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PHYSICAL AND CHEMICAL CHARACTERIZATION OF THE SERUM LIPOPROTEINS OF MARINE MAMMALS

Donald Lawrence Puppione
(Ph. D. Thesis)

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PHYSICAL AND CHEMICAL CHARACTERIZATION OF THE SERUM LIPOPROTEINS OF MARINE MAMMALS

(Abstract) Donald Lawrence Puppione

I have characterized some of the chemical and physical properties of the ultracentrifugal serum lipoprotein distributions of cetaceans (dolphins, porpoises, and whales) and pinnipeds (seals, sea lions, walruses). The complete ultracentrifugal distributions of the serum lipoproteins are presented in terms of fully corrected schlieren diagrams. Data on the chemical composition and serum concentration of the lipid moieties of the three major classes of lipoproteins (total very low density lipoproteins, VLDL, d < 1.006 gm/cc; low density lipoproteins, LDL, d: 1.006-1.063 gm/cc; high density lipoproteins, HDL, d: 1.063-1.21 gm/cc) are presented.

In these marine mammals more than 70% of the lipids were found to be associated with the HDL class. In both orders, serum concentrations of lipoproteins in the total VLDL class were low. Distinct differences in the chemical and physical properties of serum lipoproteins were found between cetaceans and pinnipeds. Serum concentrations of HDL and of total lipids were higher in the sera of pinnipeds. In both the low and the high density lipoprotein distributions, the flotation peaks of the major components of cetaceans had higher rates. Furthermore, in the case of certain cetaceans, cholesteryl ester rather than phospholipid was the predominant lipid of the HDL class. A comparison of these data with similar data for terrestial mammals was made. Differences between mammals were noted in terms of the predominant lipid in both the LDL and the HDL classes. In all the marine mammals as well as the cow, the triglyceride content of the HDL lipid moiety was very low.

I also carried out an evaluation of the relative distributions of serum lipoproteins in marine and terrestial mammals. The mammals were categorized according to their generally presumed diet in the wild state (i.e. herbivores, omnivores and carnivores). I was able to suggest that hepatic synthesis and secretion of triglycerides as influenced by the composition of the diet might be a possible factor in controlling the serum level of LDL.

The effect of fat ingestion on the chemical and physical properties of the serum lipoproteins of seals was also studied. Very little fluctuation was observed in the concentrations and the chemical compositions of LDL and HDL. The chief changes in serum lipids were increased triglyceride concentrations in the total VLDL class. A major difference between seal and human responses to a high fat intake was the extent of the increase in serum concentration of S_f° 20-400 lipoproteins. In humans about 75% of the serum concentration increase in the total VLDL class appears in the S_f° 20-400 class. This percentage ranged in values from 15% to 38% in the seals. It was suggested that a rapid uptake of chylomicron fatty acids by adipose tissue might explain this difference between man and seals.

Another difference in serum lipids between seals and humans following fat ingestion was in the chemical composition of HDL. Unlike humans, there was no significant uptake of triglycerides by seal HDL. Uptake of triglycerides by human HDL has been associated with the activity of the serum enzyme, lecithin cholesterol acyl transferase, LCAT. In vitro enzyme activity leads to a reciprocal transfer of triglycerides from VLDL to HDL and of cholesteryl esters from HDL to VLDL. The chemical compositions of HDL and VLDL of marine mammals suggest that this transfer does not take place. More unesterified than esterfied cholesterol was noted in the total VLDL class

of marine mammals. In addition, the serum concentration of triglycerides in the HDL class of fasting and fed animals was low. Incubation studies to evaluate the presence of LCAT in the sera of different marine mammals indicated that there was no significant activity.

INTRODUCTION

There are many mammals whose neutral fat depots serve both as insulators against heat loss and as energy reservoirs, especially during periods of starvation, for essential endergonic processes. Such reliance upon triglycerides, which comprise more than 99% of the depot fat, is best exemplified by two orders of marine mammals, the cetaceans and the pinnipeds, who not only live in the harsh environment of the ocean but also, in the case of certain species, fast for prolonged periods.

Because the ocean is colder than their bodies and has a thermal conductivity much higher than that of air, the need of marine mammals for heat insulation is readily apparent. To meet this need, these animals deposit triglycerides in a continuous layer of subcutaneous adipose tissue.[1] There are no intra-abdominal fat cores.[1] Pinnipeds also have a coat of hair which varies in thickness with species and affords them additional protection. Among species possessing thick fur, the blubber is not as thick, and this reliance on stored fat as an insulator appears to be less.[2]

Besides serving as an insulator, depot fat appears to be the chief source of energy for these marine mammals. This dependence on depot triglycerides is best demonstrated by those marine mammals which fast for long periods. Such animals are different from fasting, hibernating mammals, which make physiological adjustments to conserve their fat. During hibernation the temperatures of these animals drop to about 5°C, and their metabolic rates are lowered.[3] Rather than utilize their protective subcutaneous fat, they derive most of their energy from the intra-abdominal fat stores which completely disappear by the end of hibernation.[2] Hibernators also have brown adipose tissue which generates the heat necessary to warm their bodies to 37°C on arousal.[4] In contrast,

marine mammals are able to survive simultaneously both the cold of their environment and the starvation of fasting periods without the benefit of alternate fat stores or lowered body temperatures.

It should also be noted that marine mammals are not inactive as are the hibernators during their fast. Most of the observations on fasting pertain to pinnipeds. Among the family Otariidae, harem bulls of the Steller sea lion (Eumetopias jubatus), the Southern sea lion (Otaria byronia), the Hooker's sea lion (Phocartos hookeri), and the Northern fur seal (Callorhinus ursinus) have been reported to fast for as long as two months.[5] During this time, they mate frequently and defend their territory.[5,6] Examples of this behavior pattern are also found in the family Phocidae. Both the Southern and Northern elephant seal (Mirounga leonina and Mirounga angustirostris) males fast and maintain harems for periods of approximately two months. The females of this family of pinnipeds also fast while nursing, and fasting periods of two weeks are common among several species.[5,7] The Laysan monk seal (Monachus schauinslandi) is believed to fast during a lactation period of five weeks.[5] During this time, the female monk seal loses two hundred pounds while the pup gains about a hundred pounds. This loss of weight results not only from fasting but also from the production of milk with a high fat content (the percent of milk fat ranges in value from 20% to 50%).[5] Comparable data on the fasting of cetaceans are just beginning to be gathered. Several species of Mysticeti (baleen whales) are believed to fast while migrating over distances of several thousand miles.[8,9] Prolonged periods of fasting have not been reported for any member of the suborder, Odontoceti (toothed whales).

Importance of dietary fats to marine mammals

To build up the fat stores, pinnipeds and cetaceans apparently rely

on dietary fat. There is a broad similarity between the highly unsaturated fatty acid distribution of their depot fat and that of fish and krill fat.[10,11] The percentage of carbohydrate in their food is less than 1% - an amount too low to produce a significant amount of endogenous fat.[12,13] The best example of low carbohydrate ingestion can be found in pups of certain pinnipeds. The milk of the superfamily Otarioidea is low in carbohydrate.[14] There is no detectable lactose in their milk, and no other carbohydrate has been detected which could serve as a possible substitute for lactose. The major component in the diet of marine mammals is protein. Amino acids can be converted into fat. Pathways which lead to pyruvic acid can continue on to acetyl Co A from which fatty acids can be synthesized *de novo*. In an animal which has such low amounts of carbohydrate in its diet, it is more likely that the pyruvate is converted into glucose. Only leucine and isoleucine have metabolic pathways which lead to acetyl Co A. Attempts to develop obese pigs on a high protein diet have proved to be difficult.[15]

The diet of the walrus, on the other hand, consists mostly of protein and carbohydrate. The walrus is known to ingest large amounts of mollusks some of which have a glycogen content greater than 10% of the dry weight.[5, 13] The walrus most probably relies on its dietary carbohydrate for its fat stores. Although no fatty acid analysis has been done on walrus fat, the expectation would be that it would be relatively more saturated.[15]

Data from enzyme studies are also suggestive that marine mammals rely principally on dietary fat for their adipose stores. Enzyme assays have been carried out by Fried *et al*. on tissues (liver, adipose, muscle, kidney, heart, and brain) of the Weddell seal (*Leptonychotes weddelli*).[16] They found that the activity of glucose-6-phosphate dehydrogenase was

lower in the Weddell seal than in other mammals. This enzyme is involved in the first step of the shunt pathway for the direct oxidation of glucose and yields the coenzyme, nicotinamide adenine nucleotide phosphate, which is required for the synthesis of fatty acids. Although these workers recognized that this coenzyme could be generated by other pathways, their results do suggest that the tissue of the Weddell seal might not be capable of de novo fatty acid synthesis. To settle this issue, further enzyme studies certainly need to be done on other marine mammals. Nevertheless, it would appear that these animals have a limited lipogenic capacity and that their adipose cells contain primarily dietary fats.

Considerations of the diet of these animals along with the above mentioned enzymatic studies point out that marine mammals have a limited lipogenic capacity and that the fat in their adipose cells is primarily dietary in origin. The reliance of marine mammals on adipose tissue as an insulator and an energy source has already been discussed. Although detailed studies on the specific pathways in fat metabolism in marine mammals have not been done, it is reasonable to assume that comparable mechanisms which have been found in other animals also exist in marine mammals. The following is a brief discussion of some of these mechanisms relating to the ingestion and transport of dietary fats in man and animals.

Fate of dietary fats in the body

Dietary fats, which are principally triglycerides, are digested in the small intestine. In the intestinal lumen, the triglycerides are hydrolyzed mostly to unesterified fatty acids and monoglycerides through the action of a pancreatic lipase. The long chain fatty acids are absorbed into the intestinal mucosal cells and are reesterified into triglycerides. The triglycerides apparently then coalesce into a fat droplet and receive

a coat of protein. The protein coat is considered necessary not only to stabilize the fat droplet in an aqueous medium, but also, as will be discussed shortly, to somehow effect the release of the triglycerides from the cell. The coated fat droplets are released into the lymphatics and eventually enter the systemic circulation via the thoracic duct. These particles when derived from lymph or blood are called chylomicra. Several excellent reviews describing the digestion and absorption of fat have been published.[17, 18]

Once in the blood stream, these triglycerides are then transported to body tissue. Either at the capillary surface or on entering tissue cells the triglycerides are hydrolyzed through the action of lipoprotein lipase. The constituent fatty acids are then reformed into other esters or are degraded for energy purposes inside the tissue cells.

In the adipose cells, the fatty acids are reincorporated into triglycerides. Activated glycerol, alpha-glycerol phosphate, is necessary for triglyceride synthesis. In this regard, it should be noted that in the enzymatic studies of Fried et al., the activity of alpha glycerophosphate dehydrogenase in the Weddell seal was found to be roughly two and five times higher in the adipose cells than in the kidney and liver cells respectively.[16] This enzyme is necessary for the formation of activated glycerol. The activity of alpha glycerophosphate dehydrogenase in the adipose tissue of the Weddell seal was more than twice that of the adipose tissue of the mouse. This indicates that the Weddell seal is capable of a high rate of synthesis of active glycerol for the storage of fats. Depending on the energy needs of the body, these triglycerides can later be hydrolyzed. The fatty acids are released from the adipose cells into the systemic circulation and are transported in the blood in a complex with albumin. The degradation of fatty acids

for energy purposes takes place inside the tissue cells. This degradation can provide at least one-half of the energy requirements of a normal human.[19]

Hepatic synthesis of triglycerides

Blood triglycerides can also be composed of fatty acids resulting from lipogenic mechanisms. These fatty acids are synthesized from acetyl Co A derived from carbohydrates and amino acids. The esterification of these fatty acids to form triglycerides occurs primarily in the liver. Fatty acids complexed to albumin can also be taken up by the liver and esterified. The liver has the capacity either to store such triglycerides or to incorporate them into a lipoprotein complex for release into the circulation.

Physical and chemical differences between the triglyceride bearing lipoprotein released by intestinal and hepatic cells

Hepatic cells release a triglyceride containing particle which differs from chylomicra in physical and chemical properties. The data at this time indicate that in humans chylomicra have densities less than 0.94 gm/cc, range in diameter from 0.1 micron or greater, and have the following chemical composition: 81-89% triglycerides (TG), 6-10% total cholesterol (TC), 4-7% phospholipid (PL), and 1-2% protein. On the other hand, the particles containing hepatogenous triglycerides have densities between 0.94-1.006 gm/cc, range in diameter from 300-700A^{o}, and have the following chemical composition: 57-68% TG, 15-18% TC, 12-14% PL, and 5-12% protein.[20, 21]

The role of the protein moiety in triglyceride transport

Immunochemical studies have shown that at least two proteins are normally present on the triglyceride containing particles released from both the intestinal and hepatic cells. It has also been demonstrated

that these two proteins are the protein moieties of two distinct classes of lipoproteins which have densities greater than 1.006 gm/cc.[22] The A and the B proteins form the protein moieties of alpha and beta lipoproteins respectively. The use of the terms alpha and beta lipoproteins is derived from the fact that the respective lipoproteins have the same electrophoretic mobility as the alpha and beta globulins. The relation between the electrophoretic and ultracentrifugal terminology used to describe serum lipoproteins will be discussed later.

The current theory of the role of the A and B proteins in triglyceride transport is based on the following evidence. In humans, the syndrome, abetalipoproteinemia, is associated with a defect in releasing triglycerides from cells.[22] When such subjects are given dietary fats, they are able to absorb the fat and to synthesize triglycerides in the mucosal cell. However, chylomicra are not released into the lymphatics. Furthermore on a high carbohydrate diet, synthesis of triglycerides will occur in their hepatic cells, but no triglyceride containing particles are released. In both cases, the intestinal and hepatic cells become engorged with fat. A marked reduction of circulating beta lipoproteins and the development of fatty livers can also be induced in rats by administering orotic acid with their diet.[23] Orotic acid is an intermediate in the biosynthesis of pyrimidines, a basic unit of nucleic acids. Interestingly enough, their intestinal cells are apparently not affected. Oral administration of fat to these animals produced a transient hyperlipemia. Levy _et al_. contend that abetalipoproteinemics are unable to synthesize or release the B protein and hence are unable to form beta lipoproteins.[24] On the other hand, Lees has presented immunochemical evidence that a form of B protein is present in the blood system of abetalipoproteinemics.[25]

Lees feels that the defect causing abetalipoproteinemia is due to an inability of this B protein to combine with lipid at the site of glyceride production. Nevertheless, there is general agreement that the B protein has a specific function in the transport of both exogenous and endogenous glycerides from tissue cells to the blood. The role of A protein is less clear. One possible function is suggested by two observations in Tangiers disease, a human syndrome characterized by low levels of an abnormal lipoprotein.[22] In this disease, concentrations of circulating triglycerides in fasting subjects are abnormally high even on regular diets.[26] On a high carbohydrate diet, the triglyceride concentration will rise to values between 300 to 600 mg/100ml within 3 to 4 days whereas in nine normal humans the mean maximum concentration of triglycerides of 260 mg/100ml was reached in approximately six days.[26] Following fat ingestion, the triglyceride concentrations attain values that are higher than in normals, and the duration of the alimentary lipemia is also longer than in normals.[22] *In vitro* studies have shown that the presence of an A protein phospholipid complex is necessary for the enzyme lipoprotein lipase to hydrolyze a coconut oil emulsion.[27] Based on such evidence, one possible role for A protein in lipid transport is somehow to facilitate the action of lipoprotein lipase and the uptake of triglycerides by tissue cells.

Ultracentrifugal distribution of serum lipoproteins

Because serum lipoproteins contain appreciable lipid (40% or greater), their hydrated densities are much less than those of the serum proteins. Their hydrated densities range in value from 0.92 gm/cc to 1.21 gm/cc. Lipoproteins species of different densities can be conveniently isolated into specific density classes by flotation utilizing preparative ultracentrifugation.[21] Once isolated, each class can be further analyzed by appropriate physical and chemical techniques.

Three major density classes of lipoproteins are the total very low density lipoproteins, VLDL (hydrated densities less than 1.006 gm/cc), the low density lipoproteins, LDL (hydrated densities between 1.006 gm/cc and 1.063 gm/cc), and the high density lipoproteins, HDL (hydrated densities between 1.063 gm/cc and 1.21 gm/cc). The total VLDL class contain the chief carriers of triglycerides. The LDL and HDL classes correspond to the electrophoretic beta lipoproteins and alpha lipoproteins respectively. Through the use of the analytical ultracentrifuge, the concentration distribution of serum lipoproteins as a function of flotation rate can be determined for each of these density classes, with the exception of those lipoproteins with hydrated densities less than 0.94 gm/cc. This latter group of lipoproteins, sometimes referred to as the chylomicron class, can not be measured by the method of analytical ultracentrifugation. In general practice, the flotation properties and concentrations of serum lipoproteins are determined in salt solutions at two specific densities, 1.063 gm/cc and 1.21 gm/cc. The analysis of the resulting schlieren patterns and the application of a computer for calculations in this analysis have been described elsewhere.[27] A recently developed computer program at Donner Laboratory can provide a graphic representation of a fully corrected schlieren pattern.[28] Figures of ultracentrifugal distributions of serum lipoproteins to be shown in this thesis are such computer-derived representations. The resulting data from analyses performed at these two densities give two distributions, the low density and the high density distributions. Typical distributions for human males and females are shown in Figure 1. In the low density distribution, the LDL correspond to lipoproteins

in the flotation rate interval S_F^{o*} 0-20 and the VLDL minus the chylomicron class to the interval S_F^o 20-400. The high density distribution is also divided into two classes, those with flotation rates in the interval $F_{1.20}$ 3.5-9 designated HDL_2, and those in the interval $F_{1.20}$ 0-3.5 designated HDL_3. The concentrations of serum lipoproteins in specified flotation rate intervals are shown in units of mg/100 ml in the lower left hand corners of the distributions.

Statistical interrelationships between the ultracentrifugal classes of serum lipoproteins

There is considerable evidence that the protein moieties of LDL and HDL participate in the structure of serum VLDL. The presence of the A and the B proteins on the triglyceride containing particles of the total VLDL class has already been discussed. Furthermore, the proposed roles of the A and the B protein in fat metabolism were also discussed. Possible metabolic relationships among major lipoprotein classes have been suggested by statistical studies on the ultracentrifugal distributions of serum lipoproteins in normal non-fasting humans. A statistical inverse relationship has been shown to exist between the serum concentrations of VLDL and HDL in normal humans.[20] This inverse relationship is also demonstrated in several human diseases, familial exogenous hypertriglyceridemia, xanthoma tuberosum, and nephrosis. In each of these diseases, there are extremely high levels of VLDL. Exogenous hypertriglyceridemics have a deficiency of the enzyme lipoprotein lipase, and as a result, hypertriglyceridemics have very high concentration of lipoproteins with S_F^o values greater than

*S_F rate without a subscript refers to low density lipoprotein migration rate (against the centrifugal field) expressed as Svedbergs (10^{-13} cm/sec/dyne/gm) of flotation in a NaCl medium of density 1.063 gm/cc at 26° C (1.748 molal NaCl). Lipoprotein concentrations within the low density group that have been corrected for S_F versus concentration and Johnston-Ogston effects are referred to as S_F^o or "Standard" lipoprotein values. F rate denotes a flotation rate measured at any other density, signified by a subscript, e.g. $F_{1.20}$.

