ABSTRACT

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Recent interest in lung lesions seen in septic shock and demonstration of pulmonary fat embolism in experimental bacteremia prompted an experiment to assess mobilization and transport of lipid in severe bacteremia. Two groups of dogs were fasted for 24 hours, then given intravenous injections of a suspension of E. coli bacteria. When severe bacteremia with prostration was evident, thoracic duct lymph, blood, liver and lung extracts were assayed for triglyceride, cholesterol and free fatty acids.

While thoracic duct lymph showed little change, there was a significant increase in triglycerides in the other specimens.

It is postulated that in sepsis the liver is unable to handle the marked mobilization of lipid that occurs. Triglyceride with minimal or abnormal protein binding is released via the hepatic veins into the circulation. Increased lung triglyceride and pulmonary fat embolism may be explained on this basis.

LIPID METABOLISM IN BACTEREMIA

by

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ABBREVIATIONS

pO_2	= partial pressure of oxygen
pCO_2	= partial pressure of carbon dioxide
pH	= inverse of the logarythm of hydrogen ion concentration
Hct	= hematocrit
SGOT	= serum glutamic oxalo-acetic acid transaminase
LDH	= lactic acid dehydrogenase
ml	= milliliter
mg	= milligram
gm	= gram
µEq/L	= micro-equivalents per liter

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CHAPTER I. INTRODUCTION

The advent of antibiotic therapy for bacterial infections brought with it a great decrease in the mortality from infection. Today, although most instances of sepsis do not result in a life threatening situation, the number of patients that do succumb to this complication is significant. The main cause of infection in these cases is gram negative organisms which reside in the gastrointestinal tract, and which contaminate the peritoneal cavity through necrotic or perforated bowel. The occurrance of gram negative bacteremia with manipulation of the genitourinary tract also is frequent and occasionally goes on to septic shock. The main cause of late death in severe burn cases is known to be septicemia from infected wounds.

Under these conditions septicemia produces many changes in the physiological processes of the body. The most obvious are the effects on the cardiovascular system, resulting in shock. Hypoxemia also occurs in many cases without shock, and has been associated with pulmonary fat embolism at autopsy.

The term fat embolism is generally used to signify either a clinical syndrome or a patho-anatomical finding, the relationship between the two conditions not always being direct. In this thesis the term will refer to the patho-anatomical condition which can be described as the presence

of fat droplets in small vessels.

In contrast to fractures which are postulated by many to cause intravasation of marrow fat, thereby causing the fat embolism, in septicemia it is thought by some that a change occurs in the form of lipid already existing in the blood or being produced by the metabolic pathways.

CHAPTER II. HISTORICAL NOTE

A relationship between abnormalities in fat metabolism and pathology in various organs from fat embolism has been suspected for many years. Magendie (1827) was the first to realize that fluid fat would obstruct circulation. Virchow (1865) first injected oil into a dog's neck vein producing fatal fat embolism, accompanied by the well recognized symptoms and signs of pulmonary edema.

Clinical History

Among the first to realize that clinical conditions could give rise to abnormalities in the body lipids were Zenker (1862) and Wagner (1865) who both described abnormal fat droplets in the blood in some cases of osteomyelitis, pneumonia, and in one crush injury of the abdomen. On the basis of these and other case reports Grohe in 1863 put forth a theory which is still held by many today, namely that embolic droplets of fat seen at autopsy develop from serum lipids that are normally held in suspension. This change in the blood lipid was felt to be due to the altered physical properties of the blood.

Since that time many more cases of fat embolism from a variety of conditions have been reported. In 1917 Bissell implicated fat embolism as an explanation for

cases of post-operative shock. Olbrycht (1922) and Miloslavich (1930) both felt that fat embolism was common after soft tissue injury. Lehman and Moore (1927) reviewed the literature of fat embolism up to that date and in addition experimentally produced fat embolism by ether anesthesia in animals. They found that extracts of necrotic tissue caused the normal blood lipids to come out of emulsion in vitro. On the basis of this and other work they divided the causes of fat embolism into metabolic disturbances, poisoning, toxemias and tissue destruction. All have in common the general element of marked chemical and physical alteration of the blood.

More recent studies have not brought to light any evidence which would suggest that their theory was wrong. MacFarlane (1941) and Cooke (1945) both implicated fat embolism as a cause of death in cases of Clostridia Welchii infections. Grant and Reeve (1951) found that fat embolism to varying degrees was a regular feature of fractures, and 80% of open abdominal wounds showed some evidence of a change in the blood lipids. Forty percent of deaths due to burns were found to be associated with fat embolism (Levitt 1957). Complications arising from technological advances soon showed themselves as cardio-pulmonary bypass

cases were studied. Especially non membrane oxygenators seemed to predispose to fat embolism (Owens 1960). External cardiac massage commonly gives rise to fat droplets in the blood and lungs (Jackson 1965).

Acute hemorrhagic pancreatitis with fat embolism was reported by Lynch (1954). Pancreatitis is also known to be associated with the state of hyperlipemia, and diabetic patients have been reported with fat embolism without trauma (Kent 1955). This association of the pancreas with abnormalities in fat embolism would seem to indicate a role that is not fully known at present, although it is postulated that an inhibitor of lipoprotein lipase is released by the pancreas under some circumstances (Kessler 1962).

The clinical implications of fat embolism and associated disorders has initiated many experimental studies in this field. Various experimental models have been tried in an attempt to reproduce the pathological condition seen in the clinical state. These have demonstrated many facets of the problem of abnormal fat metabolism including hyperlipemia, pulmonary and systemic fat embolism, and fatty liver.

Experimental Work

Swank (1960) found that just by feeding high fat diets to rabbits, pulmonary and systemic fat embolism was produced. Death of the animals resulted from stasis of circulation and ischemia of the brain, probably due to increased viscosity of the blood. He also noted distortion and increased adhesiveness of the red cells in these rabbits. Using a similar preparation he noted thrombocytopenia (30-70% of normal) as well as an increase in the clotting time with this type of hyperlipemia (Swank 1951, 1959).

In an attempt to duplicate the effects of hyperlipidemia occuring as a result of disease or trauma, Peltier (1956) and others (Armin 1951, Connor 1963, Zbinden 1964, Wilson 1965), have infused neutral fat and free fatty acids into animals. Whereas death is produced quickly with a high dose of fatty acids, the same dose of neutral fat has little if any effect. The minimum lethal dose for neutral fat is ten times the dose for free fatty acids. Evidence suggests that the fatty acids reach the lungs before being bound to albumin, and as free fatty acids they are toxic to the capillary endothelium. Neutral fat on the other hand has no toxic action as such but may cause death by mechanical obstruction of the pulmonary circulation.

These experiments have been used to explain the "mechanical theory" of fat embolism, namely that fat from traumatized tissues escapes at the time of trauma but does not exert a toxic effect until one or two days later when hydrolysis of the fat by lung lipases, releases the free fatty acids which produce the characteristic signs.

Feigelson (1961) has shown that raising the fatty acid content of plasma by infusions of catecholamines resulted in a rise of triglycerides and also produced a fatty liver. One explanation of this finding is that an excess of fatty acids is taken up by the liver, which secretes lipoprotein at a fixed rate, thus building up the cellular fat, and producing a fatty liver. It should be noted that both glucose and insulin given concomitantly with epinephrine prevent a rise in the free fatty acid level, indicating that the response is in part due to the availability of glucose for utilization by the tissues, under the stimulus of increased metabolic demands. Other mechanisms for production of a fatty liver under this circumstance would include the direct effect of the catecholamine on liver metabolism or damage of the liver cells during the experiment. Norepinephrine infused directly into the portal vein produced no change in the fatty acid level of the blood, indicating that breakdown occurred in the liver.

Carlson (1965) repeated the above experiment, infusing for a longer period of time and found that fat droplets appear in most tissues of the body, including the lungs. His explanation for this was that the increased level of free fatty acids caused increased uptake of the fatty acids by the cells, which were then unable to metabolize the fatty acids as rapidly as they were entering the cell, thus causing a buildup of fat in the cell.

Another effect of hyperlipidemia which has been reported (Bergentz 1961) is an increased morbidity and severity of fat embolism in animals that have been fed a fatty meal just before the inciting event - trauma in most cases. Bergentz (1961) also found that within half an hour of trauma, plasma triglycerides tend to sediment with the red cell mass, indicating a trapping of triglyceride with this component. This evidence supports the theory that fat embolism is due to an altered chemical state of blood lipids.

Microscopic examination of the blood after a heavy fat meal (Swank 1951) indicates some of the changes that hyperlipidemia causes. In addition to red cell clumping, the chylomicrons tend to cluster, especially if saturated fats were ingested.

Hyperlipidemia has also been shown to be associated with experimental (Kessler 1962) as well as clinical pancreatitis (Lynch 1954). Kessler produced pancreatitis by injecting staphlococcal toxins into the pancreatic duct of rabbits and found that hyperlipidemia occurred. He showed that this was probably due to release of a lipoprotein lipase inhibitor from the pancreas.

Groves (1968) has demonstrated in our laboratory a hyperlipidemia and fat embolism in dogs subjected to repeated doses of E. coli bacteria.

Clowes (1968) recently demonstrated a lipoid pneumonitis after intra-peritoneal infection produced either by strangulation of the gut or intra-peritoneal injection of E. coli and bile.

CHAPTER III. FAT METABOLISM

In order to postulate a pathogenesis of fat embolism and hyperlipemia it is essential to understand the normal pathways of fat metabolism. The fact that many aspects of fat metabolism are contraversial and others are not known will have to be borne in mind as a scheme of normal fat metabolism is presented (Fig. 1).

Normally fat enters the body via the gastro-intestinal tract in the form of triglycerides at the rate of 1-2 gms per kilogram per day. (Frederickson 1967) This is hydrolysed in the gut to monoglycerides and fatty acids. About 75% of the ingested triglycerides are absorbed as monoglycerides (Mattson 1964).

In the mucosal cell the fatty acids and monoglycerides are re-esterified into triglyceride and along with cholesterol, phospholipids and protein are discharged into the lymphatic system as chylomicrons to be carried up the thoracic duct to the venous circulation. Although absorbed free fatty acids account for 60% of the esterified fat in chylomicrons, recently it has been shown by Karmen (1963) that about 40% of the esterified fat in chylomicrons come from endogenous fatty acids. These endogenous free fatty acids could come from serum or the breakdown products of bile in the gut (Baxter 1966). Presumably these replace

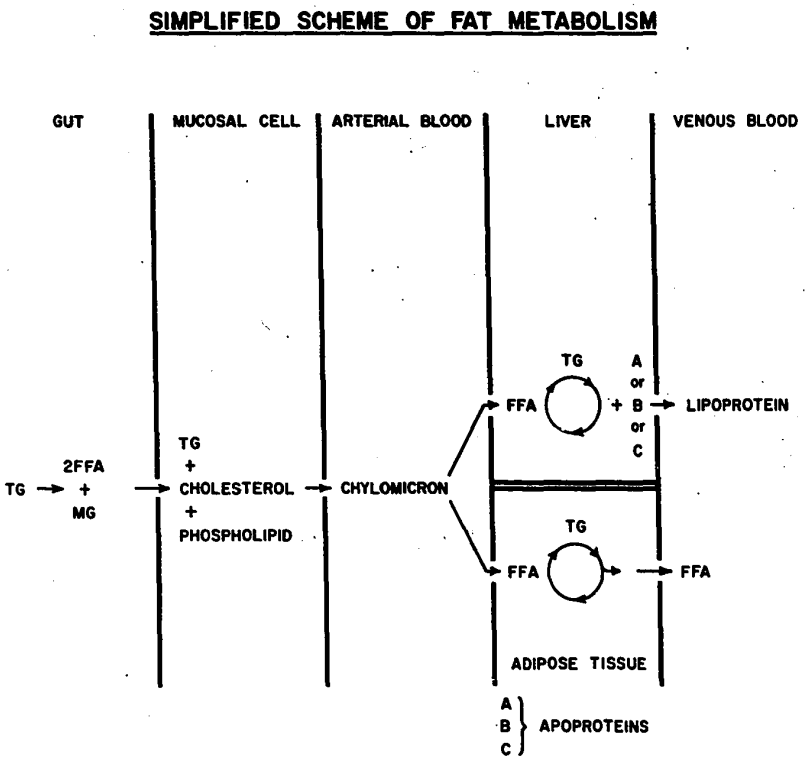

Fig. 1. Simplified scheme of fat metabolism showing formation of the chylomicron in the mucosal cell, and two of its possible routes of metabolism after reaching the arterial circulation via the thoracic duct and superior vena cava.

the absorbed short chain and medium chain free fatty acids that are taken up by the portal venous system.

