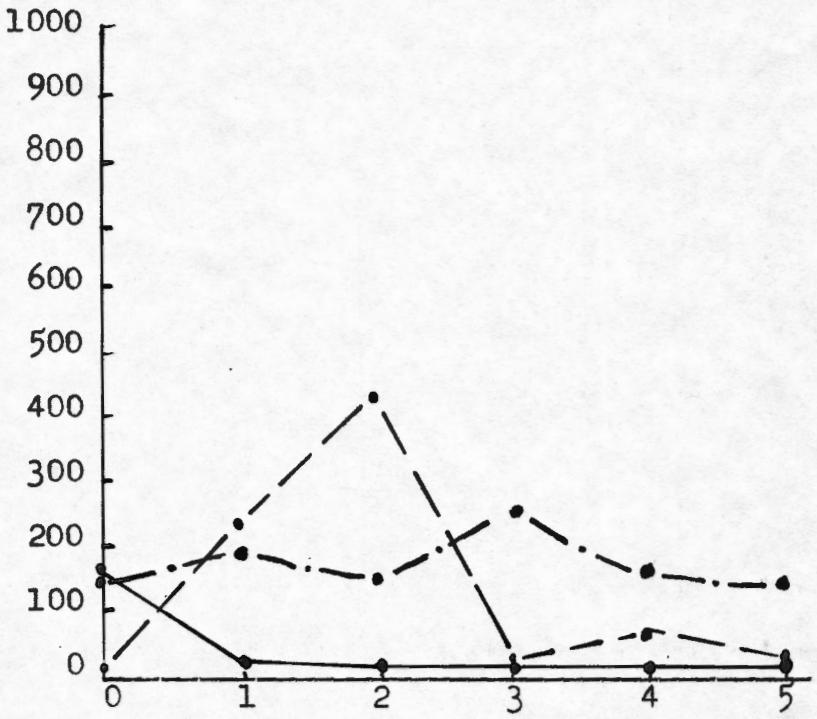
TABLE V

Abnormalities of Digestion

No	A	Wt	F	Counts						B P		P	R	T	Bld	Disease
				0	1	2	3	4	5	S	D					
42	1½	6	3	29	12	50	34	19	32			130	30	97.4	N	Steatorrhea
43	3	10	5	14	11	11	17	8	3			140	36	99	N	Fibro Cystic
43a	3	12	6	6	7	9	18	21	19			100	28	99	N	Tween 80
44	4	11	6	15	75	52	26	3	16						N	Fibro Cystic
45	33	40	20	15	4	1	1	1	.5	90	70	80	20	96	Sl	Sprue
45a	33	40	20	1	22	42	1	5	1	104	76	72	12	96.6	Sl	Tween 80
45b	33	40	20	13	18	12	23	15	14	98	70	80	16	98.8	Sl	AcTH

See tables I and II for symbols.

Comparative Graph in Non-tropical Sprue Patient



Graph X: Subject No. 45, 45a, 45b.

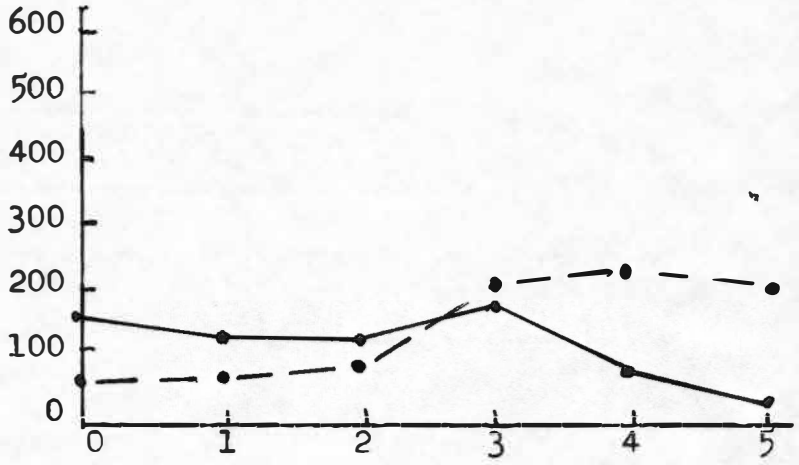
Patient gained 10 pounds in 1 month after start of AcTH; but began downhill course after hospital dismissal, which ended in death 2 months from onset of AcTH therapy.

(———) - No medication before test.

(— — —) - "Tween 80" (1.5 gm) alone before test.

(— • —) - One week of AcTH (15 mg. every 6 hours) before test.

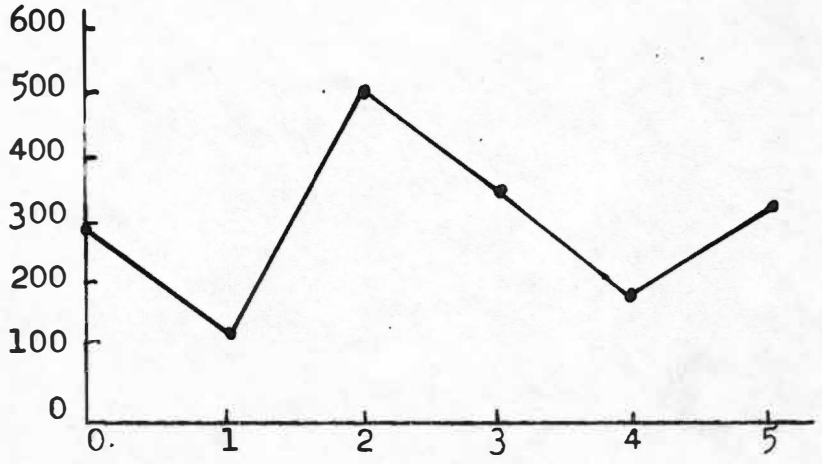
Graphs of Children with Steatorrhea



Graph Y: Subject No. 43, 43a.

Diagnosed as fibrocystic disease of pancreas. Showed no clinical improvement on "Tween 80" for 1 month.

(———) - No medication.
(– – –) - "Tween 80" (1 gm).
Note flattening and delayed peak . "Tween 80" caused slight prolongation.



Graph Z: Subject No. 42.

Diagnosed as steatorrhea, etiology unknown. Improved clinically on panteric granules. No "Tween 80" given.

This curve is interpreted as normal.

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