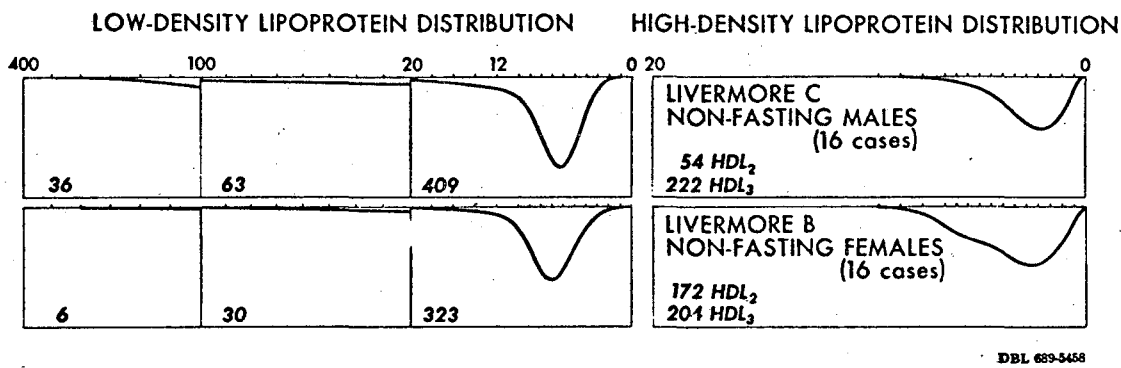

Figure 1 Average ultracentrifugal distribution of serum lipoproteins in the sera of non-fasting male and female humans. The flotation rates of the low density distribution are designated in S_f^o units along the abscissa. The flotation rates of the high density distribution are designated in $F_{1.20}$ units. The S_f^o and $F_{1.20}$ units are defined in the text.

400. The defect associated with xanthoma tuberosum has not yet been defined. In nephrosis, the build up in concentration of VLDL and the lowering in concentration of HDL appear to be correlated with proteinuria.[30] It is possible that the protein lost in the urine contains a significant amount of A protein. The lower concentrations of serum HDL encountered in each of these hypertriglyceridemic states might also be considered to give further support to the proposed role of the A protein in the tissue uptake of triglycerides.

Purpose of this study

The immunochemical studies of the protein moieties of serum lipoproteins together with statistical studies indicate interrelationships among the triglyceride containing particles of the total VLDL class and the other serum lipoproteins. Any investigation of the transport of exogenous fats in the blood stream requires information on the total distribution of serum lipoproteins. Hence, in the first phase of my investigation of fat transport in marine mammals, I characterized the chemical and physical properties of the major classes of the entire serum lipoprotein distribution. In the second phase I carried out similar studies on harbor seals and elephant seals during the course of active absorption and transport of triglycerides in the blood stream.

I embarked on this study not only to gain some insight into the mechanisms of fat transport in these animals but also to add some data to the rather meager amount of available information on serum lipoproteins in various mammals. The serum lipoprotein data obtained on marine mammals will be compared with available ultracentrifugal data on terrestial mammals.

CHARACTERIZATION OF THE SERUM LIPOPROTEINS OF MARINE MAMMALS

I. STUDIES ON SERUM LIPIDS AND LIPOPROTEINS OF MARINE MAMMALS PERFORMED PRIOR TO THIS WORK.

As was pointed out in the introduction, marine mammals apparently rely on their diet to maintain their fat stores. The important role of lipoproteins in the transport of dietary fat was also discussed. In my survey of the literature, I found a very limited amount of information on the characterization of the structure and function of the serum lipoproteins of marine mammals. Aside from the ultracentrifugal characterization of the serum lipoprotein distribution of one killer whale (*Orcinus orca*), no other such study had previously been done on marine mammals. For the killer whale, the serum lipoprotein distribution was found to be the following: 399 mg/100 ml (HDL), 45 mg/100 ml (LDL), and 54 mg/100 ml (VLDL).[31] Blood lipid studies on other marine mammals were restricted to the determination of total plasma or serum cholesterol concentrations for different cetaceans and pinnipeds. Table I lists the results of some of these studies. These data indicate the presence of higher concentrations of cholesterol in the sera of pinnipeds when compared with the sera of cetaceans. The basis for this difference will become clear when the lipid concentrations associated with the three major serum lipoprotein classes are compared in the Results Section.

Table I Total Cholesterol Concentrations in Serum /Plasma of Marine Mammals

Animal	Concentration Ranges (mg/100 ml)	References
Pinnipeds		
Southern Elephant Seal		
Female (3)	350-450	34
Male (2)	420-570	34
Weddell Seal		
Female (6)	180-460	34
Male (11)	110-350	34
Cetaceans		
Killer Whale		
Female (1)	160*	31
Male (1)	280	32
Atlantic Dolphin (7)**	140-270*	33
Pacific Dolphin (4)**	140-180*	33
Dall Porpoise (3)**	146-160*	33

* Plasma

** The sexes of the dolphins and porpoises were not cited in the reference. The numbers in parentheses represent the number of animals tested.

II. MARINE MAMMALS STUDIED IN THIS WORK

In general, the term, marine mammals, includes members of the following orders: Cetacea (dolphins, porpoises, and whales), Pinnipedia (seals, sea lions, and walruses), and Sirenia (dugongs and manatees). The sea otter, a member of the order Carnivora, is also considered to be a marine mammal. In my studies, serum lipoprotein analyses were performed only on cetaceans and pinnipeds. Because my studies were concerned with the transport of ingested fat in the blood of marine mammals, no attempt was made to obtain blood from the herbivorous sirenians. Sea otters are too rare to do any study on them.

Various factors limited me to the types of animals that were finally investigated. With the exception of the Northern fur seals, the Weddell seals, and one cetacean, a delphinus, all the animals studies were captive. The large animals are kept in zoos and oceanariums, and in these situations it was very difficult to isolate them for purposes of obtaining blood. In spite of these factors, I obtained blood samples from at least one member of each of the three families of pinnipeds and from several cetaceans belonging to the suborder, Odontoceti. I was unable to obtain blood samples from members of the suborder, Mysticeti.

Blood samples were obtained from the following pinnipeds: harbor seal, Weddell seal, California sea lion, Northern fur seal, Steller sea lion, and walrus. In addition, blood samples were obtained from a group of Steller sea lion pups. The cetaceans studies were: the Dall porpoise, the Pacific white striped porpoise, the Atlantic bottlenose dolphin, the Pacific bottlenose dolphin, the delphinus, and the killer whale.

Blood was obtained from the Northern fur seals on the Pribilof Islands and from the Weddell seal in the Antartic. The delphinus was captured off the coast of California and was bled aboard ship.

The sex, age, and weight data for all the captive animals are given in Appendix I.

III. METHODS

a. Dietary Background of Marine Mammals in This Study

Both pinnipeds and cetaceans daily were fed fish (usually mackerel and herring) in amounts roughly equivalent to 5% of their body weight. With the exception of elephant seal I, the walrus pup, and the killer whale, samples were obtained from animals that had not been fed for at least sixteen to twenty-four hours. Elephant seal I had been in captivity for two days and had not been fed before the blood samples were drawn. The Steller pups also had not been fed before blood was drawn. The walrus pup had been in captivity for twelve days. It had been fasting seven days before it arrived at the Stanford Research Institute's Biosonar Facility. Before being bled, it had been given (twice daily) for three days a formula diet.* The animal was fasting 18 hours prior to the time when the blood sample was drawn. The killer whale had delivered a still born calf six days before being bled and had refused to eat from the time following the delivery until the day she was bled. She died a week later from complications associated with pneumonia.

No attempt was made to determine the nature of the diet of the wild animals studied in this thesis. The probable diet of wild pinnipeds has been described elsewhere.[5] A reasonable expectation would be that the diet of a delphinus consists mostly of fish.

b. Bleeding Procedure

Before bleeding a pinniped, the animal was immobilized either through the use of restraints or through the use of anesthesia. Sufficient restraint was applied by two or more men holding the animal. For large animals, a safer procedure involved strapping them in a trough. Figure

*Walrus diet consisted of 600 cc of water, 100 cc of cod liver oil, 800 cc of pastry whipping cream, 90 gms of calcium caseinate, 3.5 gms of U.S.P. Salt 14, 215 mg of Tetracycline, 8 vitamin and mineral tablets, and 7.5 ozs of canned minced clams.

2 shows the various steps involved in getting the animal into the trough. The animal is first trapped inside a net, and then it is hoisted and placed in the trough. While still in the net, the animal is then strapped down. The net is opened from the top, and the animal is ready to be bled. As is demonstrated by these pictures, a 450 pound animal can be handled in this manner.

Once the pinniped has been immobilized, blood was obtained in the following way. Phocids and walruses were bled from the intra vertebral extradural vein according to the methods of Harrison.[35] The blood of otarids was obtained from the anterior vena cava. In the case of the fur seal and the Steller sea lions, their interdigital web veins of the central webs of the rear flippers are large enough to be used as a source of blood (See Figure 3). During this work, the Steller pups were the only animals bled from the interdigital web vein. The California sea lion was the only animal that was anesthetized. The anesthetic was methoxyfluorane.

It is possible to bleed cetaceans in shallow water by holding the animal's tail above water. However, if the animal can first be hoisted from its tank and placed on a cushioned table, the bleeding can be done much more easily. Unless the animal is very active, two or three men are sufficient to hold the animal steady on the table. Blood was obtained from the fluke veins (See Figure 4).

In the bleeding of all the animals except the phocids, blood was drawn with a syringe and then released slowly into a vial. An 18 guage 1 1/2 inch hypodermic needle was used in obtaining blood from the interdigital web vein, and a 16 gauge 6 inch needle was used to obtain blood from the vena cava. A 20 gauge 1 1/2 inch or an 18 gauge 1 1/2 inch

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Figure 2. Sequence of steps used in the netting and bleeding of a large pinniped.

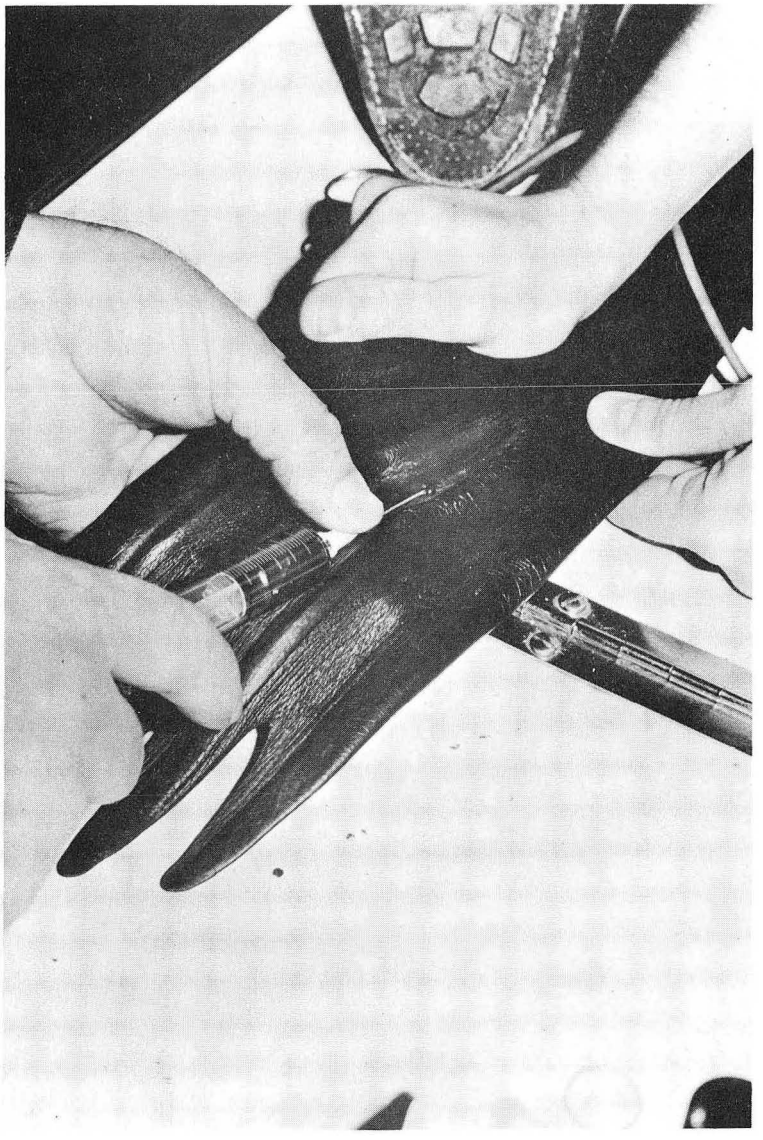

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Figure 3. The drawing of blood from the interdigital web vein of the central web of the rear flipper.

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Figure 4. The drawing of blood from the fluke vein of a dolphin.

needle were used on the fluke veins of dolphins and porpoises, and an 18 gauge 1 1/2 inch needle was used on the fluke vein of the killer whale. In the case of the elephant seals and harbor seals, blood was drawn using a disposable blood drawing set specially adapted for bleeding a seal. The set normally consisted of two stainless steel needles connected by 2 1/2 feet of plastic tubing. Since the needles in the set were too short to penetrate the blubber of the animal, one of the needles was cut away. A teflon adaptor, which formed an air tight connection, was inserted into the cut-off end. A 16 gauge 6 inch stainless steel hypodermic needly was inserted into the animal. Then 10 ml of blood were withdrawn into a disposable syringe and discarded. This was done to flush out any tissue entrapped in the needle. The teflon adapter, now attached to the tubing, was immediately inserted into the head of the hypodermic needle, and the remaining needle at the other end of the tubing was inserted into a 30 ml evacuated, rubber-stoppered glass tube.

The tubes filled with blood were then chilled with ice and transported to the laboratory. Two to three hours after the bleeding, the tubes were reamed inside with a glass stirring rod to release the clot, and they then were centrifuged at 350 x g for 25 minutes. The supernatant was pipetted off and transferred into 12 ml conical centrifuge tubes. Centrifugation at 800 x g for 25 minutes followed to remove any cells or clot fragments that might still have remained in the serum. The sera obtained from fasting animals were always clear, and no turbid layer ever floated to the top of the tubes.

Two interesting phenomena were encountered in obtaining blood from the animals. For reasons currently unknown, the red blood cells of marine mammals were found to be subject to hemolysis during the bleeding procedure. This occurred most frequently when the blood was drawn with

a syringe. But no serious difficulties were encountered in the subsequent analysis of hemolyzed serum. The other phenomenon was a long whole blood clotting time observed in bottlenose dolphins. The blood of these animals has a clotting time of four hours or more. Such prolonged exposure of the blood to room temperature could enhance the possibility of lipid oxidation. The problem can be avoided somewhat by centrifuging the blood immediately after bleeding, removing the plasma, and storing it in a chilled capped glass vial. The fibrin clot can subsequently be removed after eight hours with a glass stirring rod.

c. Lipoprotein Analysis

1. Chemical analysis of whole serum lipids

In each of the marine mammals studied, sera were set aside for determination of the lipid constituents. The lipids were extracted according to a modification of the method of Sperry and Brand.[36] In this modified procedure used at Donner, 1 ml of lipoprotein containing solution is placed in a 40 ml vial. Then 8.5 ml of methanol and 8.5 ml of choloroform are added to the vial. The vial is capped, swirled gently, and placed into a heated block for twenty minutes at a temperature between 65 and 70° C. Because marine fats contain large amounts of polyunsaturated fatty acids, heat was not used in the extracting of lipids for most of the animals in order to reduce the possibility of lipid oxidation at these temperatures.* When possible, an additional 1 ml of whole serum was extracted with the heat step included. This was done to ascertain if the lipid yields in the heat-free extraction were different. A comparison of unheated and heated extraction data is shown in Table II. The percentage difference in yield was less than 3.0%. Most of the data in this section on the chemical composition were obtained for lipids that were extracted

*As another precaution against oxidation, all solvent evaporations were done under nitrogen and at moderate temperatures (40-45°C). The lipids were also stored under nitrogen and refrigerated at -25° C.

without heat. Lipids extracted with the heat step included will be designated as such in the test.

Following extraction, the lipids were fractionated by silicic acid chromatography according to a procedure described by Hirsch and Ahrens.*[37] Each fraction was quantified by infrared analysis.[21] No unusual features were seen in the infrared spectra of the different lipids of the various marine mammals with the exception of the phospholipids from the whole serum of the killer whale. Killer whale phospholipids showed a low absorbance peak (roughly one third of the value noted for other marine mammals at the same concentration, viz. 3.0 mg/ml) at 5.8×10^3 nanometers. An absorbance peak in the region of $9.2 - 9.4 \times 10^3$ nanometers indicated the presence of phosphate esters. Because the presence of fatty acid esters would produce an absorbance peak at 5.8×10^3 nanometers, the phospholipids of this killer whale were primarily either sphingomyelin or lysolecithin, but not lecithin. No attempt was made to determine the serum concentrations of sphingomyelin or lysolecithin. The serum concentration of unesterified fatty acids was not sufficiently elevated to conclude that the absence of lecithin could have resulted from its degradation.

2. Refractometric determination of density of protein-free serum

During this work, density determinations were made on the protein-free sera of a California sea lion and an Atlantic dolphin

*1% ether in hexane was used to elute the cholesteryl esters, 100% ether was used to elute the triglycerides, the unesterified fatty acids, and the unesterified cholesterol, and 100% methanol was used to elute the phospholipids.

according to the procedure of Lindgren, et al.*[38] This was done to insure that the background density of the sera of marine mammals did not differ markedly from the background density of human serum. The procedures at this laboratory have been developed for human serum. In the analysis of human serum, the values of the index of refraction range from 1.334236 to 1.334374. The measurements were made at a temperature of 26° C and at a wavelength of 589.3 nanometers. For the California sea lion and Atlantic dolphin, the values of the index of refraction were 1.334456 and 1.334593 respectively. These values correspond to solution densities of 1.0068 and 1.0073 gm/cc. The value of the density of human serum is 1.006 gm/cc. The differences were not considered large enough to warrant a change in the procedures.

3. Ultracentrifugal Isolation of Serum Lipoproteins for Flotation and Concentration Analysis.

The procedures used for the isolation and flotation analysis of serum lipoproteins of both the low and high density distributions were those described by Lindgren et al.[28] During the ultracentrifugal isolation of serum lipoproteins for the determination of the low density distribution, the concentration of lipoproteins relative to their serum concentration is increased threefold in a medium of density 1.0630 gm/cc. During the isolation of the total high density fraction, there was no increase in the concentrations of these serum lipoproteins relative to their serum concentrations. In this case, the lipoproteins were isolated in a medium

*For this determination, 6 ml of serum were directly centrifuged for 18 hours at 114,000 x g. At the completion of the centrifugal run, the lipoportein fraction in the top 1 ml is quantitatively removed into a 1 ml standard volumetric flask. The second top ml, which is free of protein and lipoprotein, is removed in the same manner for refractometric determinations. The refractive increment (above H_2O) of this second ml fraction is then measured for estimation of solution density. The assumption is made that the second ml contains a NaCl solution.

of density 1.21 gm/cc.* The analyses of the ultracentrifugal schlieren patterns were done with the aid of the computer program mentioned earlier.

It should be noted that when the concentration of serum lipoproteins is below a certain limit a detectable schlieren pattern might not be produced. If serum lipoproteins with a broad range of flotation rates are present in the serum, as much as 40 mg/100 ml and 60 mg/100 ml may not be detected by analytical ultracentrifugation in the low density and high density distributions respectively. For a narrower range of flotation rate values, the limit of detectability is 20 mg/100 ml in the low density distribution and 30 mg/100 ml in the high density distribution. Concentration data for LDL determined by analytical ultracentrifugation often were less than the results of chemical determinations. In every case this occurred, low concentrations of LDL were encountered. The differences are probably due to the detectability limits of analytical ultracentrifugation.

4. Ultracentrifugal Lipoprotein Isolation and Chemical Analysis of the Lipid Moiety

The procedures used for the isolation of the three major density fractions were those described by Freeman et al.**[21] The same procedures

*Routine lipoprotein analyses were carried out at 52,640 rpm in a Spinco AN-D two place rotor. The temperature during the run was 26° C.