In the blood the chylomicrons are removed by the tissues of the body. Three avenues are open for the metabolism of the free fatty acids once they are released from the chylomicrons by lipoprotein lipase at the capillary wall level.

The first pathway of metabolism is oxidation of the fatty acids by the body cells for production of energy.

In the liver the chylomicron fatty acids are re-esterified to triglycerides and along with cholesterol and phospholipids are combined with one of the apo-proteins (A, B, or C) to form lipoproteins (Waddell 1953). These lipoproteins are not formed in the hepatectomized animal, a finding supporting the view that the liver is the main producer of the plasma proteins (Borgström 1961, Havel 1961). If sufficient triglycerides are present they may form small particles of fat which differ from the chylomicron particle in the ratio of the lipids they contain (Frederickson 1967) (Fig. 2).

The other course of metabolism that may be followed by the chylomicron is that of hydrolysis at the capillary level in adipose tissue (Havel 1961). In this tissue the

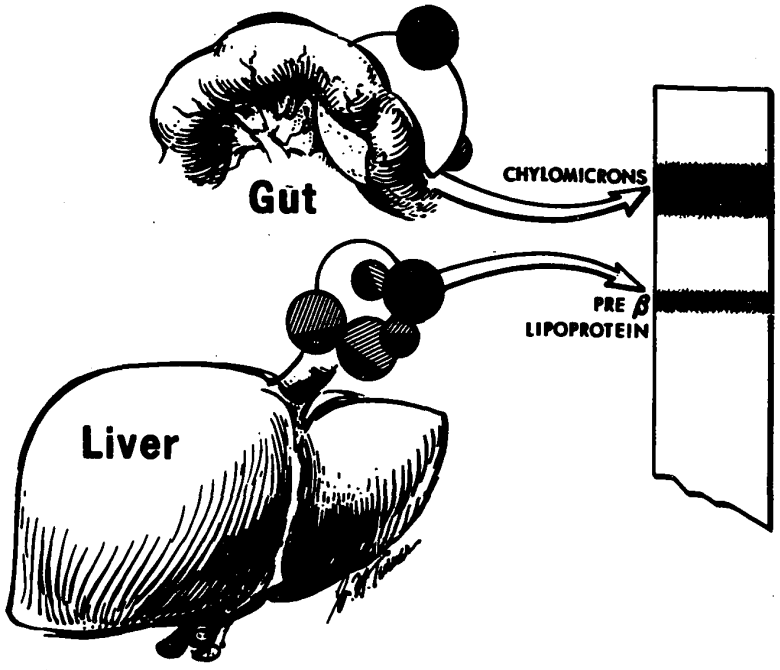

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Fig. 2. Origin of plasma glycerides and the lipoprotein in which they are formed as separated by paper electrophoresis.

fatty acids are re-esterified to triglycerides and then may be released into the blood again as free fatty acids, depending on a variety of factors as will be discussed (Borgstrom 1961).

Fatty Acid

It is generally agreed that the most active substance in normal fat metabolism is the fatty acid circulating in the blood bound to albumin. This substance, although maintained at a relatively stable concentration in the normal individual is in a constant state of flux with more than 25 grams per hour transported in the blood. Stein (1959) found that 60% of labelled palmitic acid was found in the liver 15 minutes after injection. Two thirds of this was found in the form of triglyceride, one third as phospholipid. In the post-absorption state 50-90% of the bodies energy needs are supplied by fatty acids which for the most part come from adipose tissue (Frederickson 1967). Their major contribution to the plasma lipid concentration is their conversion in the liver (Byers 1960, Borgstrom 1961) and gut (Havel 1962) to triglycerides.

Many factors alter the speed of turnover of the fatty acids, including availability of insulin (Shafrir 1959),

blood glucose level (Feigelson 1961, Baker 1968), glucagon, catacholamine release due to stress (Carlson 1968), and sympathetic nerve stimulation (Rosell 1966) and pituitary hormones. Nicotinic acid has been shown to decrease the mobilization of fatty acids, during catecholamine release (Carlson 1968).

As is true of many other substances in the blood, the fatty acid concentration is affected by both production and utilization, although it has been found that the turnover rate of plasma free fatty acids is related in a quantitative manner to the prevailing plasma free fatty acid concentration (Armstrong 1961). That is to say that the rate of uptake by the liver and other tissues is dependent on the blood level of free fatty acids. For this reason it is felt that most agents that affect the concentration do so by altering the production.

Lipoprotein

The third major component of the plasma lipid system is the lipoprotein. Although exogenous lipid in the form of chylomicrons predominate in the absorption state, the lipoproteins or endogenous lipids are most abundant in the post-absorption state. These endogenous lipids are classified in several ways each depending on the particular method used to separate them. These methods depend for their success on the varying chemical and physical properties of the

lipoproteins. Thus, whereas density of the particles is the important feature for centrifugation the electric charge on the particle is very important for electrophoresis. Electrophoresis separates the various lipids into distinct bands which are dependent on the aforementioned properties. Thus there are alpha, beta, and pre-beta lipoprotein bands, as well as the chylomicron band at the origin of the paper when albumin-containing buffer is used (Figs. 3 and 4).

Analysis of these various fractions (Frederickson 1967) would indicate that the chylomicron is a low density particle varying in size from .5 to 5 microns, average size being 1 micron, composed chiefly of triglyceride with a thin shell of protein which stabilizes the particle in its aqueous surrounding.

Alpha lipoproteins on the other hand are high density particles, the composition of which is 45-55% A-protein, 30% phospholipid, and 18% cholesterol.

Beta lipoproteins are low density particles made up of 20-25% B protein, 8% free and 35% esterified cholesterol, and phospholipid accounting for 22%. Triglyceride makes up the remaining 10%.

If the triglyceride output of the liver increases the beta protein becomes less dense and may be found in the pre-beta band which is the very low density band. This

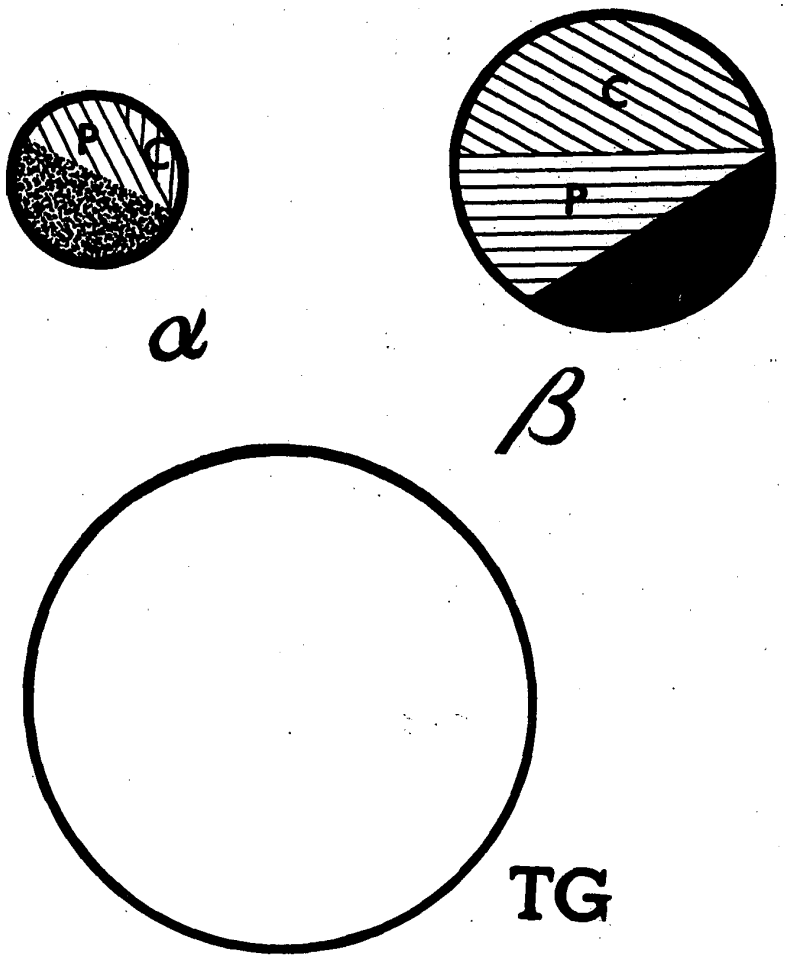

Figure 3. Symbols for the three most important factors in the simplest concept of fat transport by lipoprotein. α = Alphalipoprotein β = Beta lipoprotein TG = Triglyceride: the Phospholipid(P), Cholesterol (C) and Protein (Stippled for A Apoprotein and solid for B Apoprotein). Alloted areas in the symbols comparable to their contribution by weight to the lipoprotein. (Frederickson, D.S., Levy, R.I., Lees, R.S., New Eng. J. Med., 276:38, 1967).

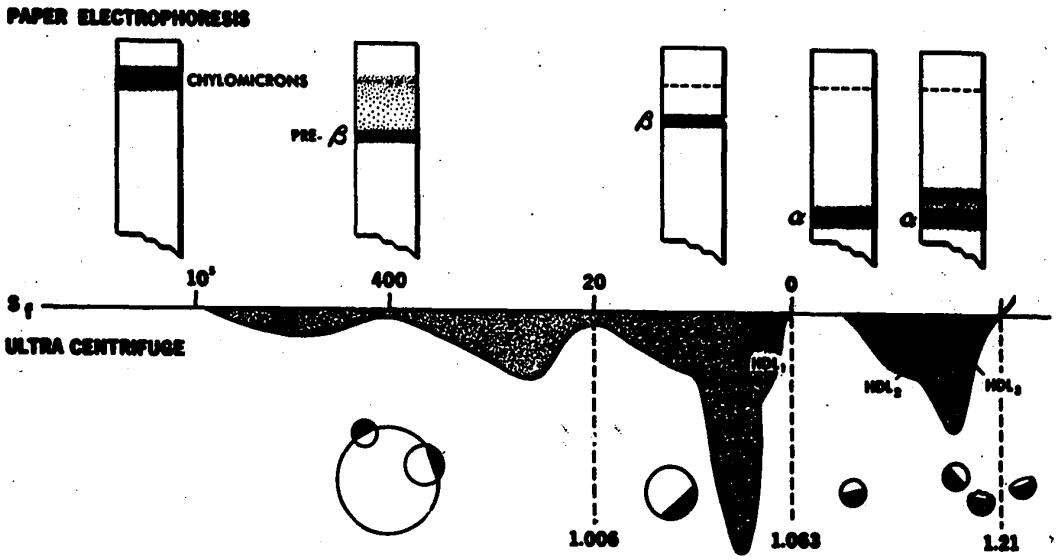

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Fig. 4. Schematic representation of the major portions of the lipoprotein spectrum as defined by paper electrophoresis and ultracentrifugation. (Frederickson, D.S., Levy, R.I., Lees, R.S. New Eng. J. Med., 276:38, 1967)

lipoprotein appears to be a combination of A, B, and C proteins, containing a high percent of triglyceride. They may form particles of lipid that can be seen microscopically, although they do not usually attain a size as large as the chylomicron.