**The procedure consists of the following steps. Serum in a 6ml centrifuge tube is centrifuged for 18 hours at 114,000 x g. Because protein-free serum has a density of 1.006 gm/cc, the lipoproteins in the total VLDL class (d<1.006 gm/cc) float to the top of the centrifuge tube. The VLDL is removed in a 1 ml volume and transferred to an appropriate vial. The second ml is removed and discarded. The remaining 4 ml in the centrifuge tube are brought to a density of 1.070 gm/cc by the addition of an appropriate solution of NaCl-NaBr. The tubes are then ultracentrifuged for 24 hours at 114,000 x g. Following this ultracentrifugation run, the low density lipoproteins are concentrated in the top 1 ml. As in the first ultracentrifugation, the top 1 ml containing the LDL is removed and saved. The second ml is also removed and discarded. Finally, another NaCl-NaBr solution is added to the 4 ml remaining in the ultracentrifuge tube to bring the density to 1.218 gm/cc. The tubes are again centrifuged for 24 hours at 114,000 x g. After this final ultracentrifugation, the high density lipoproteins are concentrated in the top 1 ml. As in the above two cases, the top 1 ml is removed and saved. In this manner the three density fractions are obtained.

used in the extraction and quantification of whole serum lipids were also applied in the analysis of these fractions.

Table II. Effect of Heating on Lipid Extraction Yield (in mg)

Animals	With Heating	Without Heating
Harbor Seal I	9.698	9.877
Harbor Seal III	11.503	11.609
Calif. Sea Lion	6.730	6.885
Weddell Fetus II	7.922	7.700
Walrus Pup	8.766	8.696
Killer Whale	4.180	4.420

IV. RESULTS

a. Analysis of Whole Serum Lipids of Pinnipeds and Cetaceans

Data on the concentration and percent lipid composition of lipids in whole serum are shown in Table III. The pinnipeds have a higher total concentration of lipids than the cetaceans. In pinnipeds, phospholipids are the predominant lipid. In cetaceans, the whole serum lipid concentration is less than 700 mg/100 ml, and the predominant lipid is cholesteryl esters. Cetaceans also show a higher percentage of triglycerides and unesterified fatty acids (UFA) than pinnipeds.

In the case of the Weddell seals, the fur seals, and the Steller sea lion, sufficient blood samples were obtained to perform only chemical analyses on the whole serum lipids. These data are presented in Table XI in Appendix II. Elevated levels of UFA were present in the sera of the fur seals and the Steller sea lion pups. The sera of the fur seals were transported from the Pribilof Islands to California without refrigeration, and the higher concentration in UFA could have resulted from the hydrolysis of fatty acid esters. The higher levels of UFA in the Steller pups could also be related to an increased energy demand at this early stage of development. This might also explain the higher levels of UFA seen in the walrus pup in Table III. Elevated UFA levels seen in young lambs have been interpreted as indicative of fat utilization related to development.[39]

The importance of UFA in human energy metabolism was mentioned earlier. Studies of the energy requirements of marine mammals are needed before these differences in the serum concentration of UFA can be understood.

Table III. Lipid Concentration and Composition of Whole Serum of Captive Marine Mammals

		Percent Lipid Composition				
Animals	Concentration (mg/100 ml)	CE	PL	TG	UC	UFA
Pinnipeds						
Harbor Seal I	1050	32.1	55.0	4.2	7.2	1.5
Harbor Seal II	897	32.8	55.9	3.2	6.9	1.1
Elephant Seal I	950	33.6	51.3	4.7	8.8	1.6
Calif. Sea Lion	717	35.4	51.1	4.5	8.4	0.6
Walrus Pup	1140	38.0	45.3	3.4	9.4	3.8
Cetaceans						
Atlantic Dolphin I	565	38.1	42.4	8.0	8.1	3.4
Atlantic Dolphin II	424	39.3	39.6	9.7	9.7	1.7
Atlantic Dolphin III	690	39.8	35.8	13.6	8.8	2.0
Pacific Dolphin	648	41.5	36.5	9.8	8.1	4.0
Killer Whale	547	46.0	27.2	9.2	13.8	3.7

Abbreviations: CE (Cholesteryl Esters), PL (Phospholipid), TG (Triglycerides), UC (Unesterified Cholesterol), UFA (Unesterified Fatty Acids).

b. Ultracentrifugal Analysis of Serum Lipoprotein Distributions

Ultracentrifugal distributions resulting from flotation analyses of the serum lipoproteins of the different marine mammals are shown in Figures 5, 6, and 7. In general, both pinnipeds and cetaceans have a predominance of high density lipoproteins (HDL) At least 70% of the distribution is in the HDL class. The walrus pup and the killer whale are exceptions. Flotation rate values for the major peaks and the concentration of LDL and HDL are shown in Table IV. In general, pinnipeds have higher concentrations of HDL than the cetaceans. The highest concentration among the cetaceans was 813 mg/100 ml, and the lowest concentration among the pinnipeds was 878 mg/100 ml.

In both the low density and high density distributions, the peak flotation rates for the cetaceans are higher than those for the pinnipeds. As can be seen from Table IV, the major peaks of cetaceans in the low density distribution have S_F^o values greater than 7.0. Among the pinnipeds, the major peaks have S_F^o values less than 3.0. The walrus pup, an exception, has a second peak with an S_F^o value of 8.1. In humans, S_F^o values of the major peaks of the S_F^o 0-12 lipoproteins have been shown to be inversely correlated with the concentration of VLDL.[28] Human males have lower S_F^o rates and higher concentrations of VLDL. The opposite is true for the females. No such correlation is obvious from the analytical ultracentrifugal data for marine mammals.

The peak flotation rate differences between these two orders of marine mammals are not as great in the high density distribution. In general the major flotation peaks of cetaceans have $F_{1.20}$ values greater than 3.0 whereas the pinnipeds have major peaks with $F_{1.20}$ values less than 3.0. The elephant seal I, the Pacific porpoise II, and the killer whale are the exceptions. It is interesting to note that Atlantic dolphin III and

Pacific porpoise II, which are males, have a higher percentage of HDL in the flotation rate interval $F_{1.20}$ 0-3 than Atlantic dolphin I and II, and Pacific porpoise I which are females. Minor secondary peaks were observed in the high density distribution of four female cetaceans, Atlantic dolphin I and II, the Pacific dolphin, and the killer whale. The number of animals studied is currently too small to explain these differences on sex alone.

Table IV Concentration and Flotation Rates of Major Peaks in the Serum Lipoprotein Distribution of Captive Marine Mammals

	LDL (mg/100 ml)	S_F^o Values of Major Peaks		HDL (mg/100 ml) $F_{1.20}$: 0-3	HDL (mg/100 ml) $F_{1.20}$: 3-20	Total	$F_{1.20}$ Values of Major Peaks	
PINNIPEDS								
Harbor Seal I	200	2.8		603 (44)	753 (56)	1356	2.8	
Harbor Seal II	138	1.9		598 (52)	545 (48)	1143	2.5	
Calif. Seal I	102	1.9		352 (40)	526 (60)	878	2.8	
Elephant Seal I	186	1.4		224 (20)	879 (80)	1103	4.4	
Walrus Pup	461	2.9	8.1	631 (72)	251 (28)	882	2.9	
CETACEANS								
Atlantic Dolphin I	44	7.5		199 (30)	457 (70)	656	3.4	9.6
Atlantic Dolphin II	96	8.5		128 (30)	304 (70)	432	3.5	7.8
Atlantic Dolphin III	159	7.3		208 (44)	270 (56)	478	3.2	
Pacific Dolphin	26	9.9		256 (31)	557 (69)	813	3.3	8.2
Pacific Porpoise I	153	8.1		147 (36)	257 (64)	404	3.4	
Pacific Porpoise II	86	7.3		140 (43)	182 (57)	322	2.8	
Killer Whale	445	8.4		67 (36)	118 (64)	185	1.9	6.9

Values in parentheses are percentages based on total lipoprotein concentration in the HDL class.

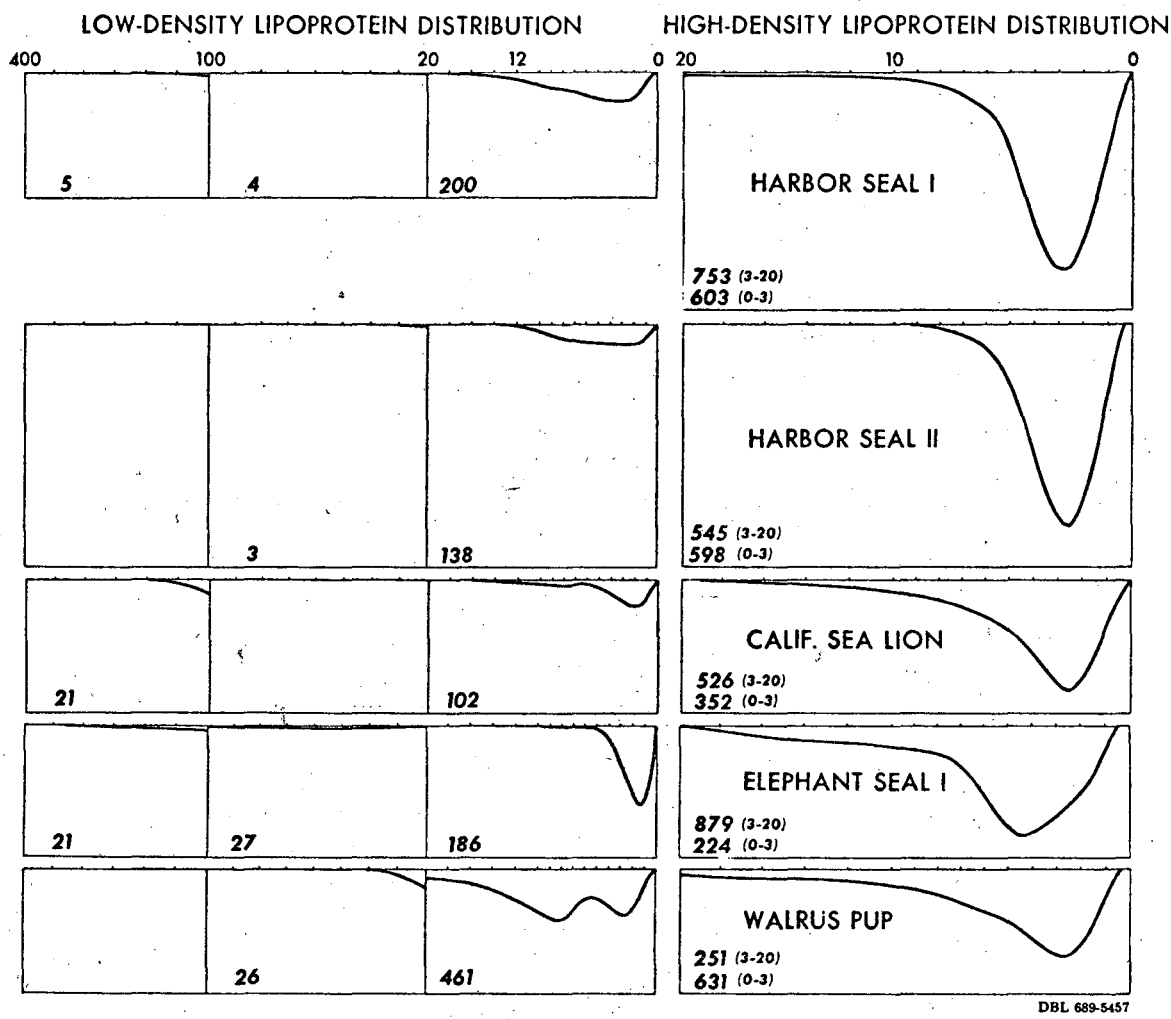


Figure 5 Ultracentrifugal distribution of lipoproteins in the sera of certain pinnipeds.

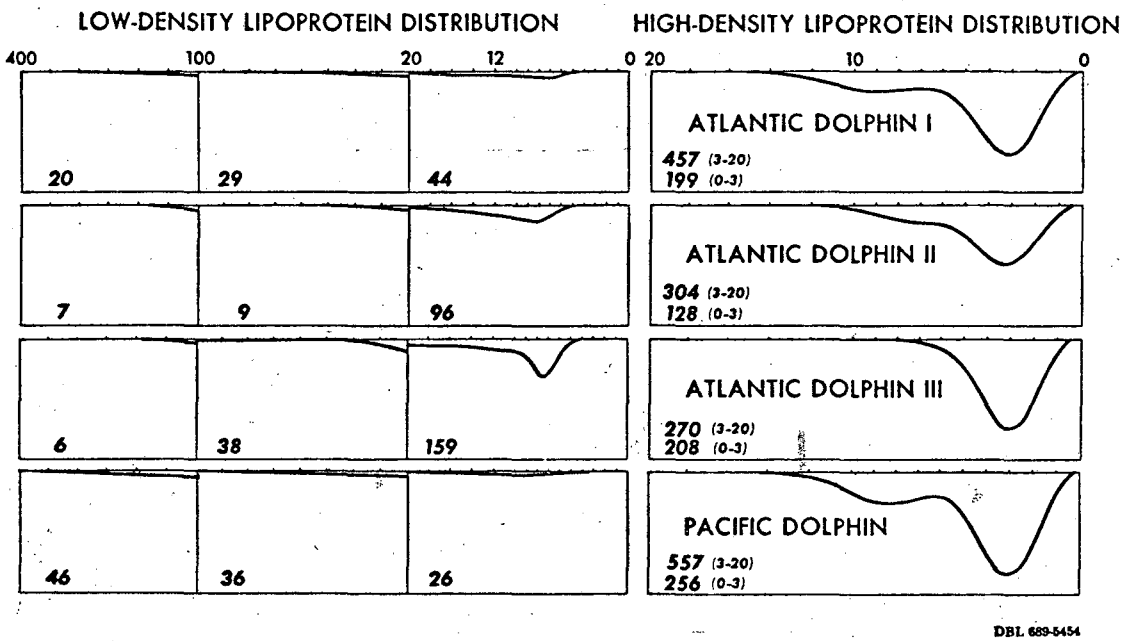


Figure 6 Ultracentrifugal distribution of lipoproteins in the sera of certain cetaceans.

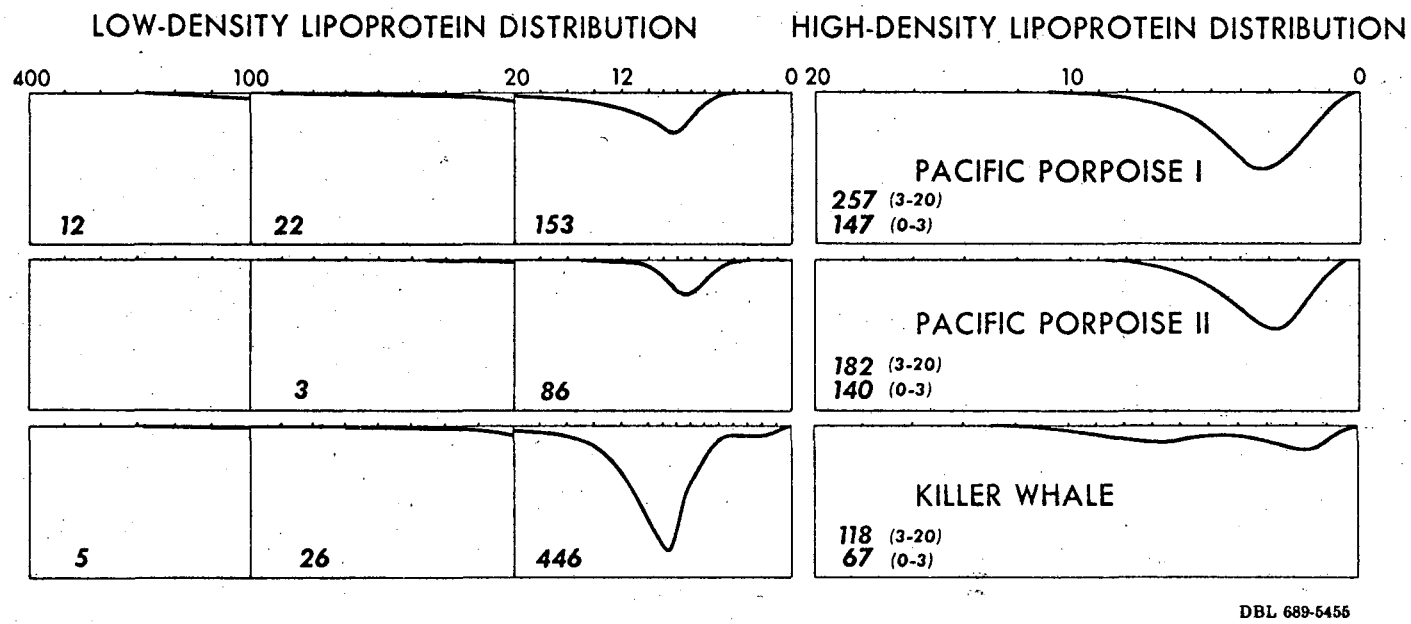


Figure 7 Ultracentrifugal distribution of lipoproteins in the sera of certain cetaceans.

c. Chemical Analysis of the Major Lipoprotein Fractions

Chemical analyses were performed on the lipid moieties of each of the three density fractions for most of the marine mammals whose serum lipoproteins distributions were described in the preceeding section. These data are presented in Table V. The chemical analyses of lipids of marine mammals not discussed in the preceeding section are in Appendix II. The chemical composition of the lipid moieties of the three major lipoprotein fractions are expressed in terms of percent of cholesteryl esters, phospholipids, unesterified cholesterol, triglycerides, and unesterified fatty acids.

Unfortunately, the concentrations of the total VLDL in some of the fasting marine mammals were too low to carry out accurate analyses. Low concentrations of LDL encountered in the serum of the Pacific dolphin also prevented adequate chemical analyses.

Among the animals studied, marked differences in chemical composition of the LDL among pinnipeds and cetaceans were seen in the sera of the walrus pup and the killer whale. Only in these two animals was the percentage of cholesteryl esters higher than that of phospholipids. Differences between the cetaceans and pinnipeds were noted in the chemical composition of the HDL lipid moiety. Pinnipeds have a high percentage of phospholipids, and cetaceans have a high percentage of cholesteryl esters. This high percentage of cholesteryl esters was not found in all the members of the order Cetacea (See Table XII, Appendix II). All of the marine mammals were found to show a low percentage of triglyceride content in the HDL lipid moiety.

Table V. Concentration and Composition of the Major Density Fractions of Serum Lipoproteins of Captive Marine Mammals

	Concentration (mg/100 ml)	Percent Lipid Composition				
		CE	PL	TG	UC	UFA
PINNIPEDS		VLDL (d< 1.006 gm/cc)				
Harbor Seal II	9*					
Elephant Seal I	61	3.5	26.2	56.5	12.7	1.1
Calif. Sea Lion	28	8.6	19.9	57.5	12.4	1.5
		LDL (d: 1.006-1.063 gm/cc)				
Harbor Seal II	130	31.4	41.8	12.9	13.6	0.3
Elephant Seal I	216	39.9	46.2	2.0	11.7	0.3
Calif. Sea Lion	131	37.4	41.7	7.0	13.5	0.3
Walrus Pup	526**	41.6	37.4	9.0	11.5	0.5
		HDL (d: 1.063-1.21 gm/cc)				
Harbor Seal II	730	31.5	61.2	0.8	6.2	0.2
Elephant Seal I	630	41.2	50.4	0.4	7.1	0.8
Calif. Sea Lion	536	36.6	54.7	0.9	7.5	0.3
Walrus Pup	495	34.2	55.7	0.6	7.5	2.1
CETACEANS		VLDL (d< 1.006 gm/cc)				
Atlantic Dolphin I	59	15.0	27.9	45.1	11.3	0.7
Atlantic Dolphin II	24*					
Pacific Dolphin	83	9.1	21.7	56.0	12.1	1.2
Killer Whale	8*					
		LDL (d: 1.006-1.063 gm/cc)				
Atlantic Dolphin I	80	31.6	40.4	13.1	14.1	0.7
Atlantic Dolphin II	100	26.4	36.0	21.6	15.6	0.4
Pacific Dolphin	34*					
Killer Whale	362	46.7	24.6	11.2	17.0	0.4
		HDL (d: 1.063-1.21 gm/cc)				
Atlantic Dolphin I	356	50.2	41.9	1.2	6.0	0.6
Atlantic Dolphin II	249	49.6	42.0	1.4	6.8	0.3
Atlantic Dolphin III	304***	52.6	39.4	1.2	6.6	0.2
Pacific Dolphin	466	54.8	36.5	0.8	6.4	1.4
Killer Whale	117	60.4	25.2	1.6	11.4	1.4

Table V cont.

* Because only a small quantity of blood was obtained, the amount of the extracted lipid was not large enough for chemical analysis. The value for concentration was determined by gravimetric means.

** These data are for lipoproteins with density less than 1.063 gm/cc. However, as can be seen from the lipoprotein distribution of the walrus pup, the concentration of lipoproteins in the VLDL is only 26 mg/100 ml. Approximately 4% of the lipid concentration cited in the table is in the VLDL class.

*** Only a small amount of serum was obtained from Atlantic dolphin III. Instead of doing chemical analyses on all of the density fractions, it was decided only to characterize the low and high density ultracentrifugal distributions of serum lipoproteins. Because the residue of the preprative ultracentrifugation performed at 1.063 gm/cc contains the HDL, it was possible to obtain the chemical data for the HDL.

V. DISCUSSION

In both fasting pinnipeds and cetaceans the serum concentrations of VLDL were low. Clearing of VLDL in these animals would appear to be very efficient. Efficient tissue uptake of triglycerides is consistent with their role in providing thermal insulation and energy to marine mammals. The low concentration of VLDL can also be considered consistent with the data that these animals do not synthesize a significant amount of endogenous fatty acids. Aside from this, the data presented in Tables III to V point out the existence of distinct differences between the pinnipeds and cetaceans in the physical and chemical properties of their serum lipoprotein distributions. Pinnipeds were found to have a higher concentration of total serum lipids and of HDL. In both the high and low density distributions, peaks of the major components of cetaceans had higher flotation rates. And in the case of the HDL of certain cetaceans, cholesteryl ester rather than phospholipid was the predominant lipid of the HDL class. It is not possible to give an explanation for these differences at this time. To see what differences exist between marine mammals and terrestial mammals I have collected lipoprotein data available in the literature on various terrestial mammals. These data are presented in Tables XIII to XIX in Appendix III. In these tables, the animals have been arranged into the general categories of herbivores, omnivores, and carnivores. Animals were categorized in this manner according to available information on their diet in the wild.

The chemical composition data in Tables XVII and XVIII indicate the existence of basic differences among the major classes of serum lipoproteins. The total VLDL class, when present, was found to be composed chiefly of triglycerides. Differences were seen in the composition of LDL and HDL. In the LDL of man, the walrus pup, and the killer whale, the percentage of cholesteryl ester was higher than that of phospholipids. In the LDL class,

other animals had a predominance of phospholipids. In the HDL of the bison, the cow, the dolphin, and the killer whale, cholesteryl ester was the predominant lipid. All the other mammals have a predominance of phospholipids in the HDL class. In all the marine mammals and in the cow, the triglyceride percentage composition of the HDL lipid moiety is low. The reason for these differences is not clear. Nor does there appear to be an explanation which can be related to the dietary patterns of these animals.

Even though the differences noted in the chemical data could not be attributed to diet, the categories were also used in Table XIX to group the analytical ultracentrifugal data. Table XIX contains the results of some recent data on certain terrestial mammals. The representations of the fully corrected schlieren patterns for these animals are in Figure 22. Comparative studies conducted in other laboratories have also appeared in the literature.[40,41] However, in these studies no measurements of the concentration of the VLDL class were made. As can be seen from the patterns in Figure 22, the beagles, like the marine mammals, have a predominance of HDL. The guinea pig, on the other hand, has no detectable HDL. Mention should be made here of the high density distribution of humans in Figure 1. Female humans have a predominance of HDL_2 whereas males have a predominance of HDL_3. Although the data in Table IV were not given in terms of HDL_2 and HDL_3, in the two cases in which comparison of sexes was possible the female Atlantic dolphins and the female Pacific porpoise had a higher percentage of serum lipoproteins in the flotation rate interval $F_{1.20}$ 3-20 than the males of these two species of cetaceans. Unfortunately the numbers of animals studied are too small to conclude that there is a sex difference among these cetaceans. In the beagle study, in which the number of animals studied was larger, there was no sex difference in the

high density distribution.

In an attempt to summarize these lipoprotein data of marine mammals together with data in Tables XIV, XV, and XIX, the following outline has been arranged in order to point out the significant features of serum lipoprotein distributions in mammals.

HERBIVORES

Artiodactyla

Bison

Average distribution of the serum lipoprotein classes: 74% (HDL) and 26% (LDL and VLDL).
Comments: None

Cow

Average distribution of the serum lipoprotein class: 96% (HDL) and 4% (LDL and VLDL).
Comments: None

Lagomorpha

Rabbit

Average distribution of the serum lipoprotein class: 77% (HDL) and 23% (LDL and VLDL).
Comments: Several investigators have reported that rabbits have a predominance of LDL.[41,42] Studies are needed to show if such differences in lipoprotein distributions exist among species.

Rodentia

Guinea Pig

Average distribution of the serum lipoprotein class: ___ (HDL), 80% (LDL) and 20% (VLDL).
Comments: HDL is undetectable in guinea pig serum. Because of the correspondence between alpha lipoprotein and HDL, one would expect that there would also be no electrophoretically detectable alpha lipoprotein. However, alpha - 1 but no beta lipoproteins have been reported to be present in the animals.[43] In order to investigate this apparent anomaly, a paper electrophoretic study of plasm serum lipoproteins was done on two male guinea pigs, and an ultracentrifugal analysis was done on their pooled plasma. The expected beta lipoprotein band corresponding to the LDL observed in the ultracentrifuge was found. There was no evidence of HDL in the ultracentrifugal study or of alpha lipoprotein in the electrophoretic study. There of course could be a species difference. Nevertheless, the absence of either

beta or alpha serum lipoprotein would make the guinea pig a most interesting experimental animal for future lipoprotein studies. In particular, such studies could possibly be most useful in understanding lipoprotein metabolism in human diseases where these lipoproteins are abnormally low.

OMNIVORES

Primates

Baboon

Average distribution of serum lipoprotein classes: 74% (HDL), 26% (LDL), and __ (VLDL).
Comments: None

Man

Average distribution of the serum lipoprotein classes: Male 32% (HDL), 50% (LDL), and 18% (VLDL); Female 42% (HDL), 49% (LDL), and 9% (VLDL).
Comments: In humans, both age and sex have been demonstrated to be factors influencing the serum lipoprotein distributions. For example, females have less LDL but more HDL_2 than men. Man has the highest value of LDL among the animals. These studies were done on an American population. Studies in another part of the world might possibly give different results.

Rodentia

Rat

Average distribution of the serum lipoprotein classes: 67% (HDL), 5% (LDL), and 28% (VLDL), total serum lipoproteins according to the Donner data in Table XIX. The HDL percentage is 82% according to the data of Hillyard et al. in Table XVIII.
Comments: The presence of serum lipoproteins with flotation rates in the interval S_F^0 20-400 might be seen only in sera obtained from non-fasting animals. Further studies are needed to clarify this point.

CARNIVORES

Carnivora

Dog

Average distribution of the serum lipoprotein classes: Male 90% (HDL), 9% (LDL), and 1% (VLDL); Female 91% (HDL), 9% (LDL), and __ (VLDL) according to the Donner beagle data in Table XIX. The HDL percentage is 91% according to the data of Hillyard et al.
Comments: None

Cetacea

Atlantic Dolphin

Average distribution of the serum lipoprotein classes: 79% (HDL), 16% (LDL), and 5% (VLDL).
Comments: There are two peaks in the HDL distribution of Atlantic dolphin I and II.

Pacific Dolphin

Distribution of serum lipoprotein classes: 87% (HDL), 3% (LDL), and 10% (VLDL).
Comments: This animal has the highest concentration of HDL among the cetaceans. There are two peaks in the HDL distribution.

Pacific Porpoise

Average distribution of the serum lipoprotein classes: 73% (HDL), 24% (LDL), and 3% (VLDL).
Comments: None

Killer Whale

Distribution of the serum lipoprotein classes: 28% (HDL), 67% (LDL), and 5% (VLDL).
Comments: The presence of high concentrations of LDL in the killer whale might have resulted from the animal's pathological condition. Unlike the earlier study of a female killer whale as well as my studies on other marine mammals, the HDL of this animal comprises a relatively low percentage of the total serum lipoproteins.

Pinnipedia

Elephant Seal

Distribution of serum lipoprotein classes: 88% (HDL), 12% (LDL), __ (VLDL).
Comments: The percentage of LDL in elephant seal I, although not high, is most interesting. Studies done on three other elephant seals indicate that there was very little LDL present in these animals (See Tables VI and XII). This could be an indication of differences in the serum lipoprotein distributions between captive and wild elephant seals.

Harbor Seal

Average distribution of the serum lipoprotein classes: 88% (HDL), 12% (LDL), __(VLDL).
Comments: The harbor seals have the highest concentration of HDL among the mammals mentioned in this work.

California Sea Lion

Distribution of the serum lipoprotein classes: 88% (HDL), 10% (LDL), and 2% (VLDL).
Comments: None

Walrus Pup

Distribution of the serum lipoprotein classes: 64% (HDL), 34% (LDL), and 2% (VLDL).
Comments: In the low density distribution, there are two peaks. The concentration of LDL in this animal is greater than the other pinnipeds. This could result from the animal being fed large amounts of saturated fats present in the whipping cream rich formula (See footnote, page 16) Polyunsaturated fats have been demonstrated to cause a reduction in serum cholesterol concentrations in both men and rats.[44,45,46] In this regard, it is interesting to note that walrus LDL contains a higher percentage of total cholesterol than the LDL of the other pinnipeds.

Some general criticisms of the data upon which this outline was based can be made. First, several authors did not specify the species, the age, or the sex of the animals. Also the data themselves might not be totally representative because of limited numbers. Furthermore, my classification of captive animals on the basis of what their diet would be in the wild is, at best, questionable. In spite of these limitations, the outline suggests that non-ruminating herbivores and man tend to have a higher percentage of LDL in contrast to carnivores. The assumption has been made that the rabbit has a predominance of LDL. A high carbohydrate diet is common to herbivores and some omnivores but not to carnivores. Dietary carbohydrate might be a factor determining the concentration of LDL in a given animal.

As was pointed out earlier, the de novo synthesis of triglycerides from carbohydrates can take place in the liver. These triglycerides then are secreted into the blood stream as part of a VLDL complex. The apoprotein of LDL is considered necessary for the secretion of triglycerides from the tissue cells into the blood. Because of the predominance of LDL in man and non-ruminating herbivores, the participation of B protein in the

secretion of triglycerides might be somehow related to the concentration of circulating LDL. The earlier mentioned study on orotic acid-fed rats indicates that there is. The inhibition of the synthesis of B protein or the complexing of B protein with triglycerides in the liver was associated with a lowering of serum levels of beta lipoproteins.

Furthermore, the additional question arises: can the low percentage of LDL in carnivores be explained by the proposed relationship? Because of their low carbohydrate diet, most of the fatty acids of triglycerides in carnivores are dietary in origin. The triglycerides are synthesized in the intestinal mucosal cells and subsequently transported by chylomicra in the blood. As in the case of endogenous triglycerides released from hepatic cells, the secretion of these dietary fats apparently also requires the B protein. Should not then the functioning of the B protein in the intestinal mucosal cells also have an effect on the concentration of LDL? The data indicate that it does not. In the study of orotic acid-fed rats, it was observed that fat ingestion produced an alimentary lipemia with no detectable increase in the concentration of beta lipoproteins.[23] Either the B protein associated with the release of chylomicra does not produce an increase in the concentration of circulating beta lipoproteins, or the amount of B protein associated with the formation of chylomicra is too low to have any noticeable effect. It was noted earlier that chylomicra have 81-89% TG and 1-2% protein, and VLDL have 57-68% TG and 5-12% protein. Using the average value of these percentages, the ratio of triglycerides to protein is 57 for chylomicra and 7 for VLDL. In other words, VLDL requires roughly eight times more protein than chylomicra to transport the same amount of triglycerides. Currently the amounts of A and B protein in these two serum lipoprotein classes are not known. Nevertheless, it would appear reasonable to assume that more B protein is

required in the transport of triglycerides in the VLDL class than in the chylomicron class.

Before concluding this discussion, several additional points should be made. By stating that the diet of carnivores is low in carbohydrates the assumption is made that these animals do not selectively eat certain organs of their prey. For example, by eating mostly muscles or livers which could be high in glycogen, these animals could conceivably ingest a higher percentage of carbohydrates than the total percentage of carbohydrate distributed in their prey. Secondly, how do the other omnivores and the ruminants fit into this scheme? The diet of the rat and the baboon (which were listed as omnivores) in the wild is often that of a carnivore.[47] For the same reasons already stated for the carnivores, it is possible that the requirement of both these mammals for B protein would be less. The cow and the bison, because they are ruminants, absorb very little dietary carbohydrate from their digestive tracts.[48] The volatile fatty acids, the major end product of ruminal fermentation, account for 70-80% of the total energy intake.[49] These volatile fatty acids are soluble in the blood, and they can be taken up by the tissue for oxidation. Propionate can also be converted into glucose in the liver, and acetate is incorporated into long chain fatty acids in the adipose tissue. As a result, the necessity for synthesizing triglycerides and the B protein might be greatly reduced in these animals. Finally, hormonal factors, genetic factors, and dietary factors are known to have an influence on the concentration of LDL, and these animals should also be considered in the development of an overall theory.

In the above I have suggested that the factors governing B protein participation in hepatic and intestinal lipoprotein synthesis and secretion might be an additional factor governing the circulating level of serum LDL. Additional studies are required to shed more light on the role of the protein.

POSTPRANDIAL STUDIES

I. INTRODUCTION

In the previous section, major classes of the serum lipoproteins of marine mammals were characterized according to their chemical and physical properties. As was mentioned earlier, this work was undertaken not only to investigate the comparative aspects of serum lipoproteins in marine mammals but also to gain some insight into the transport of triglycerides in these animals. The low serum concentrations of the VLDL class in marine mammals are consistent with the idea that triglycerides are efficiently taken up by the tissue of these animals for energy storage and for thermal insulation. Because the serum concentrations of triglycerides are so low in fasting marine mammals, ultracentrifugal and chemical characterizations were extremely difficult. To obtain adequate amounts for characterization, it was necessary to analyze the serum after meals containing high amounts of fat. From the 3rd to the 11th hours following such a meal, the concentration of serum lipoproteins of the total VLDL is sufficiently high to permit their characterization. In addition, the serum enzyme, lecithin cholesterol acyl transferase, LCAT, was also studied in several marine mammals. This enzyme has been associated with transport of triglyceride.[50]

Early postprandial studies

In 1920, Gage, using the dark field microscope, observed fat particles in the lacteals and the blood following fat ingestion. The diameter of these particles ranged in value from 0.5 to 1.0 microns. Gage proposed the term "chylomicra" to describe these particles because they originated from the chyle and had an average size of about one micron.[51]

In 1924, Gage and Fish, using a net-micrometer in the eye-piece of

the microscope, counted the number of chylomicra in a given area in the field of view.[51] By obtaining a drop of blood at different times following a fatty meal, they were able to demonstrate that the chylomicron count varied as a function of postprandial times. Postprandial studies of this type were done on humans as well as cats, dogs, and rats. The chylomicron count reached a maximum between the third and the fifth postprandial hours, and chylomicra were observed from about six to ten hours following fat ingestion. In several cases among the animals studied, a secondary rise in the chylomicron count was observed. In humans, the authors related the change in the chylomicron count to mental and physical activity. For the other animals they could only propose that the rate of flow of chylomicra into the blood was different from their rate of degradation.

Ultracentrifugal characterization of serum lipoproteins following fat ingestion

Jones *et al*. were the first to report on variations in the ultracentrifugal distributions of serum lipoproteins as a function of postprandial times.[52] Their study pointed out that a transient increase in the serum concentration of lipoproteins with S_F values greater than 60 occurred following the ingestion of a high fat meal. They also noted that as the concentration of lipoproteins of higher S_F values decreased, the concentrations of lipoproteins of lower S_F values (down to 30) increased. These observations suggested to Jones *et al*. that the clearance of glyceryl esters from the blood involves the conversion of the larger lipoproteins to the smaller species of the total VLDL class.

Havel showed that following fat ingestion the increase in triglyceride concentration observed in whole serum was due almost exclusively to increases in the concentration of triglycerides transported by serum lipoproteins of

the total VLDL class.[53] Ingestion of fat equivalent to 0.15% of the person's body weight produced triglyceride concentrations between 100 and 290 mg/100 ml. The duration of the alimentary lipemia was about eight hours. Earlier in this thesis the role of lipoprotein lipase in the hydrolysis and removal of circulating triglycerides was discussed. This enzyme hydrolyzes the triglycerides transported by chylomicra and VLDL. In a study on a subject with lipoprotein lipase deficiency, the serum triglyceride concentration was found to be in excess of 2,200 mg/100 ml from the 8th to the 24th postprandial hours.[54] The fat load was equivalent to 0.21% of the body weight. The clearing of triglycerides is thus dependent on the presence and proper functioning of the enzyme.

Increased concentrations of lipoproteins of the S_F^o 20-400 class have been noted by Nichols et al. and Harlan et al. following fat ingestion at the 5th postprandial hour.[55,56] Another study by Harlan et al. suggests that individuals with lipoprotein lipase deficiencies do not readily form VLDL following fat ingestion and that the activity of lipoprotein lipase may be related to the formation of VLDL from chylomicra.[54] These S_F^o 20-400 lipoproteins could have resulted from either of two mechanisms or a combination of both. They could be the product of sequential breakdown of chylomicra as was suggested by the work of Jones et al. Or these lipoproteins could be of hepatic origin. As has been pointed out previously, the liver can pick up fatty acids which circulate in the blood in a complex with albumin. These fatty acids can be incorporated into triglycerides subsequently secreted into the blood as lipoproteins with flotation rates in the range S_F^o 20-400. During fat ingestion, dietary fatty acids can be taken up by the liver in either of the following ways. First, the hydrolysis of chylomicron triglycerides could occur at the capillary wall. A portion of the

hydrolyzed fatty acids, instead of being taken up by the tissue, could be complexed to albumin and then transported to the liver. The tissues could also take up the fatty acids and reesterify them into triglycerides. The triglycerides could be hydrolyzed, and they could then be secreted into the blood. And the formation of a fatty acid-albumin complex would provide another route for the delivery of fatty acids to the liver. These are possible mechanisms whereby the concentration of the S_F^o 20-400 lipoproteins may increase. However, it should also be recalled that hepatogenous triglycerides can also contain fatty acids primarily derived from de novo synthesis. As a result, the S_F^o 20-400 lipoproteins appearing at the 5th postprandial hour might contain triglycerides composed of both endogenous as well as exogenous fatty acids.

It would be desirable to know the relative increases in concentration of lipoproteins in the chylomicron class and the S_F^o 20-400 class at different postprandial times. It is possible to do this by comparing the concentration of S_F^o 20-400 with the concentration of lipids in the total VLDL class. Unfortunately, both determinations have been made in only one study.[55] Blood samples were obtained just prior to and just following fat ingestion. By comparing the increases in the concentration of lipids in the total VLDL class and the increases in the concentration of serum lipoproteins in the S_F^o 20-400 interval, it was possible to estimate that approximately 75% of the serum lipoproteins in the total VLDL class have S_F^o values in the 20-400 interval at the 5th postprandial hour. Although the triglycerides are initially transported by chylomicra, the major portion is being transported by S_F^o 20-400 lipoproteins within five hours. It is obvious that a better understanding of the transport of dietary fat involves a study of the distribution within the total VLDL class.