The lipoproteins carry 90% of the cholesterol that is found in the plasma, the rest being carried by the chylomicron. Cholesterol is absorbed at the rate of 100-500 mg per day from the diet, another 1 gm being re-absorbed from the breakdown of bile in the gut. Most tissues can synthesize cholesterol and it is maintained at a steady concentration in the plasma.

The phospholipids constitute the largest mass of plasma lipids, but do not take part to any great extent in the body energy supply. Because of their physical properties they function as "biological detergents" promoting stability at the oil-water interface.

CHAPTER IV. MATERIALS AND METHODS

Preparation of Septic Animals

In the experiment two groups of mongrel dogs weighing between 20-30 kilograms were studied. In both groups dogs were fasted for 24 hours then given intravenous injection of a suspension of Echerichia coli containing 5×10^8 bacteria per milliliter in a dosage of 1 milliliter per kilogram weight. Further injections were administered daily for 2 days to produce severe bacteremia and prostration of the animals. Fasting was continued throughout the experiment. Control animals for both groups were fasted for an equivalent time but did not receive injections of bacteria.

Preparation of Echerichia Coli Suspension

Echerichia coli bacterial suspension was prepared by innoculating two 50 milliliter flasks of Trypticase Soy broth with the bacteria and incubating for 18 hours. This material was centrifuged at 3000 revolutions per minute for 30 minutes, then the organisms were washed twice in normal saline. They were then re-suspended in 100 milliliters of normal saline and a viable count was performed. Saline as indicated was then added to bring the suspension to a concentration of $5-6 \times 10^8$ bacteria per milliliter.

Experiment I

These animals consisted of 11 experimental and 11 control dogs. They were anesthetized with Nembutal (20-25 milligrams per kilogram) intravenously, intubated and placed on a volume respirator. A right thoracotomy through the 8th interspace was then performed and the thoracic duct was cannulated with PE tubing #90. The duct was cleared for 2-3 cms at the level of the 9th rib, and any accessory communications ligated. The cannula was inserted at least 3-4 cms distally, the tip lying below the level of the diaphragm. The tubing was then led out through the incision and the thoracotomy closed. A #16 red rubber catheter was left in the chest to evacuate the pneumothorax. The animal was then taken off the respirator and the tubing from the lymphatic duct was inserted into a test tube to which 10-15 centimeters of water of suction was applied (see diagram). Four to five drops of 15% liquid ethylene-diamine tetra-acetic acid was inserted into the test tube to prevent coagulation of the lymph. The first half hour drainage was discarded before starting hourly collections for analysis.

A femoral artery catheter and femoral vein catheter were inserted with the venous line being positioned close to the hepatic vein before closure of the thoracotomy incision. Blood pressure was monitored from the femoral artery by a Sanborn pressure transducer and recorder. Blood for lipid

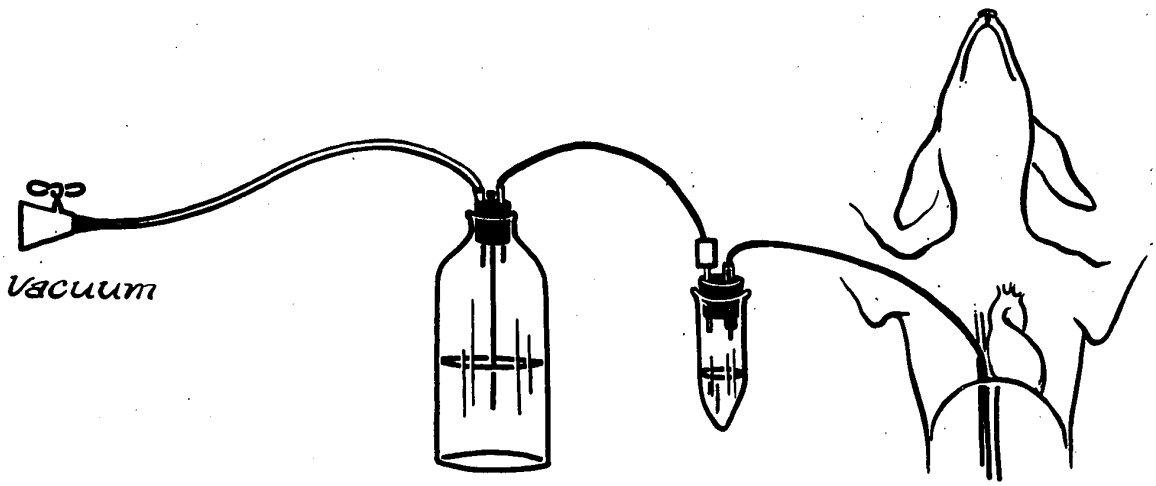


Illustration of system used for collection of thoracic duct lymph. Approximately 10 cm. of water suction was applied to the tubing in the thoracic duct, and the lymph collected without coming in contact with anything other than the plastic tubing, ensuring proper collection.

studies was taken from the venous catheter at the beginning and at the end of the experiment.

Experiment II

Twelve experimental and 12 control dogs were anesthetized as for Experiment I. Arterial blood was taken for pO_2, pCO_2 and pH, lipid analysis, Hct, SGOT and LDH. Rectal temperature was monitored. The dogs were then given a lethal dose of Nembutal and immediately through a right thoraco-abdominal incision, the lungs and a major portion of the liver were removed. Blood on the exterior of the organs was washed off. These organs were then minced for 10-15 seconds in a Waring blender with glass container. Five grams of minced tissues was put into an Erlenmeyer flask and 100 ml. of chloroform: methanol 2:1 mixture was added. Another 50 gm was weighed and the beaker put into an evaporation oven at 100°C for 24 hours.

Blood and Lymph Analysis

Specimens of blood were put immediately into vacutainer tubes containing .17 milliliters of ethylene-diamine tetra-acetic acid (E.D.T.A.). These, as well as tubes containing the lymph were then spun down in a centrifuge at 2,000 revolutions per minute for 20 minutes. The plasma was separated

and put into a cold room at 4°C as was the lymph if immediate analysis was not going to be carried out. The specimens were then analysed for free fatty acids, by Doles method, as modified by Trout, Estes and Friedberg (1956). Results were given in micro equivalents per litre. Cholesterol was analysed by Leffler's method (1962) and the results expressed as milligrams per 100 millilitres of plasma. Triglycerides were analysed as described by Van Handel (1961) and results given as milligrams per 100 millilitres of plasma.

The thoracic duct lipid output was calculated by multiplying the amount of lipid per millilitre by the volume of lymph collected per hour.

Blood gases including pO_2, pCO_2 and pH were determined on an Instrumentation Laboratory blood analyser. Hematocrit was evaluated in a Clay-Adams microhematocrit tube. SGOT studies were evaluated by the Trans-Ac technique (Babson 1962) a colorimetric assay while LDH was done by the Lacdehystrate (Babson 1965) colorimetric assay. Serum and lymph for lipid analysis was also subjected to paper electrophoresis, using the Beckman Model R electrophoresis system. The technique used was a modification of that described by Jencks and Durrum (1955) using albumin and barbital buffer with oil red 0 as the staining material.

Tissues for extraction were treated after the method of Folch (1957). After incubating one hour in an Erlenmeyer flask, with occasional stirring, the 5 grams of minced tissue and 100 ml of chloroform: methanol 2:1 mixture were strained through a filter paper into a separating funnel. Twenty mls of distilled water acidified with 1-2 drops of sulphuric acid were then added and the flask shaken vigourously. After separation of the two layers, the bottom layer was separated equally into four test tubes each containing about 16 mls. One milliliter from one of the test tubes was analysed for triglyceride, it being treated in the same manner as a plasma sample. The remaining tubes were evaporated under vacuum at 45°C.

The lipid in one tube was re-suspended in heptane for the fatty acid determination and isopropanol was the solvent used to dissolve the lipid for cholesterol estimation.

Calculations for the total lipid included correction for the various dilutions, as well as taking into account the dried weight of the sample. Basic calculations for each of the various components are as follows.

Triglycerides

$$\frac{\text{Concentration (mg/100 ml)} \times \text{volume (66 ml)}}{\text{100 ml}} \div \frac{\text{5 (gm)} \times \text{dry weight (gm)}}{\text{50 (gm)}}$$

$$= \text{weight (mg) / gm of dried tissue}$$

Free fatty acids

$$\frac{\text{Concentration } (\mu Eq/L) \times \text{vol. (6 ml)}}{1000 \text{ ml}} \div \frac{5 \text{ (gm)} \times \text{dry weight (gm)}}{50 \text{ gm}}$$

= weight μEq/gm of dried tissue

Cholesterol

$$\frac{\text{(2x) concentration (mg/100 ml)} \times 2 \text{ cc}}{100 \text{ ml}} \div \frac{5 \text{ gm} \times \text{dry weight}}{50 \text{ gm}}$$

= mg/gm dry weight

All results were subjected to the unpaired t-test for evaluation as to the significance of the differences between the experimental and control groups. This was done by a computer programme developed by others in our laboratory.

CHAPTER V. RESULTS

I. General Condition of Dogs

All experimental dogs exhibited vomiting and diarrhea within 10-15 minutes after the first injection of E. coli. Only those dogs which survived 24 hours and looked relatively healthy were given a second dose. It was noted that those that were going to get progressively worse would again vomit and have diarrhea. Approximately one third of those studied were investigated after the first injection, and the remainder after the second or third injection. One third of the dogs given the initial injection died within 12 hours.

In Experiment I, 3 dogs died on the table before the end of the collection period, going into shock over the 3-4 hours of collection. These dogs also exhibited an elevated temperature of 106-108°F and had coffee-ground material coming from their mouths and tarry stools. The rest of the dogs in both groups had normal or slightly lowered blood pressure but were not in shock at the time of study.

It was found that pO_2 and pCO_2 of both controls and experimental animals were in the same range, no significant differences being observed. (Table 1)

The temperature and hematocrits were significantly elevated in the septic dogs while the arterial blood pH was lowered in these dogs.

TABLE I

PARAMETERS MEASURED IN EXPT. II

	Control	Experimental	p Value
Arterial pH	7.347±.04	7.295±.07	< .02
Arterial pO_2 (mm Hg)	70.9±11.1	71.1±12.7	N.S.
Arterial pCO_2 (mm Hg)	37.4±5.6	34.6±7.8	N.S.
S.G.O.T. (Trans Ac units)	23.4±6.8	151.4±144.0	< .01
L.D.H. (International units)	86.8±43.8	93.7±68.4	N.S.
Temperature (°F)	101.7±1.1	103.6±2.0	<.01
Hct. (%)	40.7±7.4	46.5±7.3	<.05

Liver function tests showed a marked elevation of the S.G.O.T. in the experimental dogs, although the L.D.H. values were not changed.

Results - Experiment I

a) Thoracic duct lymph.

In our group of dogs it was noticed that volume of flow did not seem to vary with the weight of the dog to any extent. The successful placement of the catheter well down the duct, and the application of the appropriate amount of suction seemed to be the important factors for collecting the lymph. As noted in Figure 5 the flow of both groups of animals decreased with time, and there was no significant difference detected between the two groups.

One other factor which affected flow in the early studies (not reported) was that of clotting of the lymph in the tubing, where there was a metal connector. Elimination of the metal connectors assured a continuous flow of lymph. It was noted as well that whereas occasional clots would form in the control dogs lymph, even with anticoagulant in the test tube, no trouble was encountered with septic dogs' lymph.

This in spite of the hemorrhagic nature of the septic dogs lymph, which would usually become increasingly more

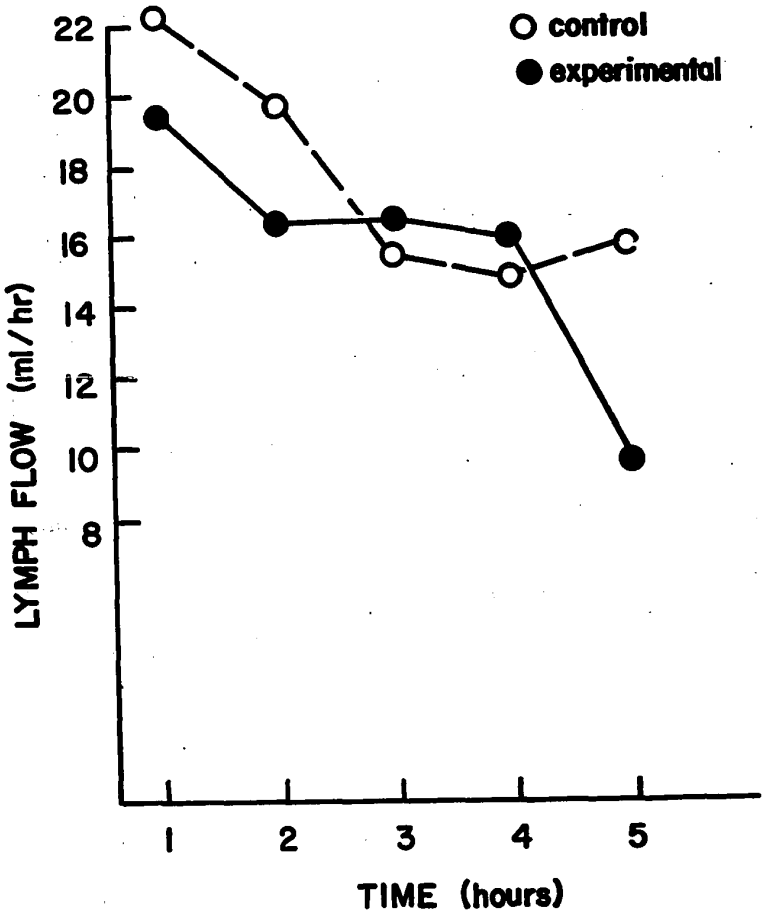


Fig. 5. Volume of lymph collected from the thoracic duct in experiment I. Collection commenced one half hour after all manipulation of the animal had ceased, and animal breathing on his own.

hemorrhagic as the experiment progressed. When spun down the lymph retained a yellow tinge, and often had a hematocrit of 1-2%.

Varying degrees of turbidity of the lymph was noted, and corresponded to the amount of triglyceride present. As seen in Table II, the concentration of triglyceride as well as the actual amount of triglyceride decreased with time, for both groups of dogs (Fig. 6).

This trend was also observed for the other components of the lymph studied, namely fatty acids and cholesterol. In spite of an increase in concentration as the collection proceeded, the output of both these components fell and in fact correlated well with the output of the control dogs (Figs. 7 and 8) (Tables III and IV).

b) Blood lipids.

Several important points are to be noted with regard to findings in the blood studies. The first of these is the higher plasma free fatty acids in the experimental animals (Table III). Although not in an excessively high range they do differ significantly from the control animals at the beginning of the lymph collection. After 5 hours however the fatty acids have fallen to within range of the controls.

Although cholesterol values are similar as seen in Table IV, it will be noted in Table II that the serum triglycerides are almost two and one half times higher in the

TABLE II

TRIGLYCERIDE CONCENTRATION - EXPT. I

LYMPH

Time (hr)	Control (mg%)	Experimental (mg%)	P Value
1	536.3	561.0	N.S.
2	456.5	533.7	N.S.
3	391.5	490.7	N.S.
4	270.8	383.6	N.S.
5	257.0	402.1	N.S.

PLASMA

1	66.4±30.5	163.8±114.5	$<.02$
5	71.6±37.0	175.9±108.9	$<.01$

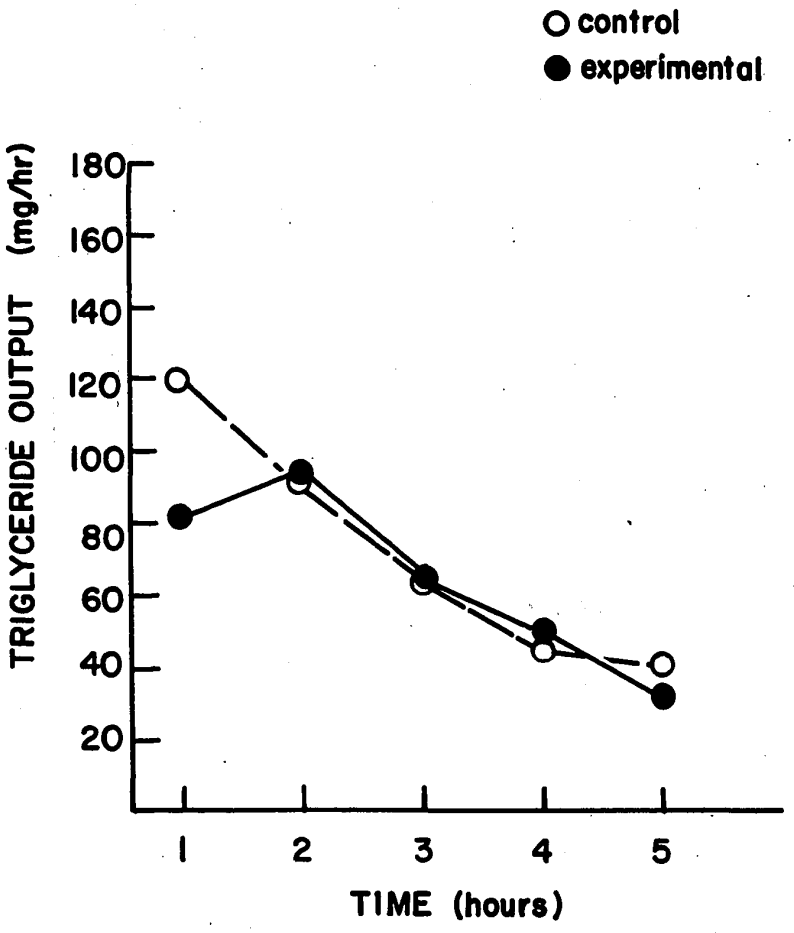


Figure 6. Triglyceride output in thoracic duct lymph in experiment I.

TABLE III

FREE FATTY ACID CONCENTRATION - EXPT. I

LYMPH

Time (hr)	Control (μEq/L)	Experimental (μEq/L)	p Value
1	969.0	739.6	N.S.
2	834.7	764.5	N.S.
3	764.8	781.3	N.S.
4	682.1	778.8	N.S.
5	764.3	828.4	N.S.

PLASMA

1	501.6±119.6	779.1±157.3	$< .01$
5	551.8±93.9	611.9±247.6	N.S.

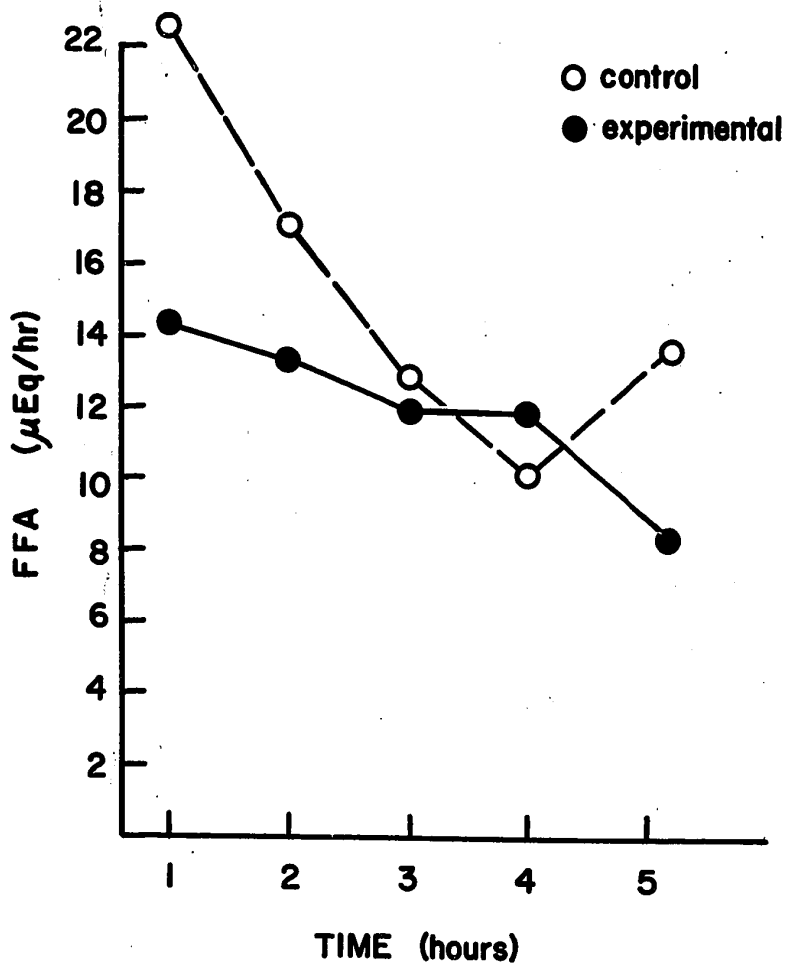


Figure 7. Fatty acid output in thoracic duct lymph in experiment I.

TABLE IV

CHOLESTEROL CONCENTRATION - EXPT. I

LYMPH

Time (hr)	Control (mg%)	Experimental (mg%)	p Value
1	109.0±28.9	140.5±55.3	N.S.
2	105.4±25.5	139.8±49.9	N.S.
3	99.5±26.6	141.8±60.0	$<.05$
4	92.3±33.0	135.0±49.7	$<.05$
5	95.8±33.5	169.8±61.3	$<.01$

PLASMA

1	139.8±45.9	259.8±170.5	N.S.
5	158.2±51.8	209.7±89.9	N.S.

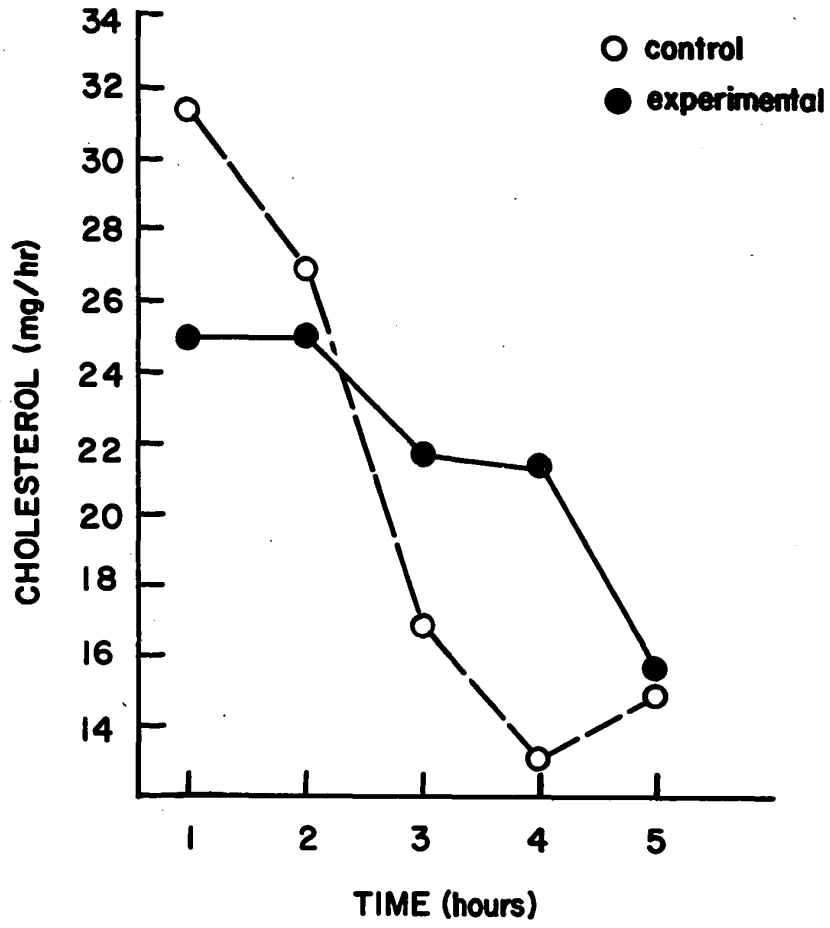


Figure 8. Cholesterol output in thoracic duct lymph in experiment I.

experimental group, both at the beginning and the end of the experiment. In spite of the loss of triglyceride via the lymph, the mean value actually is higher at the end of the experiment, and the difference between groups is slightly more significant.