Postprandial variations in concentration following fat ingestion have also been observed in the other portions of the serum lipoprotein distributions. These variations were observed in the flotation ranges S_F^o 8-20 and $F_{1.21}$ 4-8.[55] In association with these concentration changes, the chemical compositions of the lipid moieties of the LDL and HDL classes, particularly HDL, have been reported to vary as a function of postprandial times. Increases in phospholipids and triglycerides have been found in the HDL class.[53,55] The triglyceride increments in HDL represent 7.2% of the triglyceride increments observed postprandially in the whole serum of humans. In the dog, an animal normally exhibiting a high concentration of HDL, the triglyceride increments in the HDL class were approximately 22.5% of the triglyceride increments in the whole sera of dogs at the 5th postprandial hour.[57]

Lindgren *et al*. have also reported that the percentage triglyceride composition of the HDL lipid moiety in the sera of humans is significantly related to the serum concentration of VLDL.[58] This uptake of triglycerides by HDL is thought to be associated with the action of the serum enzyme, LCAT.[50]

LCAT and its relation to triglyceride transport

The enzyme, LCAT, catalyzes the reaction: Lecithin + Cholesterol → Lysolecithin + Cholesteryl ester. Glomset has suggested that the reaction catalyzed by LCAT is the major source of cholesteryl esters circulating in the blood.[59] This reaction has been observed only during *in vitro* studies on both plasma and serum. The *in vitro* rate of esterification agrees well with the calculated rate of esterification *in vivo*.[59]

In vitro incubation studies on human serum, rich in VLDL, have shown that triglyceride transfer to HDL occurs in association with a reciprocal transfer of cholesteryl esters to VLDL.[50] These studies have shown that

the transfers are increased through the action of LCAT. As a result, it has been suggested that the LCAT reaction may be related to *in vivo* uptake of triglycerides by HDL. That this transfer does take place *in vivo* is suggested by the chemical compositions of HDL and VLDL in individuals with xanthoma tuberosum. The percentage of cholesteryl esters in the VLDL class and the percentage of triglycerides in the HDL class are 32% and 20% respectively, a little greater than twice the normal values in each case.[60] The serum concentration of lipoproteins in the S_F^o interval 20-100 and 100-400 are very elevated in these subjects.[61,62]

In marine mammals my data show the percentage triglyceride composition of the HDL lipid moiety to be less than 2% (See Table V). To determine if this low percentage of triglycerides in the HDL class is due to an absence of LCAT, the sera of different marine mammals were incubated to test for the activity of this enzyme.

II. METHODS

a. Postprandial Procedures

To minimize any possible trauma for the animals as well as the handlers, the animals were bled no more than three times a day. At each bleeding sixty ml of blood were obtained. In order to estimate the duration of the alimentary lipemia, a 10th hour blood sample was first obtained from each animal. Two weeks later the animal was again bled. On the first day of this second series of bleedings, the 9th and the 11th hour postprandial samples were obtained, and on the next day the 3rd, 5th, and 7th hour samples were withdrawn. Unless noted otherwise in the tables, the animals were fed 5% of their body weight in herring. Samples at each of the above postprandial times were obtained from both harbor seals. Two elephant seals which also were to have been studied died unexpectedly, and analyses were done on only the 10th hour sample. The bleeding procedures and the subsequent serum lipoprotein analyses were essentially the same as those described in a previous section with only two minor modifications.

In order to determine the recovery of lipid during silicic acid chromatography, it was decided during the postprandial study also to weigh each fraction and to compare the sum of the column eluates with the amount of lipid placed initially on the column. The average percentage difference between the two values was less than 3%. However, when the values for each fraction were compared with the results of the infrared analysis, the gravimetrically determined values for the phospholipid fraction were always greater than the value determined by infrared spectrophotometry. To reduce this difference, a second change was made in the procedure.* The discrepancy between the gravimetric results and

*According to these procedures, after the eluting solvent has been evaporated, the lipid fraction is dissolved in chloroform and transferred from a 40 ml vial to an 8 ml vial. The latter is then capped and stored for subsequent infrared analysis. In preparing samples for infrared

the infrared results was associated with the use of insufficient solvent for dissolving the phospholipids prior to infrared analysis. As a result, the dilution factor was changed from 4.5 mg/ml to 3.0 mg/ml. The average percentage difference between the gravimetric results and the infrared results was 10% for harbor seal I (before the change) as compared with a value of 5% for harbor seal III.

In the chemical analysis of the lipid fractions obtained from harbor seal III, a second rise in triglyceride concentration was found to occur between the 7th and 11th hours. Since the 7th hour sample was obtained on the day after the 9th and 11th hour samples were obtained, the experiment was repeated in part. This time the blood samples were drawn at the 7th, 9th, and 11th hour of the same day. An increase in the triglyceride concentration was again observed between the 7th and 11th hours. The lipoprotein distributions for the 7th, 9th, and 11th hours, shown in the results section, correspond to this second set of serum samples.

b. Evaluation of LCAT Activity

In order to evaluate LCAT activity in the sera of marine mammals, aliquots of whole serum slightly in excess of 1 ml were placed in 8 ml vials. The vials were then capped and placed in a water bath at 37° C for twenty-four hours. Following the incubation, 1 ml aliquots were then placed in extraction vials, and the lipids were extracted and quantified

*(Cont. from page 53) analysis, the 8 ml vials are placed in a heat block, at temperatures between 40 to 45° C, and the chloroform is evaporated under nitrogen. Once all the chloroform has been evaporated, the phospholipids are mixed with a measured amount of carbon tetrachloride to bring the concentration to approximately 4.5 mg/ml. 0.4 ml of the resulting solution is then transferred to an absorption cell.

according to the procedure described in the previous section.

LCAT studies were done on serum samples obtained from fasting animals: harbor seals I and II, the walrus pup, and the killer whale. A lipemic serum sample obtained from harbor seal I, four hours after a meal, was also incubated to determine the influence of an elevated serum triglyceride concentration on LCAT activity.

III. RESULTS

a. Postprandial Studies

Table VI contains data that resulted from the analyses of sera obtained from seals at the 10th postprandial hour. The ultracentrifugal distributions of the serum lipoproteins are shown in Figure 8. Tables VII and VIII contain additional harbor seal data on the chemical composition of the lipid moiety for other postprandial times. The ultracentrifugal distributions for these other postprandial times are shown in Figures 9 and 10.

In fasting pinnipeds, the whole serum concentration of triglycerides was roughly 50 mg/100 ml or less. At the 10th postprandial hour, the data in Table VI indicate that only harbor seal I has a triglyceride concentration (78 mg/100 ml) near this value. In each of the seals in Table VI, the increases in triglyceride concentrations result exclusively from increases in the lipid concentration in the total VLDL class. The analytical data in Figure 8, however, show the serum concentrations of VLDL to be quite low in each seal. The major portions of these lipoproteins have flotation rates in the range S_F^o 100-400. Assuming that the percentage lipid composition of S_F^o 20-400 lipoproteins is 90%, the lipid moieties of these lipoproteins represent less than 42% of the total lipid increment in the total VLDL class of harbor seal I. In the other seals, values for this percentage were even lower: 25% in harbor seal III, 14% in elephant seal II, and 16% in elephant seal III. In other words, the major portion of the lipid concentration in the total VLDL class is most probably in the S_F^o 400 and greater class (the chylomicron class). Data on the chemical composition of the total VLDL class indicate that as the percentage of S_F^o 20-400 lipoproteins in the total VLDL class increases, the percentage of triglycerides decreases.

Table VI Lipid Concentration and Composition of the Three Major Density Classes and of Whole Serum for the Harbor Seal (Phoca vitulina) and Elephant Seal (Mirounga angustirostris) at the 10th Postprandial Hour.

		Percent Lipid Composition				
	Concentration (mg/100 ml)	CE	PL	TG	UC	UFA
			VLDL (d< 1.006 gm/cc)			
Harbor Seal I*	65	8.3	13.2	69.1	8.9	0.4
Harbor Seal III*	202	3.7	8.6	78.7	9.0	—
Elephant Seal II	497	1.3	6.3	84.4	8.0	—
Elephant Seal III	248	1.4	7.1	83.5	7.6	0.4
			LDL (d: 1.006-1.063 gm/cc)			
Harbor Seal I	232	37.5	42.4	6.1	13.7	0.3
Harbor Seal III	215	38.4	40.7	7.4	13.3	0.2
Elephant Seal II	38	25.8	44.2	14.6	14.5	0.9
Elephant Seal III	31	17.5	39.2	29.3	13.2	0.9
			HDL (d: 1.063-1.21 gm/cc)			
Harbor Seal I	824	33.3	58.5	0.7	7.0	0.5
Harbor Seal III	741	34.2	57.6	1.6	6.2	0.4
Elephant Seal II	664	34.0	56.8	2.3	6.3	0.6
Elephant Seal III	465	33.1	58.8	2.3	5.4	0.4
			Whole Serum			
Harbor Seal I	1160	32.9	51.3	6.7	8.1	1.0
Harbor Seal III	1160	31.9	45.7	14.8	7.1	0.6
Elephant Seal I	1220	20.7	39.1	32.4	7.0	0.8
Elephant Seal III	898	19.0	37.9	34.9	7.5	0.7

* Harbor Seal III was fed herring equivalent to 9% of its body weight.

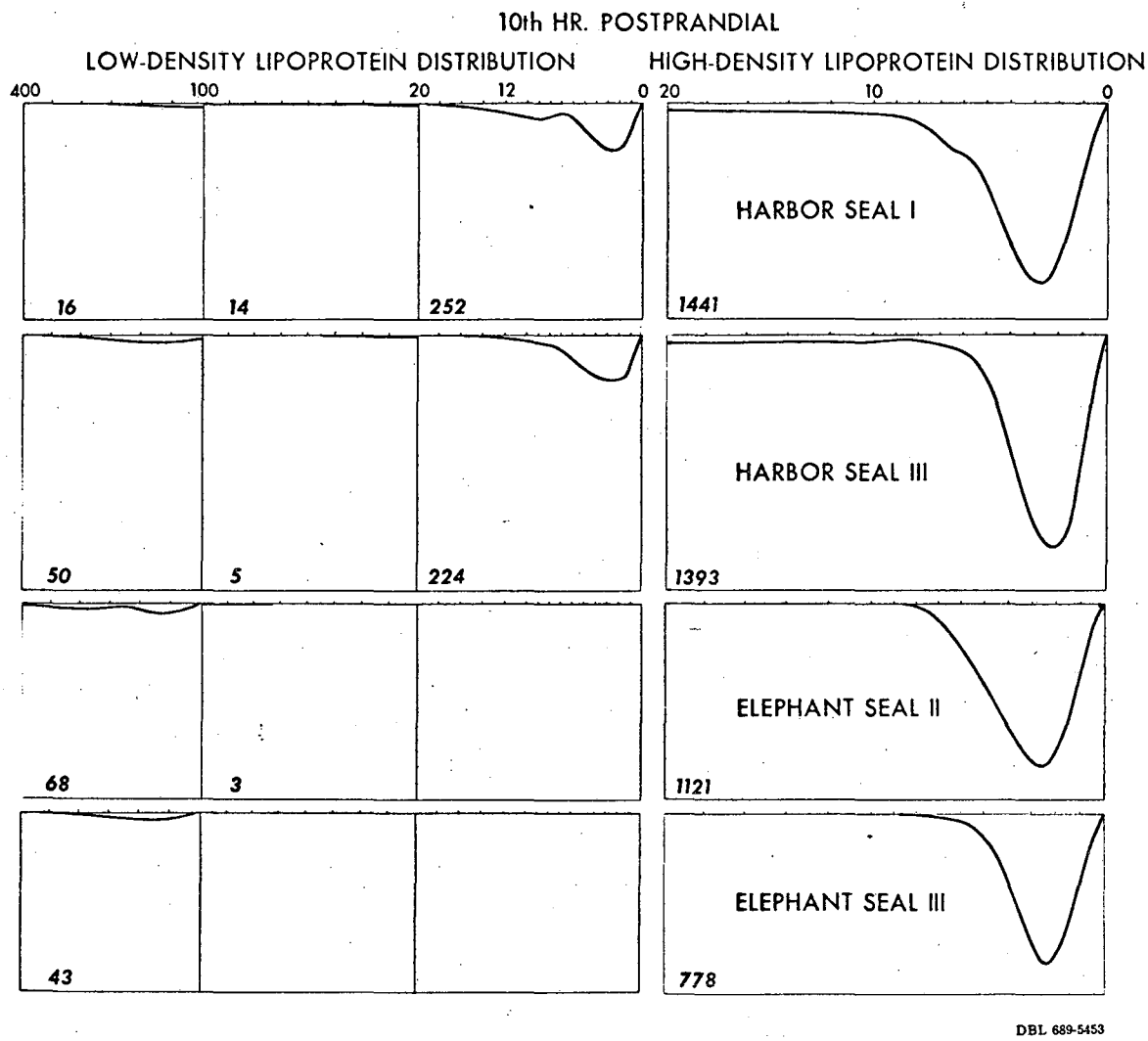


Figure 8 Ultracentrifugal distribution of lipoproteins in the sera of seals at the 10th postprandial hour.

This is in agreement with the chemical data on the compositions of the chylomicron and S_F^o 20-400 classes in humans.[20] Comparison of data of Table VI with Table V shows little or no differences between the LDL and HDL of fasting and fed pinnipeds. As indicated in Figure 8, no LDL was detected in either of the elephant seals. As was pointed out earlier, concentrations of serum lipoproteins as high as 40 mg/100 ml may not be detected by the method of flotation analysis. Electrophoresis in agarose gel was done on a postprandial serum sample obtained from elephant seal II. A pre-beta band, but no beta band, was detected. Apparently these animals can form pre-beta lipoproteins without the subsequent accumulation of beta lipoproteins. These electrophoretic data are in Appendix IV.

In the postprandial study of the two harbor seals, very little fluctuation in the concentrations of lipids of the LDL or HDL classes was noted on a given bleeding day. It is interesting to note that within a two-week period the concentration of HDL in harbor seal III increased by roughly 300 mg/100 ml. A concentration increase of roughly 100 mg/100 ml in the HDL class was observed in harbor seal III from the 3rd to the 5th postprandial hour. The increase was associated almost exclusively with an increase in the phospholipid concentration. However, concentration increases occur primarily in the total VLDL class. In harbor seal I a maximum concentration of lipids in the total VLDL class was reached at the 7th hour, but in harbor seal III it occurred at the 5th hour. As was mentioned earlier, a second maximum was observed in harbor seal III at the 11th postprandial hour. In both instances, the secondary rises in the concentration of triglycerides were associated with changes in the percent lipid composition of the total VLDL class. At the first observation, the triglyceride percentage of the lipid moiety rose from 69% for the 9th hour to 75% for the 11th hour. The

Table VII Lipid Concentration and Composition of the Three Major Density Classes and of Whole Serum for Harbor Seal I at Different Postprandial Times

			Percent Lipid Composition				
Postprandial	Hour	Concentration (mg/100 ml)	CE	PL	TG	UC	UFA
			VLDL (d< 1.006 gm/cc)				
3rd		128	4.7	8.8	78.3	7.8	0.4
5th		188	4.3	7.5	78.4	9.1	0.7
7th		222	4.3	8.0	75.3	11.8	0.6
9th		115	5.8	11.0	74.8	7.9	0.5
11th		89	6.7	12.0	68.3	12.3	0.7
			LDL (d: 1.006-1.063 gm/cc)				
3rd		211	36.9	43.9	6.2	12.8	0.2
5th		219	35.9	44.2	6.6	12.9	0.4
7th		225	35.7	44.1	7.0	12.9	0.4
9th		237	37.6	42.5	6.0	13.7	0.3
11th		221	37.2	42.8	5.8	14.0	0.3
			HDL (d: 1.063-1.21 gm/cc)				
3rd		739	35.1	57.7	0.7	6.1	0.5
5th		709	33.6	58.3	0.9	6.3	0.9
7th		730	33.8	58.2	0.8	6.4	0.8
9th		740	35.4	57.4	0.7	6.2	0.4
11th		725	35.5	57.0	0.6	6.2	0.4
			Whole Serum				
3rd		1250	29.0	48.8	13.6	7.8	0.9
5th		1290	27.7	45.2	18.3	7.6	1.2
7th		1280	28.0	43.2	20.1	7.7	1.1
9th		1160	32.0	48.6	11.0	7.8	0.6
11th		1130	31.6	51.4	8.2	8.1	0.7

Table VIII Lipid Concentration and Composition of the Three Major Density Classes and of Whole Serum for Harbor Seal III at Different Postprandial Times

		Percent Lipid Composition				
Postprandial Hour	Concentration (mg/100 ml)	CE	PL	TG	UC	UFA
		VLDL (d< 1.006 gm/cc)				
3rd	165	3.4	11.2	76.1	8.7	0.6
5th	203	4.6	11.6	78.1	5.1	0.5
7th	171	5.5	11.6	74.0	8.3	0.6
9th	128	5.5	16.5	68.5	8.8	0.7
11th	179	3.7	11.4	75.1	9.1	0.8
7th*	233	2.5	11.1	75.7	9.9	0.8
9th*	179	3.0	12.5	73.3	10.6	0.8
11th*	242	2.8	11.6	75.7	9.1	0.8
		LDL (d: 1.006-1.063 gm/cc)				
3rd	142	37.4	42.8	7.2	12.3	0.3
5th	148	37.0	42.4	7.5	12.6	0.5
7th	158	36.1	43.8	7.0	12.8	0.5
9th	137	33.6	43.9	8.6	13.5	0.4
11th	121	34.6	42.6	8.7	13.8	0.3
7th*	197	34.6	43.9	9.8	11.3	0.4
9th*	203	34.0	43.1	10.2	12.2	0.5
11th*	203	34.8	42.8	10.5	11.5	0.3
		HDL (d: 1.063-1.21 gm/cc)				
3rd	701	38.2	54.9	1.2	5.2	0.6
5th	805	34.2	57.9	1.5	5.2	1.2
7th	742	34.6	58.2	0.9	5.4	0.9
9th	685	35.1	57.7	0.5	5.9	0.7
11th	661	34.5	58.9	0.5	5.8	0.3
7th*	859	34.0	59.3	0.8	5.2	0.7
9th*	856	33.7	58.9	1.0	5.4	0.9
11th*	876	34.7	58.4	0.8	5.2	1.0

Table VIII for Harbor Seal III (cont.)

		Whole Serum				
Postprandial hour	Concentration (mg/100 ml)	CE	PL	TG	UC	UFA
3rd	1170	29.2	48.2	15.1	6.5	1.0
5th	1270	26.4	48.4	18.3	5.2	1.7
7th	1220	28.0	48.0	15.4	7.1	1.5
9th	1090	30.0	49.9	12.1	6.8	1.3
11th*	1160	28.2	47.1	17.5	6.7	0.6
7th*	1430	26.1	47.7	17.9	7.4	1.0
9th*	1400	27.6	47.9	15.1	8.2	1.3
11th*	1540	27.1	45.6	19.0	7.6	0.7

* As was described in the method section, these samples were obtained two weeks later.