Electrophoresis of plasma detected a change in the lipoprotein pattern of the plasma after bacteremia (Figure 9). Whereas normal fasting dogs showed no lipid at the origin, bacteremic dogs showed a definite "chylomicron-like band", that was the same as that expected after a fatty meal in normals. The rest of the electrophoretic strip was essentially normal except for a questionably thickened beta band in some dogs. It should be noted that there was no increased lipid in the pre-beta band, the usual place to find "endogenous" triglycerides. Electrophoretic strip of lymph detected lipid at the origin in most controls in which this was done, therefore this was not carried out on the experimental dog's lymph.

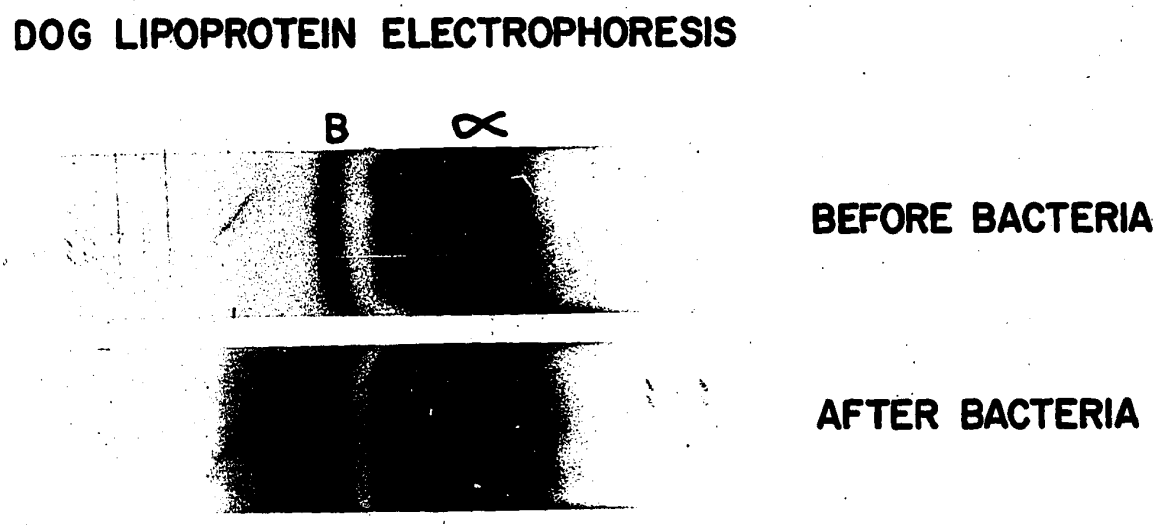


Figure 9. Example of the findings on paper electrophoresis of the plasma in a normal control and a bacteremic dog. Note increase in density in the chylomicron band at origin of paper after bacteria.

Results - Experiment II

a) Blood Lipids

The same change in blood triglyceride levels was observed in these experimental dogs as in the first group. That is the septic dogs had triglyceride levels two and one half times higher than controls (Table V). There was a small increase in the serum levels of cholesterol and free fatty acids in the experimental animals, however, the increase was not significant.

It is to be noted that although the relative increase of serum triglycerides in the experimental animals corresponds to the relative increase in liver triglyceride content, there was a relatively less increase in lung triglyceride.

Serum cholesterol levels changed very little compared to the ratio of increase in liver cholesterol content in the experimental animals.

b) Organ extraction

At the time of removal, the lungs of the septic dogs often had petichial hemorrhage, and two had areas of gross hemorrhage into the lung substance.

Analysis of the lungs showed a significant increase in the triglyceride content of the septic dogs. Fatty acid and cholesterol analysis failed to reveal any significant change in content (Tables V, VI and VII.)

Liver analysis (See Table V) showed a three-fold increase of triglycerides in the septic livers, while a decrease in the amount of cholesterol was noted. Free fatty acid analysis showed no change. At the time of removal of the livers it was often possible to predict that an increased amount of fat would be found because of the yellow colour of the liver.

TABLE V

TRIGLYCERIDE EXTRACTION EXPT. - DOGS

	Control	Experimental	p Value
Serum triglycerides (mg%)	88.7±42.6	235.0±157.1	< .01
Liver triglycerides (mg/Gm D.W.)	43.4±33.2	136.4±80.9	< .01
Lung triglycerides (mg/Gm D.W.)	23.9±9.9	38.7±20.8	< .05

TABLE VI

FREE FATTY ACID EXTRACTION - DOGS

	Control	Experimental	p Value
Serum F.F.A. (μEq/L)	591.9±344.6	622.2±400.8	N.S.
Liver F.F.A. (μEq/Gm D.W.)	20.7±7.4	18.1±6.9	N.S.
Lung F.F.A. (μEq/Gm D.W.)	23.23±14.8	21.31±11.5	N.S.

TABLE VII

CHOLESTEROL EXTRACTION - DOGS

	Control	Experimental	p Value
Serum cholesterol (mg %)	196.6±128.06	223.3±72.4	N.S.
Liver cholesterol (mg/Gm D.W.)	9.4±5.1	16.0±6.2	$< .01$
Lung cholesterol (mg/Gm D.W.)	17.2±7.1	22.1±12.2	N.S.

CHAPTER VI. DISCUSSION

Although the effects of gram-negative bacteremia and endotoxemia have been studied from many different aspects, few have documented the nature and extent of changes in fat metabolism. This subject has significance because of the pathological deposits of fat seen in various organs after these conditions. The lymphatics are important in the over-all metabolism of fat and a few workers have studied the characteristics of lymph after endotoxin.

Ballin (1959) investigated intestinal lymph flow in the dog after endotoxin, and found that although there was a direct correlation between lymph flow and portal pressure in control dogs, in dogs given endotoxin the flow was greater than expected and showed no correlation with the portal pressure. This effect presumably was due to an increased capillary permeability in the gut.

Alican (1961) also investigated lymphatic flow after a lethal dose of endotoxin and found that shortly after the administration of endotoxin the hepatic lymph flow increased markedly, correlating with the rise in portal venous pressure. The intestinal component on the other hand did not increase until a short time later when the portal pressure was back to normal. When he measured the

total flow from the thoracic duct, these two components were measured as a 4-5 fold increase in flow commencing shortly after endotoxin and continuing while the animal was in endotoxin shock, gradually decreasing as the animal succumbed.

It is not possible to compare these results directly with our animals as they were obtained within a short time of the administration of the endotoxin. These experiments however might lead one to believe that the lymphatic flow would be increased in our septic dogs. This was not the case as the mean flow in our experimental dogs was not significantly different from controls. One explanation for this finding is the fact that the dogs were somewhat dehydrated, as indicated by the higher mean hematocrit in the experimental dogs of experiment II. If capillary permeability was normal or only slightly increased at the time of measurement the results are as might be expected.

Concerning the lipid content of the lymph, much work has been done in the normal but very little on the septic animal.

Morris (1956) found that hepatic lymph cholesterol concentration was 85% of plasma, and hepatic lymph flow constituted 30% of the total flow of thoracic duct lymph.

Friedman (1961) found the hepatic and intestinal lymph cholesterol concentrations about half that of plasma, although Bollman (1951) states that cholesterol concentration was the same in both hepatic lymph and serum. As noted in our results, the cholesterol concentration of the lymph varied between 60 - 78% of the plasma concentration in controls and 54 - 80% of the plasma concentration in experimental dogs.

The source and amount of the lymph lipids is somewhat disputed, in that different percentages are given for the amount of endogenous versus exogenous lipid. Possibly one reason for the differing results is indicated by the finding of Rampone (1961), who found that whereas dogs fed by duodenal tube had increased amounts of lipid in the thoracic lymph for 13 hours, those fed by gastric tube had elevated levels for 24-48 hours. Thus the common practice of starving animals for 12 hours or even 24 hours before obtaining basal lymph values will produce false results in some cases. After 24-48 hours starving, he found that the average rate of lipid transport was 124 mg/hour.

Shrivastava (1967) reported an 80% decrease in the esterified fatty acids of thoracic duct lymph in fasting rats with a bile fistula. He therefore suggested that

the phospholipids of bile released fatty acids in the gut which in turn were absorbed and formed a large portion of esterified fatty acid in thoracic lymph. Karmen (1963) found that a full 40% of esterified fat in lymph was endogenous, that is synthesized by the mucosal cell, from non-ingested fat. Baxter (1966) on the other hand felt that 50% of fatty acids in lymphs were from bile lipid and only a little circulating free fatty acid was incorporated into lymph lipid by the cell. Rampone has found that only 1.2% of re-infused chylomicrons are found going back up the thoracic duct, indicating a slow plasma-lymph exchange in this form.

Our results would tend to support the findings of those who found that a large portion of esterified fatty acids in the thoracic duct are not exogenous in origin, as our dogs, both control and experimental, had been fasted for at least 2 days and yet had a high concentration of triglyceride in the lymph. Presumably this is explained by the contributions of the bile fatty acids as well as endogenous fatty acids being esterified in the gut.

Our observations that the lymph was hemorrhagic in the experimental dogs confirms the finding made by Alican (1961) who noted that both intestinal and hepatic lymph became bloody after endotoxin. These observations are explained

by microscopy findings of the intestinal and hepatic lymphatic systems.

Baez (1955) studied the intestinal lymphatics and found that transavasation of red cells occurred in this tissue, after endotoxin. De Palma (1967) studied the liver ultra-structure by electron microscopy and found after endotoxin that several changes were evident, within 2 hours of the injection. There was centrilobular necrosis as well as a generalized depletion of glycogen. Swelling of the Kupfer cells was evident, and it was noted that red cells were in the space of Disse.

These observations would fit in with the findings of Hardaway (1966) and Gans (1960) who describe a process of intravascular coagulation with resultant fall in fibrinogen and platelets. This process results in prolongation of the clotting and bleeding time, increasing the possibility of red cells getting into the lymphatic channels.

The anatomic alteration of the liver is reflected in abnormal enzyme production as determined histochemically. Coscorano (1961) studied the effect of E. coli endotoxin and found depletion of succinic dehyrogenase in the centrilobular area within 10 minutes of injection. This picture did not change appreciably with time over the 24 hours studied.

Hirsch (1964) found that B.S.P. retention increased to 5 - 20% within 2 hours of injection of endotoxin.

These studies are in agreement with our finding of elevated SGOT levels, and perhaps indicate why this abnormality was found.

Pulmonary Fat Embolism

Fat embolism is well known as a complication of skeletal trauma, and has been shown to occur in sepsis. Few studies have attempted to quantitate this phenomena in sepsis.

Nelson (1951) reported the effects of parcolon bacteremia, which were similar to our own in that metabolic acidosis, elevated hematocrit and rise in temperature were noted. He also mentioned that histological section of the lung showed dilated capillaries with hemolysed blood in them.

Lillehei and MacLean (1958) found that pulmonary lesions were more common in dogs that survived 24 hours than those dying sooner after endotoxin. The typical gut lesions were somewhat less in these dogs than in those dying sooner. The pulmonary lesion in these dogs consisted of clinical pulmonary edema with hemorrhage and congestion being found at autopsy.

Hirsch (1964) also noted hemorrhage and pulmonary edema in rabbits that had been given two doses of endotoxin 24 hours apart.

Groves (1968) and Allardyce (1968) in our laboratory both found histological evidence of pulmonary fat embolism

in dogs and rabbits respectively, after bacteremia and endotoxin. In the studies, the degree of fat embolism was impossible to estimate and not all experimental animals had fat embolism on histologic section. Our results suggest that fat embolism is a regular feature of bacteremia as an increase in the triglyceride content of the lung was found in 80% of experimental animals. Possibly because of the hyperlipemia present in our dogs the values are much higher than the hyperlipemic dogs described by Bergentz (1962). His results indicate that the amount of embolic fat is determined to some extent by the serum triglycerides.

Lequire (1959) saw birefringent crystals similar to those of cholesterol in 9 patients and in rats that had fat embolism when subjected to rapid decompression from hyperbaric conditions. The embolic fat contained free and esterified cholesterol in greater amounts than normal adipose tissue. In our study there was no significant increase in cholesterol in lung tissue despite demonstration of fat embolism and increased lung triglycerides.

Fatty Liver and Pulmonary Embolism

Clinically, fat embolism after trauma is felt to be more common among patients who have a fatty liver and cirrhosis due to alcoholism (Lynch 1957). This fact has prompted the proposal that fat globules, unbound to

protein and therefore insoluble in blood, rupturing out of the liver tissue and are the responsible agent in these cases. A similar theory was postulated for the septic dogs by Groves (1968).

Hartroft (1951) observed fat globules breaking into bile canaliculi and hepatic sinusoids of rats that had fatty livers because of choline deficiency. He also showed fat embolism in lung and kidney and believed that this fat represented embolization from fatty liver (1955).

In our experiments there was an association between increased liver and lung triglycerides in an experimental model which had been shown previously to be associated with pulmonary fat embolism. If lung embolisation does occur from liver it is unlikely to occur via the lymphatics since the lipid output of the thoracic duct was the same for control and experimental dogs. We cannot rule out fat embolization via the hepatic veins however.

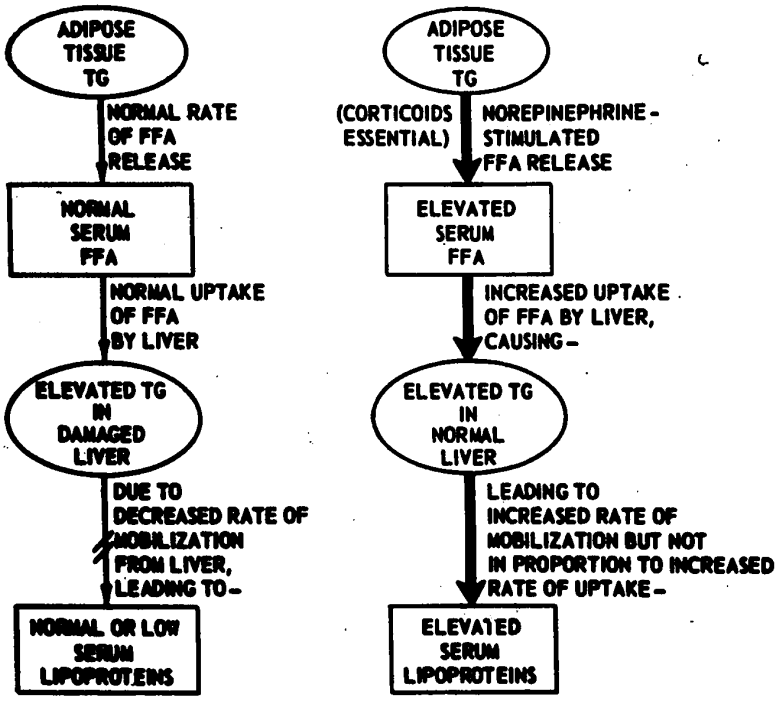
Fatty Liver and Hyperlipedemia

That there is an association between fatty liver and hyperlipedemia has been appreciated for many years. Hypertriglyceridemia and fatty liver have been produced in several experimental studies. Both Feigelson (1961) and Carlson (1965) demonstrated hypertriglyceridemia in dogs following intravenous infusions of norepinephrine. In

addition Carlson found fat droplets in the lung, liver and muscle tissue and noted that the dogs exhibited tachpnea and hyperpyrexia, signs associated with clinical fat embolism. Fatty livers are known to be associated with increased fatty acid mobilization and these results suggest that lipid mobilization with increased catecholamine levels is an important mechanism in the production of fatty livers. The elevation of the fatty acids and triglycerides in our dogs would suggest that there was a greatly increased mobilization over a fairly long period, at least 8 hours since it has been shown that fatty acids must be elevated for at least 6 - 8 hours before a fatty liver can be induced.

Mechanism of Fat Embolism

From the above evidence several mechanisms explaining the observed changes can be postulated (see Fig.10). It should be stated first however that despite several plausible mechanisms whereby hyperlipidemia and fat embolism might occur, hyperlipidemia per se has no direct cause and effect relationship to fat embolism. Clinically (Frederickson 1967) and experimentally (Morris 1956) hyperlipemia can exist without the pathological changes that we have found. Under the conditions of our experiment however it seems probable that a relationship does exist, and may occur by one of the following mechanisms.



Feigelson et al I967

Figure 10. Theory explaining increased liver triglyceride and elevated serum lipoproteins as prepared by Feigelson et al. (J. Clin. Invest., 40:2171, 1961).

The first is that proposed in principle by Feigelson (1961) for a healthy liver, that is, the elevated catecholamines and adrenal corticoids release free fatty acids from the adipose tissue which in turn go to the liver. The liver increases its production of lipoprotein and after 6 or more hours of elevated fatty acids, a hyperlipidemia is produced. At this point the liver has accumulated fat in the cells since fatty acid is arriving faster than the cells can synthesize lipoprotein. In pathological conditions such as bacteremia or endotoxemia it is possible that larger or abnormal lipoproteins are formed which could form the fat droplets seen as fat embolism. The electrophoresis results also could be explained by this mechanism since the larger particles might behave as chylomicrons in this situation, rather than the lipid that normally forms the pre-beta band. The mechanism for precipitation of these larger fat particles possibly involves some change in the "detergent" of the serum, namely the phospholipid fraction of lipids.

The second possibility is that instead of the liver fat being released as lipoprotein, it is being released into the hepatic venous system as unbound fat droplets from the fatty cysts or damaged liver cells. This type of lipid would also have physical properties different from

the normal lipoproteins, thus increasing the possibility of embolization and also explaining the changes in electrophoresis.

One possibility that our results do not exclude is that of excess lipid from the periphery coming up the thoracic duct. Since our experiments were carried out on anesthetized dogs, most of the lymph flow was from the abdominal viscera (Morris 1956). In the normal situation a larger percentage would come from the periphery, as Fuchsig pointed out.

Another possible explanation might be the mechanism as found in pancreatitis, namely an inhibition of lipoprotein lipase by a substance released by the pancreas or other body tissues. This in effect would decrease the utilization of lipid, causing hypertriglyceredemia.

Triton WR-1339 has been used to study fat metabolism under various conditions (Morris 1956, Friedman 1957). Its action is to change the form of the blood lipids such that they are not utilized by the tissues for 24 - 36 hours after injection. It is possible that a similar mechanism takes place in sepsis, due to some as yet unknown factors.

CHAPTER VII. SUMMARY

E. coli bacteremia was induced in dogs by intravenous injection after a 24 hour fast. Investigations of two types were carried out on the surviving dogs at least 24 hours following injection. These revealed hypertriglyceredimia and elevated fatty acids in association with increased lipids in the chylomicron range on lipoprotein electrophoresis. Extraction of lung and liver demonstrated an elevated triglyceride content, and a decreased cholesterol content of the liver. Thoracic duct lipid output was unchanged, indicating that increased triglyceride release by the liver, if occurring, was not via lymphatics.

It is postulated that catecholamine increase in bacteremia results in mobilization of increased amounts of free fatty acids, these in turn being inadequately handled by the liver such that fat accumulates in the hepatic cells. Release of this fat, whether by lipoprotein synthesis or by breakdown of the fat globules is via the hepatic vein. Increased triglyceride content of liver and lung was correlated with increased plasma lipids. These results support previous work showing pulmonary fat embolism as part of the lung lesion of bacteremia.

VIII. ADDITIONAL TABLES SHOWING CRUDE DATA

EXPERIMENT II. ORGAN EXTRACTION

Controls

Dog #	LDH	SGOT	Ht	Temp.	pO_2	pH	pCO_2
9	45	18	36	101	80	7.30	34
14	69	34	37	102	70	7.35	30.5
15	107	20	38	104	66	7.36	34.5
16	60	16	42	100	62	7.37	41
17	40	24	38	101	70	7.32	36.5
24	48	28	57.5	101	78	7.40	34.5
25	160	23		101	70	7.33	29.5
26	123	18	32	102	80	7.29	38.5
27	80	15	34	102	75	7.31	47
29	170	32	33	101	70	7.39	41
30	69	33	48	101	65	7.37	39
31	71	20	46	102	63	7.29	41.5

Experimental

28	277	470	60	103	883	7.38	31.5
23	43	45	44	101	83	7.33	43.0
22	71	350	41	102	65	7.33	34.5
21	46	23	38	102	85	7.35	35.0
20	86	140	51	103	43	7.25	45.0
19	55	73	46	103	46	7.25	49
18	79	114	52	103	82	7.32	32.5
13	85	138	36	102	70	7.22	34.0
11	123	73	39.5	106	63	7.35	28
10	72	88	58	102	65		39
8	85	156	45	108	82	7.31	25
7	95	144	43	103	80	7.30	36

EXPERIMENT II. ORGAN EXTRACTION - CONTROLS

Serum Values

Dog #	Triglyceride mg/100 ml	Free Fatty Acid µEq/L	Cholesterol mg/100 ml
14	25	572	80
15	71	797	160
16	71	655	125
17	76	417	113
24	40	375	196
25	151	1350	575
26	52	788	220
27	71	550	120
29	125	500	245
30	122	900	170
31	156	638	178
9	105.	761	178

EXPERIMENT II. ORGAN EXTRACTION - EXPERIMENTAL DOGS

Serum Values

Dog #	Triglyceride mg/100 ml	Free Fatty Acids μEq/L	Cholesterol mg/100 ml
28	442	1565	290
23	87	352	200
22	545	1042	245
21	121	572	118
20	168	782	310
19	119	500	179
18	202	359	238
13	180	473	275
11	189	785	155
10	155	421	225
8	520	864	350
7	150	897	209

EXPERIMENT II. ORGAN EXTRACTION - CONTROLS

Lung Content

Dog #	Triglyceride mg/gm	Free Fatty Acid μEq/gm	Cholesterol mg/gm
14	24.5	14.5	24.0
15	12.0	15.5	21.5
16	28.3	15.0	21.5
17	31.0	15.0	27.0
24	28.5	32.5	16.0
25	17.3	37.0	20.1
26	11.4	16.7	15.5
27	8.8	14.5	17.0
29	19.3	44.5	8.0
30	31.0	47	11.0
31	39.5	44.0	2.5
9	35.0	32.5	22.5

EXPERIMENT II. ORGAN EXTRACTION - EXPERIMENTAL DOGS

Lung Content

Dog #	Triglyceride mg/gm	Free Fatty Acids μEq/gm	Cholesterol mg/gm
28	90.0	12.4	24.0
23	22.5	22.0	16.0
22	33.0	28.5	13.5
21	29.3	25.5	17.0
20	56.0	22.2	15.5
19	30.0	28.0	11.0
18	16.2	17.8	6.5
13	18.5	45.0	22.5
11	30.6	27.0	7.5
10	26.5	17.7	37.0
8	45.0	26.8	34.5
7	64.0	22.0	39.5