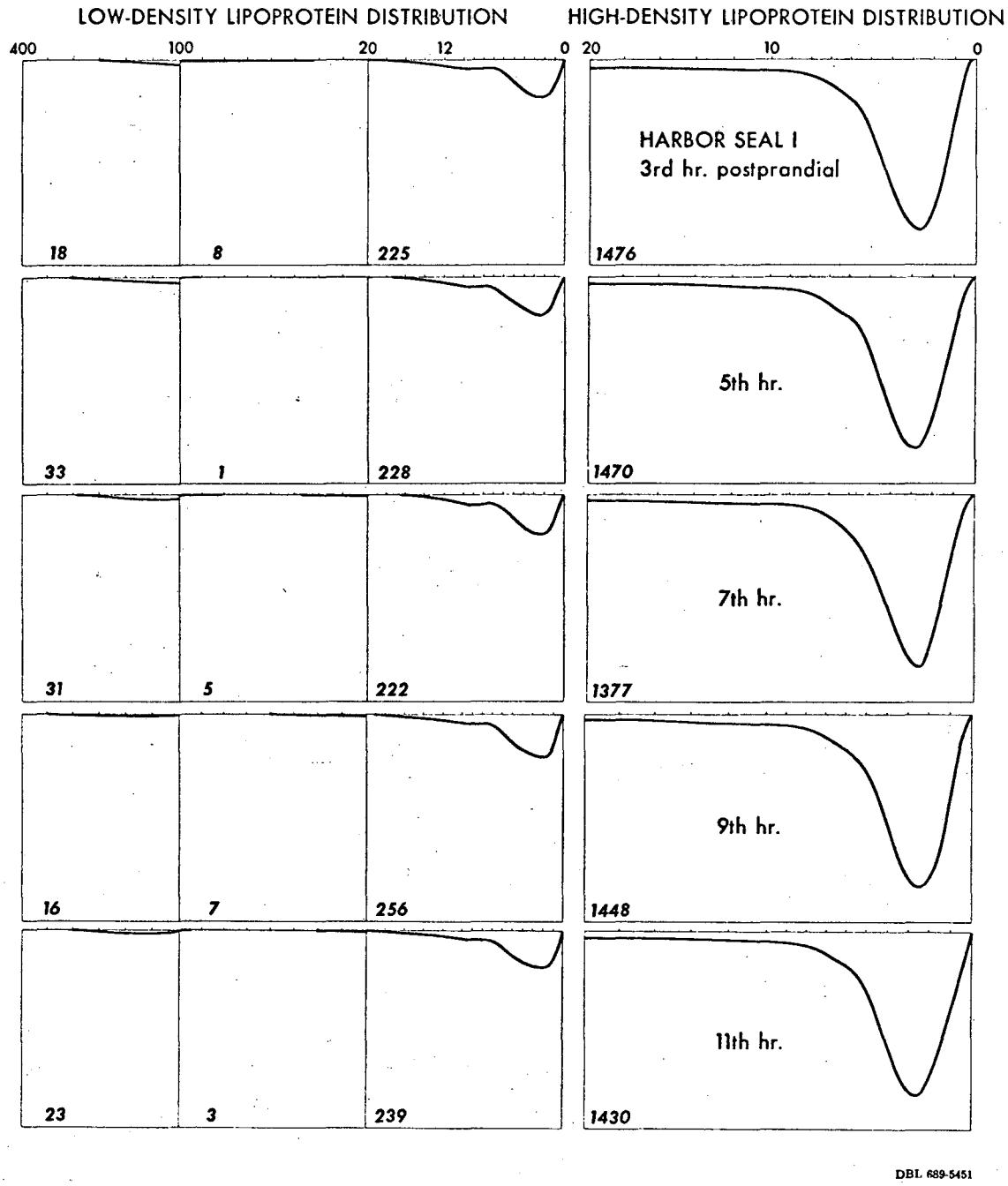


Figure 9 Ultracentrifugal distribution of lipoproteins in the serum of harbor seal I at different postprandial times.

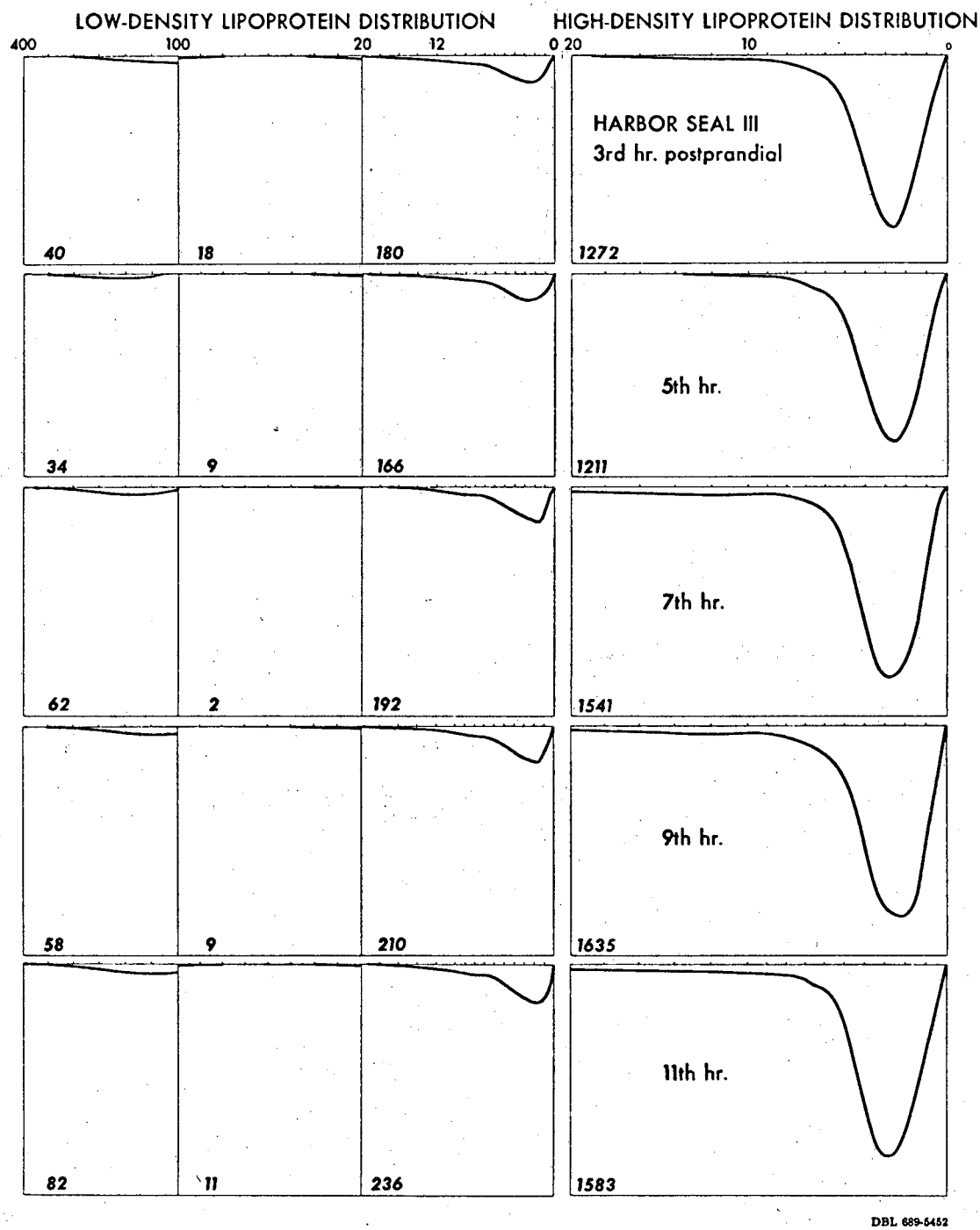


Figure 10 Ultracentrifugal distribution of lipoproteins in the serum of harbor seal III at different postprandial times.

second time, the rise in this percentage was 73% to 76%. The chemical composition for the 9th hour on the first bleeding day suggests that there might be a higher percentage distribution of serum lipoproteins in the S_F^o 20-400 interval. The flotation analyses done on sera obtained two weeks later indicate that approximately the same percentage of serum lipoproteins in the total VLDL class are in the S_F^o 20-400 interval at both the 9th and 11th postprandial hours. Unfortunately, no flotation analyses were done when the greater rise in the triglyceride percentage was detected.

Although the triglyceride composition of the lipoproteins in the total VLDL class agree well with human data, the cholesterol composition is quite different. In particular, the ratio of free to esterified cholesterol in humans is roughly 0.6. In the harbor seals, the values of this ratio, according to the data in Tables VI, VII, and VIII, range in values from 1.2 to 4.5. For the two elephant seals, the value of the ratio is 5.8 for elephant seal III and 6.1 for elephant seal II. Calculations of these ratios were based on the assumption that cholesterol comprises 60% of cholesteryl esters.

b. LCAT Studies

The results of the LCAT study are given in Table IX in terms of the concentration of the lipid constituents for the control and the incubated serum samples. The increases in the concentration of cholesteryl esters and the decreases in the phospholipid concentration and in the unesterified cholesterol concentration suggest that an LCAT reaction does take place in the sera of these animals. However, with the exception of the percentage changes in unesterified cholesterol noted in the serum of harbor seal III, the changes are within the overall accuracy of ± 5% associated with the measurements. The results of LCAT studies on human serum are included for the purpose of comparison.

Table IX Evaluation of LCAT Activity : Concentration (mg/100 ml) of Serum Lipids Before and After Incubation

	CE	PL	TG	UC	UFA
Harbor Seal I					
Control	338	579	45	76	15
Incubation	349	566	46	73	18
4th Hour Postprandial					
Control	339	640	201	95	18
Incubation	345	629	205	92	22
Harbor Seal II					
Control	294	502	29	62	10
Incubation	308	487	29	55	13
Walrus Pup*					
Control	428	515	36	111	45
Incubation	445	502	36	106	46
Killer Whale*					
Control	259	156	52	78	21
Incubation	263	150	50	77	25
Human (Males, 26-41 yrs)					
Control	283	263	154	67	9
Incubation	328	242	149	42	14

*The samples were analyzed in duplicates.

IV. DISCUSSION

a. Postprandial Studies

In the postprandial studies, differences were seen between harbor seal I and III in the time course of triglyceride concentrations and in the duration of the alimentary lipemia after the meal. Such individual variations have been also seen in postprandial studies on humans.[53,54] The chief difference between humans and seals is the build up of S_F^o 20-400 lipoproteins after a fat load. In humans about 75% of the increment in the total VLDL class appears in the S_F^o 20-400 class, while approximately 20% appears in the S_F^o 20-400 class in harbor seals at the 5th postprandial hour. At the 11th postprandial hour, this percentage increased to 29% in harbor seal I and 38% in harbor seal III. At the 10th postprandial hour, this percentage in both elephant seals was roughly 15%. This lack of build up of S_F^o 20-400 lipoproteins in seals suggests that these lipoproteins might be broken down more quickly in seals. Another possibility is that fatty acids, which result from the hydrolysis of chylomicron triglycerides, are taken up by the adipose tissue rather than being incorporated into a VLDL complex. This is consistent with the findings of Fried *et al*.[16] Their demonstration of the high activity of alpha glycerophosphate dehydrogenase in the adipose tissue of the Weddell seal suggests that these animals could form triglycerides at a higher rate than other mammals. If this is the case, seals would be able to store a very high percentage of chylomicron fatty acids without the subsequent formation of S_F^o 20-400 lipoproteins as observed in humans.

The participation of the B protein in hepatic and intestinal lipoprotein synthesis and secretion was discussed previously. It was then suggested that the ingestion of dietary fat could minimize the participation of B protein in triglyceride transport. This appears to be the case

in seals. Man, on the other hand, is apparently unable to hydrolyze chylomicron triglycerides without the subsequent formation of a large percentage of S_F^o 20-400 lipoproteins. Because more protein is present on the S_F^o 20-400 lipoprotein, the expectation would be that more B protein would be involved in the transport of dietary fats in humans than in seals. If that is the case, the ingestion of carbohydrate as well as fat might then be related to the serum concentrations of human LDL.

Nothing is currently known about the rate of chylomicra flow into the systemic circulation or the rate of tissue uptake of triglycerides in seals. The secondary rise in triglycerides in the total VLDL class, which was seen in harbor seal III following the 9th postprandial hour might be due to a difference in these two rates. In the animals studied by Gage and Fish, a secondary rise occurred between the 5th and 8th postprandial hours.[51] But just as these investigators did not observe this secondary rise in every animal studies, the secondary rise was noted in only one of the harbor seals.

Another difference between human and seal responses to fat ingestion was in the chemical composition of HDL. Unlike the studies done on dogs and humans, there was no marked triglyceride uptake by seal HDL after fat ingestion. However, as was reported in humans, an increase was also noted in the phospholipid concentration in the HDL class of harbor seal III. The lack of triglyceride uptake by seal HDL together with the higher ratio of free to esterified cholesterol in the total VLDL class suggests that the transfers of triglycerides and cholesteryl esters associated with LCAT do not occur in the sera of seals.

The lack of ultracentrifugally measurable amounts of lipoproteins with S_F^o values in the interval 0-20 is most interesting. Chemical analyses indicate that a small amount of lipid is present in the LDL class of both

these animals. As was pointed out earlier, concentrations of serum lipoproteins as high as 40 mg/100 ml may not be detected by the method of flotation analysis. Electrophoresis in agarose gel was done on a postprandial serum sample obtained from elephant seal II. A pre-beta band, but no beta band was detected. Apparently these animals can form pre-beta lipoproteins without the subsequent accumulation of beta lipoproteins.

b. LCAT Study

The chemical compositions of the total VLDL and HDL classes suggest that the transfer associated with serum enzyme LCAT in humans does not take place in the sera of marine mammals. The incubation studies failed to detect significant LCAT activity in the sera of these animals. Under the test conditions used for the determination of LCAT activity, the concentrations of the necessary reactants, lecithin and unesterified cholesterol (See Table III), were considered adequate for the detection of the enzyme. The killer whale with a low concentration of lecithin is an exception. The concentration differences between the control and incubated serum samples are markedly higher in human serum than the differences observed in the sera of marine mammals.

In a report on three humans lacking transferase activity, the plasma cholesteryl ester concentration was found to be negligible and the plasma alpha lipoprotein was undetectable by immuno-electrophoresis.[63] In marine mammals, however, both cholesteryl esters and HDL are present in serum in substantial amounts. As was mentioned earlier, Glomset has suggested that the plasma transferase reaction is the major source of esterified cholesterol in human plasma. Because cholesteryl esters are present in the sera of marine mammals, it would appear from my data that esterification of cholesterol would have to occur primarily in body tissue.

The possibility exists that in my studies the enzyme could have been inhibited as a result of the blood drawing and handling procedures. An LCAT inhibitor might also be present in the blood of marine mammals. Although further studies are needed to study the nature of LCAT in the sera of marine mammals, the low activity demonstrated by the results in Table IX is consistent with the observed absence of triglyceride uptake by HDL following fat ingestion.

SUMMARY

To survive cold temperatures in the ocean and, in the case of certain species, periods of starvation, marine mammals rely on their adipose fat. Both dietary and limited enzymatic studies suggest that marine mammals must consume diets high in fat to maintain their adipose stores. In order to obtain some insight into the mechanisms involved in the transport of triglycerides in marine mammals, the chemical and physical properties of their serum lipoproteins were investigated. Studies were done on members of the orders Cetacea and Pinnipedia. In both orders more than 70% of the lipids were associated with the HDL class. In both orders, the serum concentration of lipoproteins in the total VLDL class was low. However, distinct differences in the chemical and physical properties of serum lipoproteins were found between cetaceans and pinnipeds. Pinnipeds were found to have a higher concentration of HDL and of total serum lipids. In both the low and the high density lipoprotein distributions, the flotation peaks of the major components of cetaceans had higher rates. Furthermore, in the case of certain cetaceans, cholesteryl ester rather than phospholipid was the predominant lipid of the HDL class. The data on the chemical composition of the lipoproteins showed some similarities as well as differences when comparisons were made between marine and terrestial mammals. In man as well as in the killer whale and in the walrus pup, the percentage of cholesteryl esters was higher than that of phospholipids in the LDL class. The data for the killer whale and the walrus pup must be viewed with caution because the killer whale was sick, and the walrus pup was on an artificial diet. In the LDL class, the other animals had a predominance of phospholipids. The chemical composition of the cow HDL like that of some cetacean

HDL was predominantly cholesteryl ester. In all the marine mammals as well as the cow, the percentage of triglycerides in the HDL class was very low. There were also similarities and differences in the ultracentrifugal distribution of serum lipoproteins of terrestial and marine mammals. The dog, a carnivore like marine mammals, was also found to exhibit a predominance of HDL. The guinea pig, a herbivore, had no detectable HDL. Almost all of the lipoproteins of the guinea pig were found to be in the LDL class.

From an evaluation of the relative distributions of serum lipoproteins in the three categories of herbivores, omnivores, and carnivores, I would like to suggest that a possible relationship might exist between the circulating levels of LDL and the participation of B protein in the secretion of triglycerides. I have made several assumptions in formulating this relationship. The first assumption was that more B protein was present on lipoproteins in the S_F^0 20-400 class than in the chylomicron class. The assumption was also made that herbivores ingest a diet high in carbohydrates, and carnivores ingest a low carbohydrate diet. These assumptions were then combined with the findings of Windmueller _et al_. on orotic acid-fed rats to suggest the above relationship. Their studies showed the following: 1) the inhibition of the synthesis of B protein or the complexing of B protein with triglycerides in the liver was associated with a lowering of the serum level of LDL and 2) the ingestion of fat produced an alimentary lipemia with no detectable increase in the concentration of LDL. If the inhibition of the participation of B protein in the secretion of triglycerides was associated with a decrease in the concentration of LDL, perhaps the participation of B protein would be associated with an increase in the concentration of LDL. Herbivores convert a substantial fraction of dietary carbohydrate into

fat in the liver. The B protein is required for the secretion of hepatogenous triglycerides. This would, according to my suggestion, tend to elevate the concentration of LDL. The ingestion of fat in carnivores, judging from the results of the study on orotic acid-fed rats, would have little effect on the concentration of LDL. As was noted above, the guinea pig, a non-ruminating herbivore, had a predominance of LDL, and the dog and marine mammals, all carnovores, had a low percentage of LDL. Omnivorous man, whose diet is high in carbohydrates, also had a predominance of LDL. The suggested relationship between the participation of the B protein and the concentration of LDL was extended to other omnivores, the rat and the baboon, and to ruminants, the cow and the bison. It was pointed out that in the wild the diets of the rat and the baboon are often that of carnivores. Both these animals had a predominance of HDL. As for the ruminants, volatile fatty acids account for 70-80% of the total energy intake. These volatile fatty acids are soluble in the blood, and they can be taken up by the tissue for oxidation. As a result, the necessity for synthesizing triglycerides and the B protein might be greatly reduced in these animals. Both the bison and the cow had a predominance of HDL.

Although fasting levels of triglycerides are low in marine mammals, an alimentary lipemia was observed in each of the seals on which post-prandial studies were done. Very little fluctuation was observed in the concentrations and the chemical composition of LDL and HDL. The chief changes were associated with increases in the triglyceride concentration of the total VLDL class. A difference between the seal and human responses to a high fat intake was the extent of the build up of S_F^o 20-400 lipoproteins. In humans about 75% of the increment in the total VLDL class appears in the S_F^o 20-400 class. This percentage ranged in values from 15% to 38% in seals. It was suggested that the rapid uptake of chylomicron

fatty acids by adipose tissue might explain the lower build up of the S_F^o 20-400 lipoproteins in seals as compared to man.

Another difference between seals and humans was in the chemical composition of HDL following fat ingestion. Unlike humans, there was no significant uptake of triglycerides by seal HDL. The uptake of triglycerides has been associated with the activity of the serum enzyme LCAT. The enzyme is thought to be involved in the reciprocal transfer of triglycerides from VLDL to HDL and of cholesteryl esters from HDL to VLDL. The chemical compositions of HDL and VLDL of marine mammals suggest that this transfer does not take place. Incubation studies to evaluate the presence of LCAT in the sera of different marine mammals indicated that there was no significant activity.