EXPERIMENT II. ORGAN EXTRACTION - CONTROLS

Liver Content

Dog #	Triglyceride mg/gm	Free Fatty Acid μEq/gm	Cholesterol mg/gm
14	39.5	14.3	7.0
15	32.0	15.0	11.5
16	47.5	25	15.0
17	25.5	11.5	10.0
24	47.0	19.5	5.5
25	21.8	17.7	8.0
26	38.2	21.8	12.0
27	15.8	9.9	8.0
29	41.5	25.0	4.0
30	144.0	33.5	2.5
31	27.3	24.5	4.5
9	41.0	31.0	20.5

EXPERIMENT II. ORGAN EXTRACTION - EXPERIMENTAL DOGS

Liver Content

Dog #	Triglyceride mg/gm	Free Fatty Acid µEq/gm	Cholesterol mg/gm
28	147.0	11.7	7.0
23	20.9	16.3	11.5
22	155.0	28.0	14.5
21	110.0	18.5	16.0
20	314.0	16.2	15.5
19	114.0	22.8	15.0
18	118.0	5.4	9.0
13	147.0	16.0	12.5
10	114.0	21.2	16.5
8	114.0	12.5	27.5
7	118.0	22.1	22.5

EXPERIMENT I. LYMPH FLOW (ml/Hr)

Controls

Dog #	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
44	32	28	13	17	25
36	14	14	8	9	10
29	27	14	12	11	10
28	27	14	14	12	18
26	22	22	21	10	13
25	17	21	9	9	7
24	27	21	13	24	18
45	22	23	24	24.5	31
46	18	25	25	19	19
47	16	13	17	16	6
48	13	22	13	14	

Experimental

35	14	12	12	14	12
30	20	14	14	14	11
37	11	9	8	10	10
33	13	12	10	9	9
22	11	15	15	15	5
38	16	30	18	13	
42	25	17.5	15.2	12	12
43	43	33.2	34.5	33	
49	3.6	6.5	8	7	7
50	25	37	30	32	
40	33	23	19	16	13

EXPERIMENT I. BLOOD LIPID LEVELS

Controls

Dog #	Triglycerides mg/100 ml. Hour 1	Hour 5	Cholesterol mg/100 ml. Hour 1	Hour 5	Free fatty acids mEq/L Hour 1	Hour 5
44	58	43	141	188	487	526
36	53	44	74.5	86	688	488
29	85	88	166	172	489	489
28	119	118				
26	130	135	215	230	420	417
25	119	118	200	209	542	456
24	48	65	165	167	247	662
45	48	62	112	160	595	620
46	50	41	166	132	440	643
47	38	28	66	63	520	685
48	45	47	165	175	506	532

Experimental

35	450	305	212	275	732	778
30	206	185	220	199	891	771
37	331	260	280	270	960	622
33	105	115	142	147	635	397
38	121	128	195	203	596	511
42	84	36	210	150	874	130
43	103	82	198	159	1000	721
49	188	210	329	400	659	1050
50	150	100	212	220	867	605
40	198	348	98	74	577	534

EXPERIMENT I. LYMPH CHOLESTEROL CONTENT

CONTROL

Dog #	1st Hr.		2nd Hr.		3rd Hr.		4th Hr.		5th Hr.	
	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.
47	104	16.7	101	13.2	92	15.6	42.5	6.8	51	3.0
48	130	17.0	132	29.0	130	17.0	133	18.7		
45	75	16.5	85	19.5	80	19.2	96	23.0	135	42.0
46	146	41.0	130	32.5	126	31.5	99	18.8	119	22.6
44	108	34.5	102	28.8	94	12.2	77	13.1	75	18.7
36	66	92.5	57	80.0	50	4.5	49	4.4	64	6.4
29	106	28.5	102	14.3	93	11.2	94	10.4	88	8.8
28	68.5	18.5	74.5	10.4	71.5	10.0	64	7.7	62	11.2
26	135	29.5	123	27.0	120	25.2	116	11.6	114	14.8
25	142	24.2	137	28.8	135	12.1	149	13.4	151	10.5
24	119	25.0	116	15.1	104	25.0	96	17.3	99	14.9

EXPERIMENT I. LYMPH CHOLESTEROL CONTENT

EXPERIMENTAL

Dog #	1st Hr.		2nd Hr.		3rd Hr.		4th Hr.		5th Hr.	
	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.
50	150	37.5	166	61.5	149	44.7	136	43.5		
49	160	5.75	154	10.0	210	16.8	200	14.0	202	14.1
43	90	38.7	93	30.8	96	32.6	74	24.5		
42	84	21.0	98	17.6	103	15.5	109	13.1	86.5	10.4
40	98	32.5	114	26.2	109	20.8	124	38.5	125	16.3
38	150	24.0	138	41.4	115	20.8	128	16.7		
37	230	25.4	208	18.7	242	19.3	200	20.0	242	24.2
35	166	23.3	145	17.4	128	15.5	136	19.0	145	17.5
33	120	15.6	117	14.0	103	10.3	99	8.9	108	9.7
30	230	46.0	236	33.0	236	33.0	212	29.5	208	23.0
27	67	7.4	69	10.4	69	10.4	68	10.2	242	12.1

EXPERIMENT I. LYMPH TRIGLYCERIDE CONTENT

CONTROL

Dog #	1st Hr.		2nd Hr.		3rd Hr.		4th Hr.		5th Hr.	
	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.
44	590	190.0	335	94.0	276	36.0	124	19.0	62	15.5
36	474	66.5	492	69.0	302	24.2	244	22.0	144	14.4
29	440	119.0	163	22.0	182	22.0	191	21.0	159	15.9
28	400	108.0	515	72.0	164	22.0	109	12.5	159	28.0
26	368	81.0	374	82.5	330	69.5	138	13.8	254	33.0
25	268	45.6	455	95.5	310	27.9	316	28.4	410	28.6
24	474	128.0	492	104.0	302	39.2	244	58.5	144	26.5
45	305	67.0	205	47.0	170	41.0	355	87.0	400	124.0
46	1100	295.0	785	195.0	775	194.0	550	105.0	640	120.0
47	1070	170.0	845	110.0	1250	212.0	440	71.0	198	11.8
48	410	53.0	360	79.0	245	32.0	268	37.5		

EXPERIMENT I. LYMPH TRIGLYCERIDE CONTENT

EXPERIMENTAL

Dog #	1st Hr.		2nd Hr.		3rd Hr.		4th Hr.		5th Hr.	
	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.	conc. mg/100 ml.	amount mg.
35	1020	142.0	780	94.0	460	55.5				
30	535	107.0	666	93.0	755	106.0	496	69.5	310	34.2
37	1700	187.0	1320	119.0	1600	128.0	710	71.0	760	76.0
33	556	22.5	530	64.0	346	34.6	225	20.2	372	33.5
27	205	22.5	215	32.3	228	34.2	232	35.0	214	10.7
38	570	91.0	580	174.0	260	47.0	175	21.2		
42	205	51.5	490	86.0	365	54.0	418	50.0	220	26.5
43	340	146.0	290	96.5	250	86.0	220	72.5		
49	290	10.5	350	23.0	700	56.0	1000	70.0	760	53.2
50	560	14.0	490	180.0	260	78.0	205	66.0		
40	198	65.0	160	37.0	184	35.0	155	24.8	179	23.4

EXPERIMENT I. LYMPH FREE FATTY ACID CONTENT

CONTROL

Dog #	1st Hr.		2nd Hr.		3rd Hr.		4th Hr.		5th Hr.	
	conc. µEq/L	amount µEq	conc. µEq/L	amount µEq	conc. µEq/L	amount µEq	conc. µEq/L	amount µEq	conc. µEq/L	amount µEq
47	1025	16.5	722	9.4	925	15.7	875	14.0	506	3.0
48	659	8.6	506	11.1	532	6.9	557	7.8		
45	643	14.1	620	14.3	833	20.0	1000	24.0	1432	44.5
46	1950	54.5	1775	44.0	1595	40.0	1080	20.6	1575	30.0
44	2250	72.0	1345	38.0	895	11.6	605	10.3	552	13.8
36	1067	15.0	1188	16.7	910	7.2	645	5.8	997	9.9
29	567	15.3	534	7.5	511	6.1	622	6.8	545	5.4
28	922	25.0	711	9.9	600	8.4	577	6.9	577	10.4
26	452	9.9	381	8.4	381	8.0	436	4.3	436	5.7
25	686	11.7	900	18.9	743	6.6	642	5.7	657	4.5
24	439	9.2	500	6.5	488	11.7	464	8.4	366	5.5

EXPERIMENT I. LYMPH FREE FATTY ACID CONTENT

EXPERIMENTAL

Dog #	1st Hr.		2nd Hr.		3rd Hr.		4th Hr.		5th Hr.	
	conc. µEq/L	amount µEq	conc. µEq/L	amount µEq	conc. µEq/L	amount µEq	conc. µEq/L	amount µEq	conc. µEq/L	amount µEq
50	552	13.8	659	24.4	500	15.0	632	20.2		
49	342	1.2	526	3.4	764	6.1	946	6.6	895	6.2
43	802	34.5	710	23.5	710	24.2	710	23.5		
42	663	16.5	732	13.1	710	10.7	802	9.6	780	9.3
40	577	19.0	489	11.2	689	13.0	622	10.0	845	11.0
38	532	8.5	447	13.4	383	6.9	575	7.5		
37	1460	16.2	1391	12.5	1431	11.4	1040	10.4	986	9.8
35	1110	15.6	1089	13.1	910	10.9	932	13.0	922	11.0
33	586	7.6	667	8.0	650	6.5	507	4.5	476	4.2
30	1000	20.0	970	13.6	1321	18.5	1262	17.7	1108	13.2
27	512	5.6	730	11.0	526	7.9	539	8.1	615	3.0

IX. BIBLIOGRAPHY

1. Alican, F. and Hardy, J.D. Mechanism of Shock as Reflected in Studies of Lymph of Abdominal Organs. Surg. Gynec. and Obstet., 120:743, 1961.

2. Allardyce, D.B. Unpublished data. 1968.

3. Armstrong, D.T., Steele, R., Altszuler, N., Dunn, A., Bishop, J.S., Debodo, R.C. Regulation of Plasma Free Fatty Acid Turnover. Am. J. Physiol., 201:9, 1961.

4. Armin, J., and Grant, R.T. Observations on Gross Pulmonary Fat Embolism in Man and the Rabbit. Clin. Sci., 10:441, 1951.

5. Babson, A.L., Shapiro, P.O., Williams, P.A.R. and Phillips, G.E. Trans-Ac Method for Colorimetric Assay of Serum Glutamic-oxalacetic Transaminase. Clin. Chim. Acta 7:199, 1962.

6. Babson, A.L. and Phillips, G.E. Lac-dehystrate Method for Colorimetric Lactic Dehydrogenase Assays. Clin. Chim. Acta 12:210, 1965.

7. Baez, S. Lymphatic Adjustments in Shock. Transactions of the Fifth Conference on Shock and Circulatory Homeostasis. Ed. by H.D. Green, Princeton, N.J., 1955.

8. Baker, N., Garfinkel, A.S. and Shotz, M.D. Triglyceride Secretion in Relation to Lipogenesis and Free Fatty Acid Mobilization in Fasted and Glucose-refed Rats. J. Lipid Res., 9:1, 1968.

9. Ballin, H.M., and Meyer, M.W. Intestinal Lymph Flow in Dogs After Endotoxin. Proc. Soc. Exp. Biol., 103:93, 1959.

10. Bollman, J.L. and Flock, E.V. Cholesterol in Intestinal and Hepatic Lymph in the Rat. Am. J. Physiol.,164:480, 1951.

11. Baxter, J.H. Origin and Characteristics of Endogenous Lipid in Thoracic Duct Lymph in Rats. J. Lipid Res., 7:158, 1966.