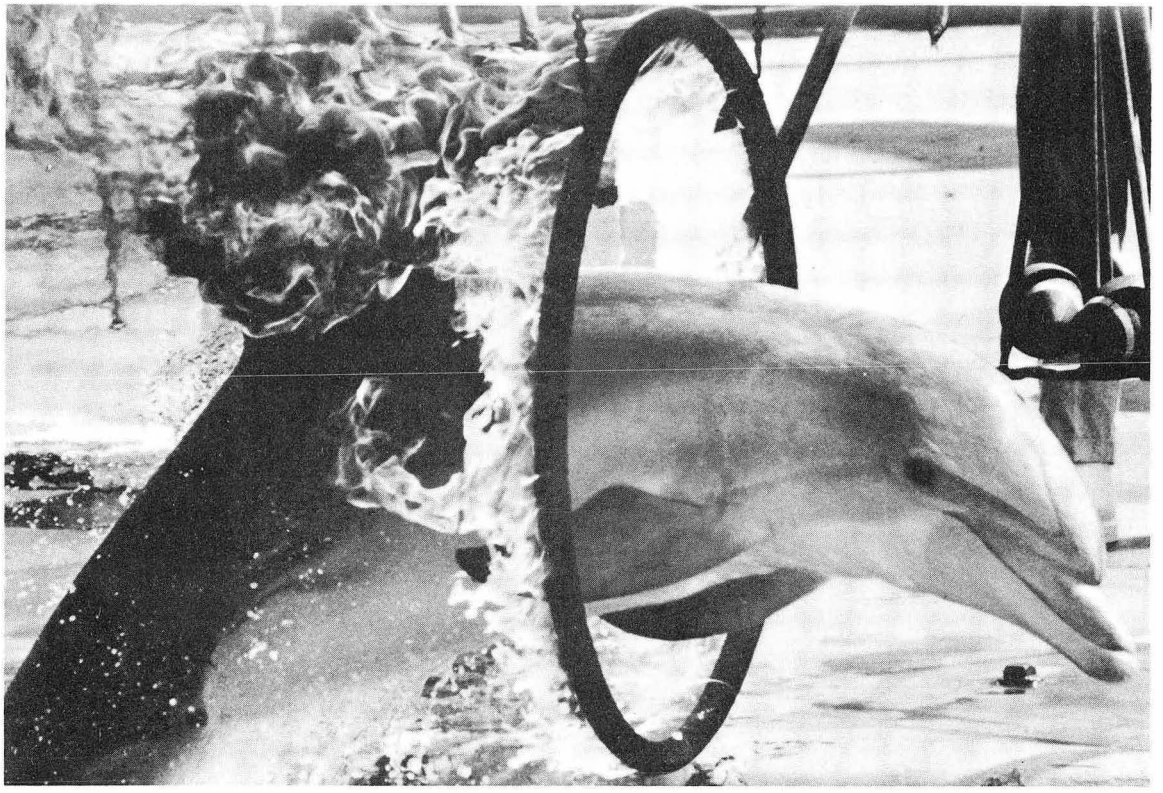
APPENDIX I

Table X Information on the Sex, Weight and Age of Captive Marine Mammals

	Sex	Weight (lbs.)	Age
Cetacea			
Atlantic Dolphin (*Tursiops truncatus*)			
I	F	350	13-15 yrs.
II	F	250	8 yrs.
III	M	280	young adult
IV	F	310	old
Pacific Dolphin (*Tursiops truncatus gilli*)			
	F	450	8-9 yrs.
Dall Porpoise (*Phocoenoides dalli*)			
	M	270	old
Pacific Porpoise (*Lagenorhyncus obliquidens*)			
I	F	210	young adult
II	M	200	young
Killer Whale (*Orcinus orca*)			
	F	6,000	7-8 yrs.
Pinnipedia			
Elephant Seal (*Mirounga angustirostris*)			
I	M	235	10 mo.
II	M	350	21 mo.
III	M	460	33 mo.
IV	F	210	10 mo.
Harbor Seal (*Phoca vitulina*)			
I	F	90	21 mo.
II	F	175	7-8 yrs.
III	F	137	7-15 yrs.
Steller Sea Lion (*Eumetopias jubatus*)			
Male Pups			
I		50	1 mo.
II		58	1 mo.

Table X cont.

	Sex	Weight (lbs.)	Age
III		46	10 days
IV		45	10 days
Female Pup			
I		40	5 days
II		34	3 days
III		30	5 days
IV		31	10 days
V		39	2 days
VI		43	10 days
VII		45	1 month
VI		33	1 month
Walrus Pup (*Odobenus rosmarus*)			
	F	110	3 weeks



XBB 688-5213

Figure 11. Atlantic dolphin.

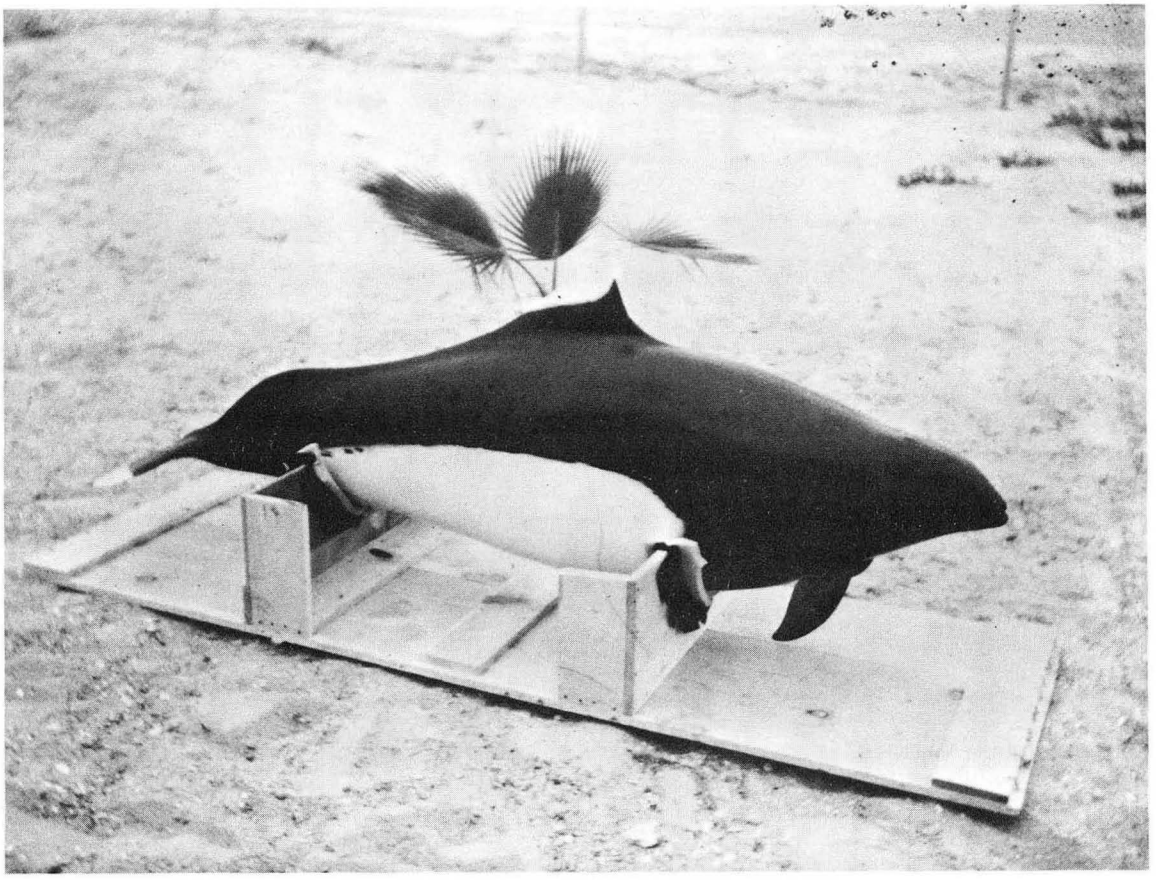


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Figure 12. Scale model of a Dall porpoise.

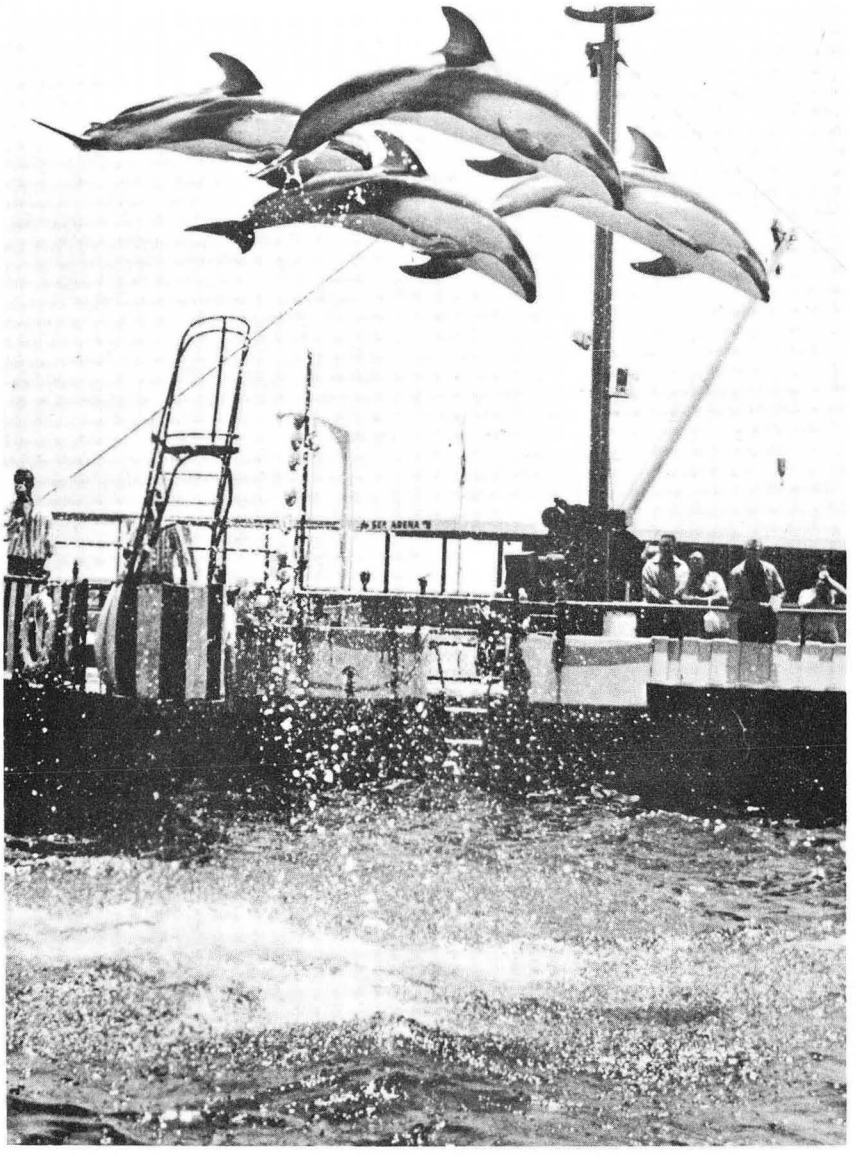

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Figure 13. Pacific porpoises.

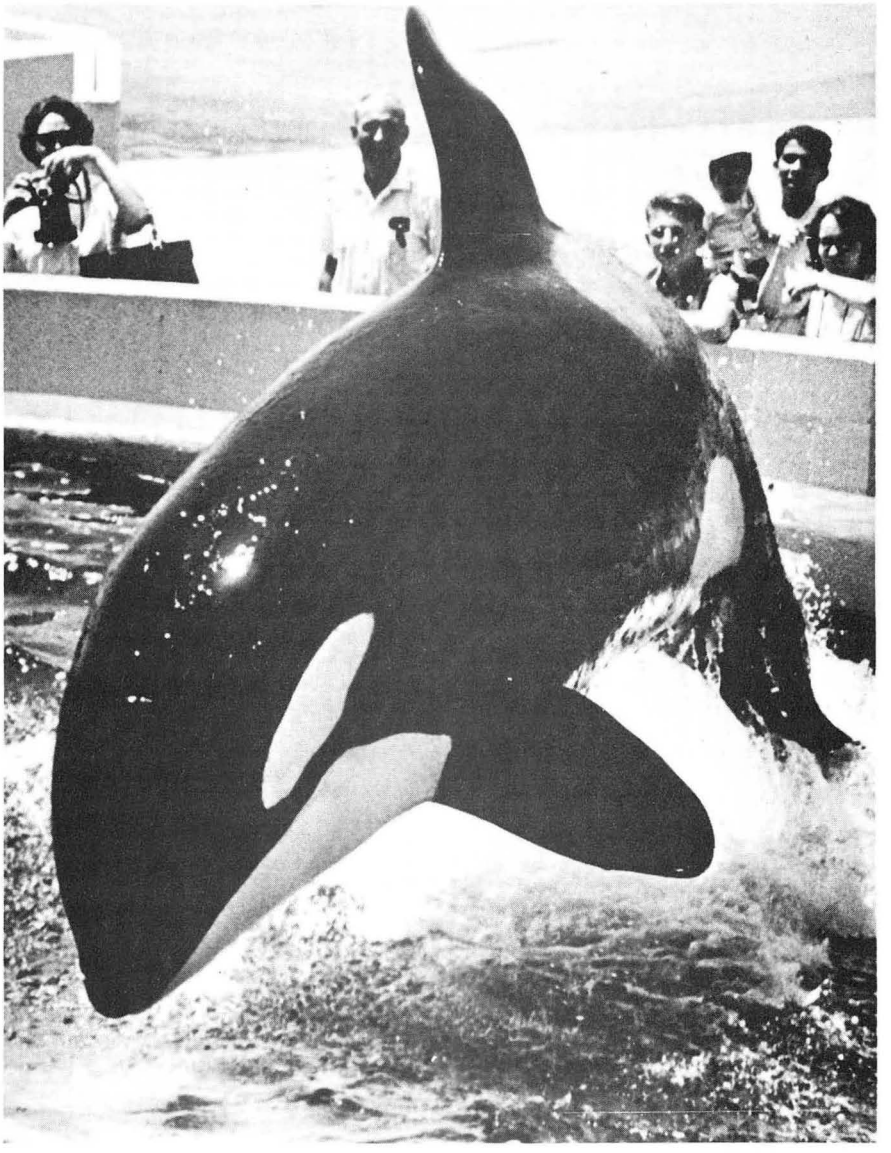

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Figure 14. Killer Whale.

XBB 6811-6933

Figure 15. Elephant seal.

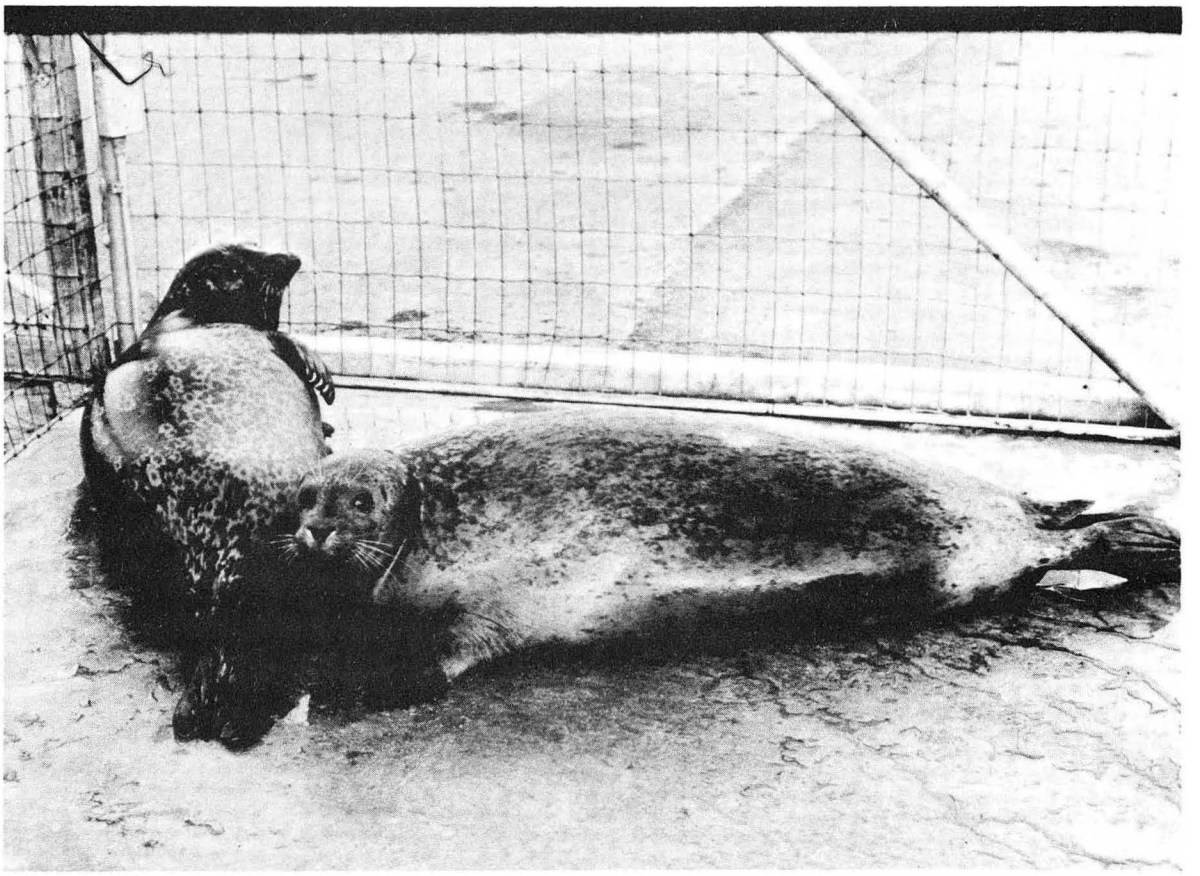

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Figure 16. Harbor seals.

XBB 6811-6932

Figure 17. Weddell seals.

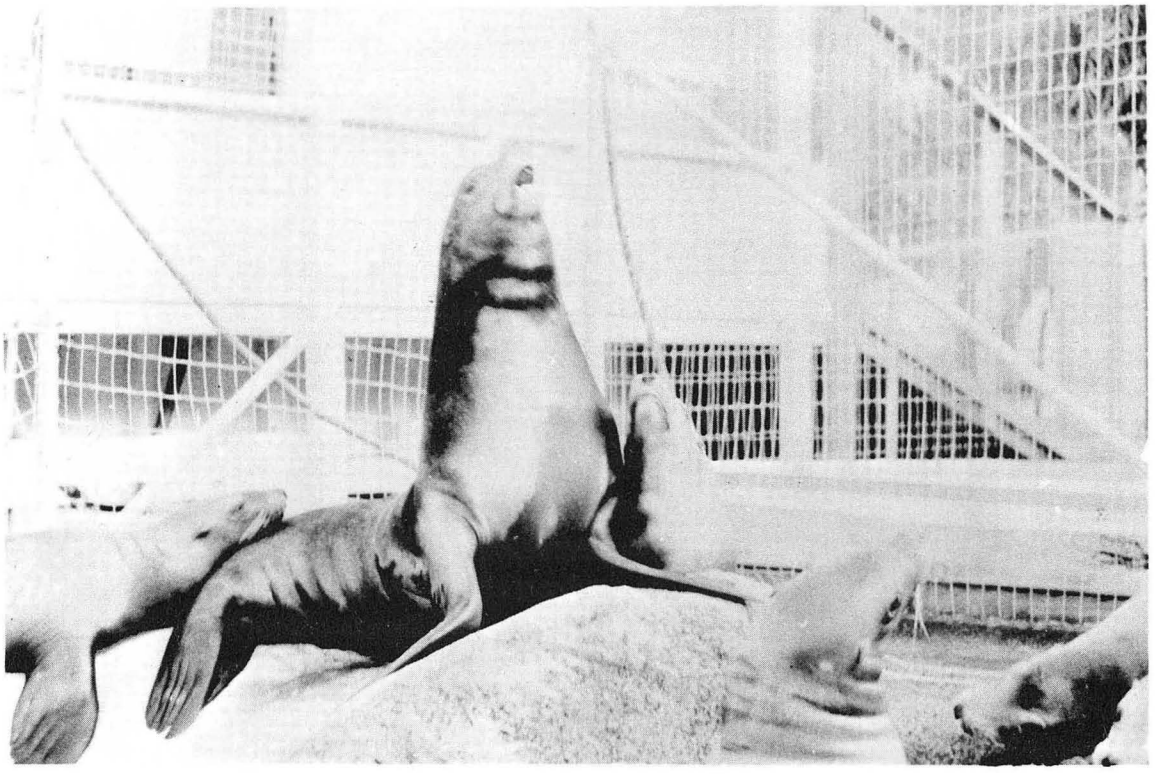

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Figure 18. California sea lion in the center.

XBB 6811-6935

Figure 19. Northern fur seals.

XBB 6811-6934

Figure 20. Steller sea lion pups.