12. Bergentz, S.E. Fat Embolism. Acta Chir. Scand. (Suppl.) 182, 1961.

13. Bergentz, S.E., Gelin, L.E., Hallgreb, B., Rudenstorm, C.M. and Svanborg, A. Influence of Trauma on the Lipids in Blood and Lung Tissue. Acta Chir. Scand., 124:377, 1962.

14. Bissell, W.W. Pulmonary Fat Embolism: a frequent cause of post-operative shock. Surg. Gynec. and Obstet., 25:8, 1917.

15. Borgstrom, B., Oliverrona, T. The Metabolism of Palmitic Acid-1-C^{14} in Functionally Hepatectomized Rats. J. Lipid Res., 2:263, 1961.

16. Byers, S.O., Friedman, M. Site of Origin of Plasma Triglyceride. Am. J. Physiol., 198:629, 1960.

17. Carlson, L.A., Liljedahl, S.O. and Wirsen, C. Blood and Tissue Changes in the Dog During and After Excessive Free Fatty Acid Mobilization. Acta Medica Scand., 178:81, 1965.

18. Carlson, L.A., Levi, L., Oro, L. Plasma Lipids and Urinary Excretion of Catecholamines in Man During Experimentally Induced Emotional Stress and Their Modification by Nicotinic Acid. J. Clin. Invest., 47:1795, 1968.

19. Coscarano, J., Rubin, A.D., Neumann, A.K. and Zweifach, B.W. Effect of Bacterial Endotoxemia on Succinic Dehydrogenase of Liver and Kidney of Rabbit. Am. J. Physiol., 201:16, 1961.

20. Clowes, H.A., Zuschneid, W., Turner, M., Blackburn, G., Rubin, J., Toole, P. and Green, G. Observations on the Pathogenesis of the Pneumonitis Associated with Severe Infections in other parts of the Body. Annals of Surgery 167:630, 1968.

21. Connor, W.E., Hook, J.C. and Warner, E.D. Massive Thrombosis Produced by Fatty Acid Infusion. J. Clin. Invest., 42:860, 1963.

22. Cooke, W.T. Clostridial Infections in War Wounds. Lancet 1:487, 1945.

23. De Palma, R.G., Coil, J., Davis, J.H., Holden, W.D. Cellular and Ultrastructural Changes in Endotoxemia: a light and electron microscopic study. Surgery 62:505, 1967.

24. Feigelson, E.B., Pfaff, W.W., Karmen, A. and Steinberg, D. The role of plasma Free Fatty Acids in Development of Fatty Liver. J. Clin. Invest., 40:2171, 1961.

25. Folch, J.I., Lees, M. and Sloane, S.G.H. A Simple Method for the Isolation and Purification of Total Lipids From Animal Tissues. J. Biol. Chem., 226:497, 1957.

26. Frederickson, D.S., Levy, R.I. and Lees, R.S. Fat Transport in Lipoproteins. New Eng. J. Med., 276:34, 1968.

27. Friedman, M., Byer, S.O. and Omoto, C. Some Characteristics of Hepatic Lymph in the Intact Rat. Proc. Soc. Exp. Biol. Med., 108:11, 1961.

28. Friedman, M., Byer, S.O. Mechanism Underlying Hypercholesteroiemia Induced by Tritan WR-1339. Am. J. Physiol., 190:439, 1957.

29. Gans, H. and Krivitz, W. Effect of Endotoxin Shock on the Clotting Mechanism of Dogs. Annals of Surgery 152:69, 1960.

30. Grant, R.T., Reeve, E.B. Observations on the General Effects of Injury in Man. Medical Research Council Special Series 277. His Majesty's Stationary Office, London, 1951.

31. Grohe, F. Bericht uber Leistungun in der patoto-geschen anatomie, In Constatt's Jahresbericht uber die Fartschritte der gesant en Medium in allen Landern in Jahre 1862 Bd. 1, p. 45, Wurzberg 1863.

32. Groves, A.C. Unpublished data. 1968.

33. Hartroft, W.S. and Ridout, J.H. Pathogenesis of the Cirrhosis Produced by Choline Deficiency. Escape of Lipid from Fatty Hepatic Cysts into the Biliary and Vascular Systen. Am. J. Path., 27:951, 1951.

34. Hartroft, W.S. Fat Emboli in Glomerular Capillaries of Choline Deficient Rats and of Patients with Diabetic Glomerulosclerosis. Am. J. Path., 31: 381, 1955.

35. Havel, R.J., Felts, J.M. and Van Duyne, C.M. Formation and Fate of Endogenous Triglycerides in Blood Plasma of Rabbits. J. Lipid Res., 3:297, 1962.

36. Havel, R.J. and Goldfien, A. Role of the Liver and of Extra-hepatic Tissues in Transport and Metabolism of Fatty Acids and Triglyceride in Dogs. J. Lipid Res., 2:389, 1961.

37. Hardaway, R.M., and Elovitz, M.J. Endotoxin Shock as a Syndrome of Disseminated Intravascular Coagulation. Oklahoma State Medical Association J., 59: 451, 1966.

38. Hirsch, R.L., McKay, D.G., Trovers, R.I. and Skraly, R.K. Hyperlipidemia, Fatty Liver and BSP Retention in Rabbits Injected Intravenously with Bacterial Endotoxin. J. Lipid Res., 5:563, 1964.

39. Jencks, W.P. and Durram, E.L. Paper Electrophoresis as a Quantitative Method: The Staining of Serum Lipoproteins. J. Clin. Invest., 34:1437, 1955.

40. Jackson, C., Grundykn, R.M. Pulmonary and Cerebral Fat Embolism After Closed-chest Cardiac Massage. Surg. Gynec. and Obstet., 120:25, 1965.

41. Karmen, A., Whyte, M. and Goodman, D.S. Fatty Acid Esterification and Chylomicron Formation During Fat Absorption: I. Triglycerides and Cholesterol Esters. J. Lipid Res., 4:322, 1963.

42. Kent, S.P. Fat Embolism in Diabetic Patients Without Physical Trauma. Amer. J. Path., 31:399, 1955.

43. Kessler, J.I., Finkel, M., Pek-Ping, Ho and Jonowitz, H.D. Lipoprotein Lipase Inhibition in Rabbits with Experimental Pancreatitis. Proc. Soc. Exp. Biol. Med., 110:24, 1962.

44. Leffler, H.H. and McDougald, C.H. Estimation of Cholesterol in Serum by Means of Improved Techniques. Techn. Bull. Regist. Med. Techn., 33:19, 1963.

45. Lehman, T., Moore, R. Fat Embolism - Including Experimental Production Without Trauma. Arch. Surg., 14: 621, 1927.

46. Lequire, V.S., Shapiro, J.L., Lequire, C.B., Cobb, C.A.Jr., and Fleet, W.F.Jr. A Study of the Pathogenesis of Fat Embolism Based on Human Necropsy Material and Animal Experiments. Amer. J. Path., 35:999, 1959.

47. Lillehei, R.C. and MacLean, L.D. The Intestinal Factor in Irreversible Endotoxin Shock. Annals of Surgery 148:513, 1958.

48. Lynch, M.J.G. Nephrosis and Fat Embolism in Acute Hemorrhagic Pancreatitis. A.M.A. Arch. Int. Med., 94:709, 1954.

49. Lynch, M.J.G. Fat Embolism in Chronic Alcoholism. A.M.A. Arch. Path., 67:68, 1957.

50. Macfarlane, R.G., Oakley, C.L. and Anderson, C.G. Hemolysis and the Production of Opalescence in Serum and Lecithovitellin by the Alpha-toxin of Clostridium Welchii. J. Path. Bact., 52:99, 1941.

51. Magendie (noted by Lehman and Moore) 1827.

52. Mattson, F.H., Volpenheim, R.A. The Digestion and Absorption of Triglycerides. J. Biol. Chem., 239:2772, 1964.

53. Morris, B. The Hepatic and Intestinal Contribution to the Thoracic Duct Lymph. Quant. J. Exp. Physiol., 41:318, 1956.

54. Miloslavich, E.L. Fat Embolism: Medico Legal Aspects. Wisc. Med. J., 29:139, 1930.

55. Morris, B. and Courtice, F.C. Origin of Chylomicrons in the Cervical and Hepatic Lymph. Quant. J. Exp. Physiol., 41:341, 1956.

56. Morris, B. Exchange of Protein Between Plasma and the Liver and Intestinal Lymph. Quart. J. Exp. Physiol., 41:326, 1956.

57. Morris, B. and Courtice, F.C. Effect of Intravenous Injections of Tritan WR-1339 on the Lipids of the Plasma and Liver Lymph of Rabbits. Quart. J. Exp. Physiol., 41:349, 1956.

58. Nelson, R.M. Metabolic Effects of Paracolon Bacteremia. Annals of Surgery 134:885, 1951.

59. Olbrycht, J. Experimentelle Beitrage zur Lebre von der Fettembolie der Lungen mit lusanderer Berucksichtigung ihrer gerichlsarz Hichen Bedentang. Dtscg. Z. ges. gerichtl. Med., 1:642, 1922.

60. Owens, G., Adams, J.E., Scott, H.W. Embolic fat as a Measure of Adequacy of Various Oxygenators. J. Appl. Physiol., 15:999, 1960.

61. Peltier, L.F. Fat Embolism - toxic Properties of Neutral Fat and Free Fatty Acids. Surgery 40: 665, 1956.

62. Rampone, A.J. Rate of Fat Uptake by Intestinal Lymphatics. Proc. Soc. Exp. Biol. Med., 108:278, 1961.

63. Shafrir, E., Susman, K.E., Steinberg, D. Nature of the Epinephrine-induced Hyperlipidemia in Dogs and its Modification by Glucose. J. Lipid Res., 1:109, 1959.

64. Shrivastava, B.K., Redgrave, T.G. and Simmonds, W.J. Source of Endogenous Lipid in the Thoracic Duct Lymph of Fasting Rats. Quart. J. Physiol., 52:304, 1967.

65. Stein, Y., Shapiro, B. Assimilation and Dissimilation of Fatty Acids by the Rat Liver. Am. J. Physiol., 196:1238, 1959.

66. Swank, R.L. Changes in Blood Produced by a Fat Meal and by Intravenous Heparin. Am. J. Physiol., 164: 798, 1951.

67. Swank, R.L. Changes in Blood of Dogs and Rabbits by High Fat Intake. Am. J. Physiol., 196:473, 1959.

68. Swank, R.L., Gliusman, W. Production of Fat Embolism in Rabbits by Feeding High Fat Meals. Surg. Gynec. and Obstet., 110:9, 1960.

69. Trout, D., Estes, E.H.Jr. and Friedberg, S.J. Titration of Free Fatty Acids of Plasma: a Study of Current Methods and a New Modification. J. Lipid Res., 1:199, 1960.

70. Van Handel, E. Suggested Modifications of the Micro Determination of Triglycerides. Clin. Chim. Acta 7:249, 1961.

71. Virchow (1865) noted by Lehman and Moore,

72. Waddell, R.G., Geyer, R.P., Clarke, E. and Store, F.J. Role of Various Organs in the Removal of Emulsified Fat from the Blood stream. Am. J. Physiol., 175: 299, 1953.

73. Wagnern E. Die Fettembolie der lungencapillaren. Arch. Heilk, 6:146, 1865.

74. Wilson, L.W., Powers, D., Soloff, L.A. Hemodynamic Effects of Infusion of Long Chain Saturated Fatty Acids into Dogs. Am. J. of the Medical Sciences, 249:75, 1965.

75. Zbinden, G. Transient Thrombopenia After Intravenous Injection of Certain Fatty Acids. J. Lipid Res., 5:378, 1964.

76. Zenker, P. Beitrage zur Normalen und Pathologischen Anatomie der Lungen. Dresden, Brennsdorf 1862.