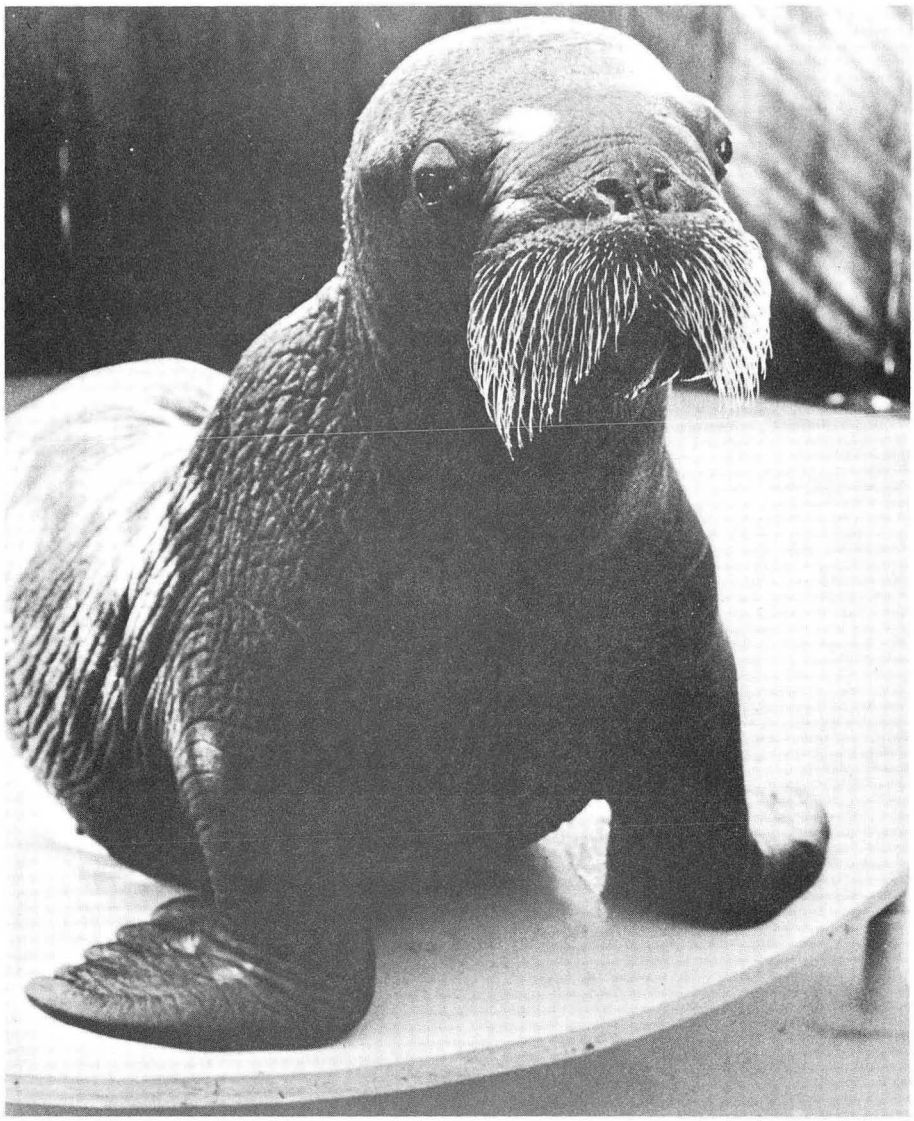

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Figure 21. Walrus pup.

APPENDIX II

Table XI Mean Concentration and Mean Composition of Whole Serum Lipids of Certain Pinnipeds

Animals	No	Concentration (mg/100 ml)	Percent Lipid Composition				
			CE	PL	TG	UC	UFA
Weddell Seals (*Leptonychotes weddelli*)							
Females	4	1320 (±70)	39.5 (±1.0)	47.7 (±1.0)	3.4 (±1.0)	8.1 (±0.3)	1.8 (±0.6)
Fetuses	4	832 (±340)	36.5 (±4.0)	51.3 (±3.2)	2.6 (±1.5)	9.0 (±1.2)	0.7 (±0.2)
Northern Fur Seals* (*Callorhinus ursinus*)							
Males	5	798 (±190)	34.0 (±2.9)	50.6 (±2.6)	4.1 (±0.7)	7.5 (±0.7	3.0 (±2.0)
Steller Sea Lion (*Eumetopias jubatus*)							
Males	4	755 (±67)	34.4 (±0.8)	49.0 (±2.0)	4.7 (±2.0)	8.6 (±0.5	3.4 (±0.8)
Females	8	752 (±120)	32.5 (±2.9)	49.0 (±1.5)	3.1 (±2.6)	9.7 (±1.2)	2.9 (±2.1)

The values in parentheses are standard deviations from the mean values.

*Heat was used in the extraction of lipids.

Table XII Lipid Concentration and Composition of the Three Major Density Classes of Other Marine Mammals

Animals	Concentration* (mg/100 ml) of the Three Density Classes			Percent Lipid Composition of HDL				
	VLDL**	LDL**	HDL	CE	PL	TG	UC	UFA
Delphinus bairdi (male)	71	122	763	44.8	45.1	2.4	7.1	0.7
Phocoenoides dalli (male)***	167	39	278	38.7	50.0	3.1	6.5	1.8
Mirounga angustirostris (IV, female)	38	41	501	38.1	53.0	1.3	5.8	1.8

*Heat was used in the extraction of lipid in each case.

**Because only a small amount of blood was obtained, the amount of the extracted lipid was not large enough for chemical analysis. The value for concentration was determined by gravimetric means.

***Postprandial sample.

APPENDIX III

Tables XIII, XIV, and XV show data on the concentration of lipid constituents in whole serum and in different density classes for various terrestial mammals. The concentrations of protein and total lipid were not determined in every case. Instead, assumed values underlined in the tables were used. These values were based on the following assumptions. For lipoproteins with density less than 1.019 gm/cc, the protein moiety was assumed to comprise 10% of the lipoprotein. For lipoproteins with density less than 1.063 gm/cc and for those with density between 1.063 and 1.019 gm/cc, the percentage of protein in the lipoproteins was assumed to be 20%. In the HDL class, the protein moiety was assumed to comprise 50% of the lipoproteins of the cow and bison. In cases where it was possible, the percent lipid composition of these constituents were calculated. These data are in Tables XVI, XVII, and XVIII.

Table XIII Lipid Concentration (mg/100 ml) of Whole Serum

	No	CE	UC	TC	PL	TG	UFA	TL	Ref.
HERBIVORES									
Bison (Female *Bison bison*)	2	65	11	50*	25	29	3.7	134	64
Bovine (Female Holstein)	2	244	30	175*	157	13	9.1	453	65
Hamster	N.A.			121	269				66
Rabbit	N.A.			41	93				66
OMNIVORES									
Man (Males; 26-41 yrs)	6	280	69	235*	261	153	8.9	772	67
Man (Males; 20-30 yrs)	10			171	215				66
Man (Females; 20-30 yrs)	10			187	237				66
Monkey	N.A.			173	295				66
Pig	N.A.			106	161				66
Rat	N.A.			50	114				66
Rat	6			62	102	51			68
CARNIVORES									
Dog (Female Beagles)	5	202	46	166*	363	31	19	661	69
Dog (Male Beagles)	5	166	40	139*	309	29	14	558	69
Dog	N.A.			150	362				66

* This value is an estimate. It is based on the assumption that cholesterol comprises 59.5% of the cholesteryl ester. ** 18% of this concentration value for triglycerides is monoglycerides and diglycerides. Abbreviations: CE (Cholesteryl Esters), UC (Unesterified Cholesterol), TC (Total Cholesterol), PL (Phospholipid), TG (Triglycerides), UFA (Unesterified Fatty Acids), TL (Total Lipid), N.A. (Data were not available in the reference cited). These abbreviations will be used in subsequent tables.

Table XIV Lipid Concentration (mg/100 ml) of Serum Lipoproteins of Density Less than 1.063

	No	Density Class	CE	UC	TC	PL	TG	TL	Pro	LP	Ref.
HERBIVORES											
Bison (Female Bison bison)	2	<1.063 gm/cc	21	5.7	18*	9.5	17**	53	13	66	64
Bovine (Female Holstein)	2	<1.063 gm/cc	9.3	1.0	6.5*	8.4	6.8	26	7	33	65
Hamster	N.A.	<1.019 gm/cc			25	44		189	21	210	66
Hamster	N.A.	1.019-1.063 gm/cc			29	34		88	22	110	66
Rabbit	N.A.	<1.019 gm/cc			15	18		126	14	140	66
Rabbit	N.A.	1.019-1.063 gm/cc			9	11		48	12	60	66
Rabbit	6	<1.063 gm/cc	19	6	17*	21	36	82	17	99	70
OMNIVORES											
Male (20-30 yrs)	10	<1.019 gm/cc			26	31		153	17	170	66
Female (20-30 yrs)	10	<1.019 gm/cc			19	24		153	17	170	66
Males (20-30 yrs)	10	1.019-1.063 gm/cc			97	69		240	60	300	66
Female (20-30 yrs)	10	1.019-1.063 gm/cc			108	78		240	60	300	66
Monkey	N.A.	<1.019 gm/cc			17	20		117	13	130	66
Monkey	N.A.	1.019-1.063 gm/cc			64	64		208	52	260	66
Pig	N.A.	<1.019 gm/cc			8	12		90	10	100	66
Pig	N.A.	1.019-1.063 gm/cc			51	37		144	36	180	66
Rat (Sprague-Dawley)	22	<1.063 gm/cc	11	4	11*	14	32	61	13	74	70
Rat	N.A.	<1.019 gm/cc			6	17		108	12	120	66
Rat	N.A.	1.019-1.063 gm/cc			10	13		68	17	85	66
Rat	6	<1.063 gm/cc			13	18	29	60	15	75	68
CARNIVORES											
Dog	3	<1.063 gm/cc	9	7	12*	17	32	65	21	86	70
Dog	N.A.	<1.019 gm/cc			2	10		81	9	90	66
Dog	N.A.	1.019-1.063 gm/cc			10	19		68	17	85	66

Values in parentheses are percentages based on total lipid concentration.

*This is an estimate. It is based on the assumption that cholesterol comprises 59.5% of the cholesteryl ester. ** 17% of this concentration value for triglycerides is monoglycerides and diglycerides. Abbreviations: PRO (Protein), LP (Lipoprotein). The underlined values are estimates based on the values in the text.

Table XV Lipid Concentration (mg/100 ml) of Serum Lipoproteins of Density Greater than 1.063 gm/cc

	No	Density Class	CE	UC	TC	PL	TG	TL	Pro.	LP	Ref.
HERBIVORES											
Bison (Female *Bison bison*)	2	1.063-1.21 gm/cc	53	8.1	40*	18	16**	95	95	190	64
Bovine (Female Holstein)	2	>1.063 gm/cc	249	29	177*	155	6.6	440	440	880	65
Hamster	N.A.	>1.063 gm/cc			61	176					66
Rabbit	6	1.063-1.22 gm/cc	31	10	28*	75	24	140	196	336	70
Rabbit	N.A.	>1.063 gm/cc			17	69					66
OMNIVORES											
Man (Males; 20-30 yrs)	10	>1.063 gm/cc			43	111					66
Man (Females; 20-30 yrs)	10	>1.063 gm/cc			54	134					66
Monkey	N.A.	>1.063 gm/cc			80	206					66
Pig	N.A.	>1.063 gm/cc			41	102					66
Rat (Sprague-Dawley)	22	1.063-1.22 gm/cc	69	13	54*	91	11	184	156	340	70
Rat	N.A.	>1.063 gm/cc			31	78					66
Rat	6	1.063-1.21 gm/cc			42	43		87	87	174	68
CARNIVORES											
Dog	3	1.063-1.22 gm/cc	135	28	108*	233	47	443	414	857	70
Dog	N.A.	>1.063 gm/cc			127	325					66

* This value is an estimate. It is based on the assumption that cholesterol comprises 59.5% of the cholesteryl ester. ** 24% of this concentration value for triglycerides is monoglycerides and diglycerides.

Table XVI Lipid Composition of Whole Serum

	Percent Lipid Composition							
	No	CE	UC	PL	TG	UFA	TL (mg/100 ml)	References
HERBIVORES								
Bison (Female *Bison bison*)	3	48	8.2	19	22	2.8	134	64
Bovine (Female Holstein)	2	54	6.6	35	2.9	2.0	453	65
OMNIVORES								
Man (Males; 26-41 yrs)	6	36	8.9	34	20	1.1	772	67
CARNIVORES								
Dog (Female Beagles)	5	30	7.2	55	5.2	2.5	558	69

Table XVII Lipid Composition of Serum Lipoproteins of Density Less than 1.063 gm/cc

			Percent Lipid Composition					
	No	Density Class	CE	UC	PL	TG	TL (mg/100 ml)	Ref.
HERBIVORES								
Bison (Female *Bison bison*)	2	<1.063 gm/cc	39	11	18	31	53	64
Bovine (Female Holstein)	2	<1.063 gm/cc	34	3.7	31	25	26	65
Rabbit	6	<1.063 gm/cc	23	7.3	26	44	82	70
OMNIVORES								
Man (Males; 35-55 yrs)	N.A.	<1.006 gm/cc	13-16	4-6	16-25	63-75	143	19
Man (Females; 35-55 yrs)	N.A.	<1.006 gm/cc	13-16	4-6	16-25	63-75	50	19
Man (Males; 35-55 yrs)	N.A.	1.006-1.063 gm/cc	46	13	26	15	296	19
Man (Female; 35-55 yrs)	N.A.	1.006-1.063 gm/cc	46	13	26	15	242	19
Rat (Sprague-Dawley)	22	<1.063 gm/cc	18	6.5	23	52	61	70
CARNIVORES								
Dog	3	<1.063 gm/cc	14	11	26	49	65	70

The underlined values are estimates based on the values stated in the text.

Table XVIII Lipid Composition of Serum Lipoproteins of Density Greater than 1.063 gm/cc

			Percent Lipid Composition					
	No	Density Class	CE	UC	PL	TG	TL (mg/100ml)	References
HERBIVORES								
Bison (Female *Bison bison*)	2	1.063-1.21 gm/cc	56	8.5	19	17	95	64
Bovine (Female Holstein)	2	>1.063 gm/cc	56	6.6	35	1.5	440	65
Rabbit	6	1.063-1.22 gm/cc	22	7.1	54	17	140	70
OMNIVORES								
Man (Males; 35-55 yrs)	N.A.	1.063-1.21 gm/cc	40	4	48	8	138	19
Man (Females; 35-55 yrs)	N.A.	1.063-1.21 gm/cc	40	4	48	8	218	19
Rat (Sprague-Dawley)	22	1.063-1.22 gm/cc	37	7.1	49	6.0	184	70
CARNIVORES								
Dog	3	1.063-1.22 gm/cc	30	6.3	52	11	443	70

Table XIX Ultracentrifugal Distributions of Serum Lipoproteins in Different Terrestial Mammals

Animals	No*	Serum Concentration (mg/100ml)		
		HDL	LDL	VLDL
HEBIVORES				
Guinea Pigs (Albino Strain Males; 12-17 wks)	7		182	47**
OMNIVORES				
Babbons (*Papio cyanocephalus* Males; 15 mo.)	2	345	120	2**
Humans (30-39 yrs.)***				
Males	834	255	406	142
Females	99	316	365	64
Rats (Buffalo Strain Male Pool)	5	417	29	171
CARNIVORES				
Dogs (Beagles; 1-2 yrs)				
Males	5	705	69	6**
Females	5	826	77	0

*The value in the numbers column represents the number of animals that were tested to obtain the corresponding data.

**Several animals in the group tested had no dectectable VLDL. The highest concentration value among the animals tested is shown in the table.

*** Reference 61

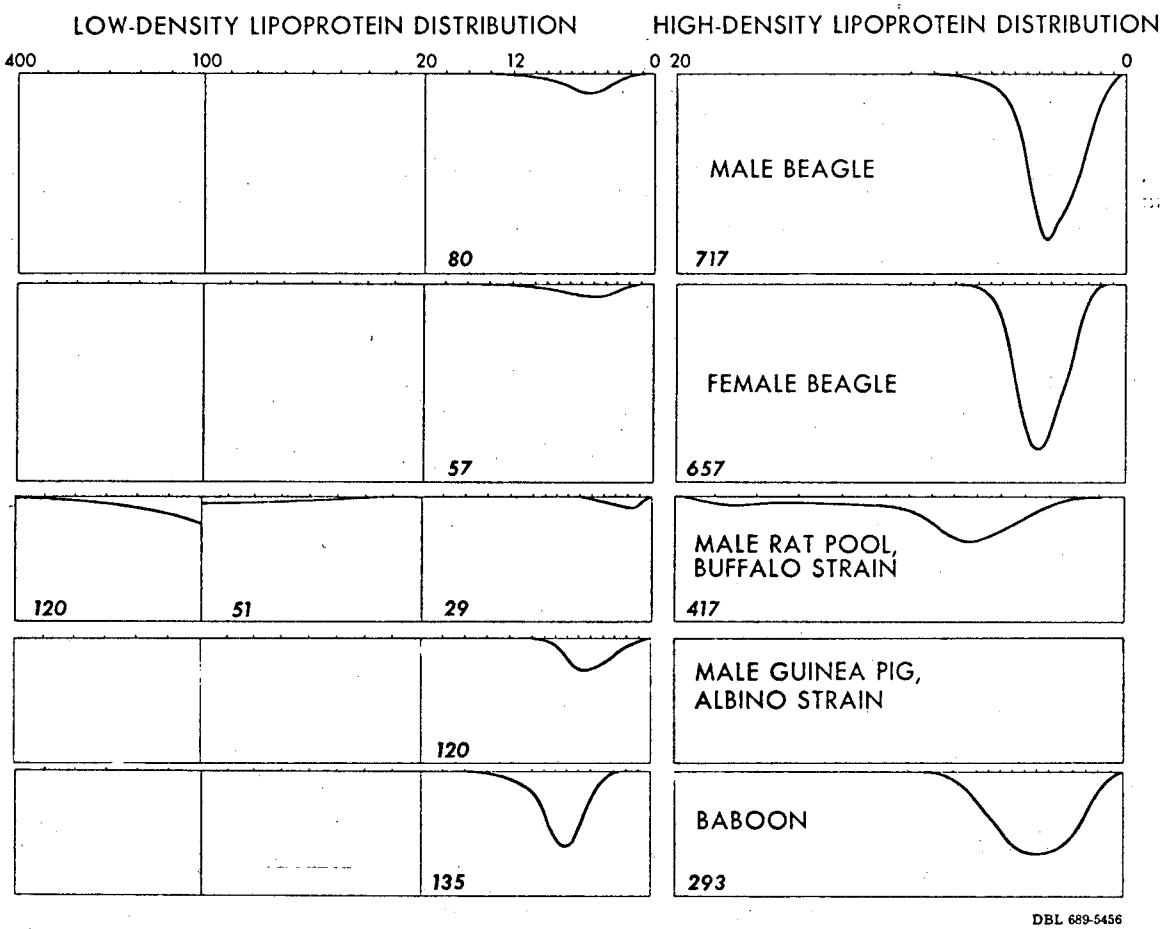

Figure 22 Representative ultracentrifugal distributions of lipoproteins in the sera of certain terrestial mammals.

APPENDIX IV

The correspondence between the ultracentrifugal distribution of serum lipoproteins and the electrophoretic patterns was discussed earlier in the Introduction Section. This correspondence illustrated in Figure 22 for human serum lipoproteins exists also in marine mammals. Electrophoretic studies were done by Dr. F. T. Hatch and J. A. Mazrimas of the Biomedical Division of the Lawrence Radiation Laboratory, Livermore, California. The electrophoresis was done in agarose gel at pH 8.6 according to the procedure of Noble _et al_.[71]

Alpha lipoproteins were present in the sera of each of the animals. No beta lipoproteins were detected in the sera of the Pacific dolphin or elephant seal II, and no pre-beta lipoproteins were detected in the sera of harbor seal II. The difference in the alpha band mobility of pinnipeds and cetaceans suggests that the slower bands might correspond to the HDL of pinnipeds with low flotation rates and the faster band to the HDL of cetaceans with higher flotation rates (See Figures 5 and 6 and Table IV). The postprandial sample from elephant seal II shows a very heavily stained band in the starting well, which represents chylomicra and $S_F^o > 400$ lipoproteins that are too large to migrate in the gel.

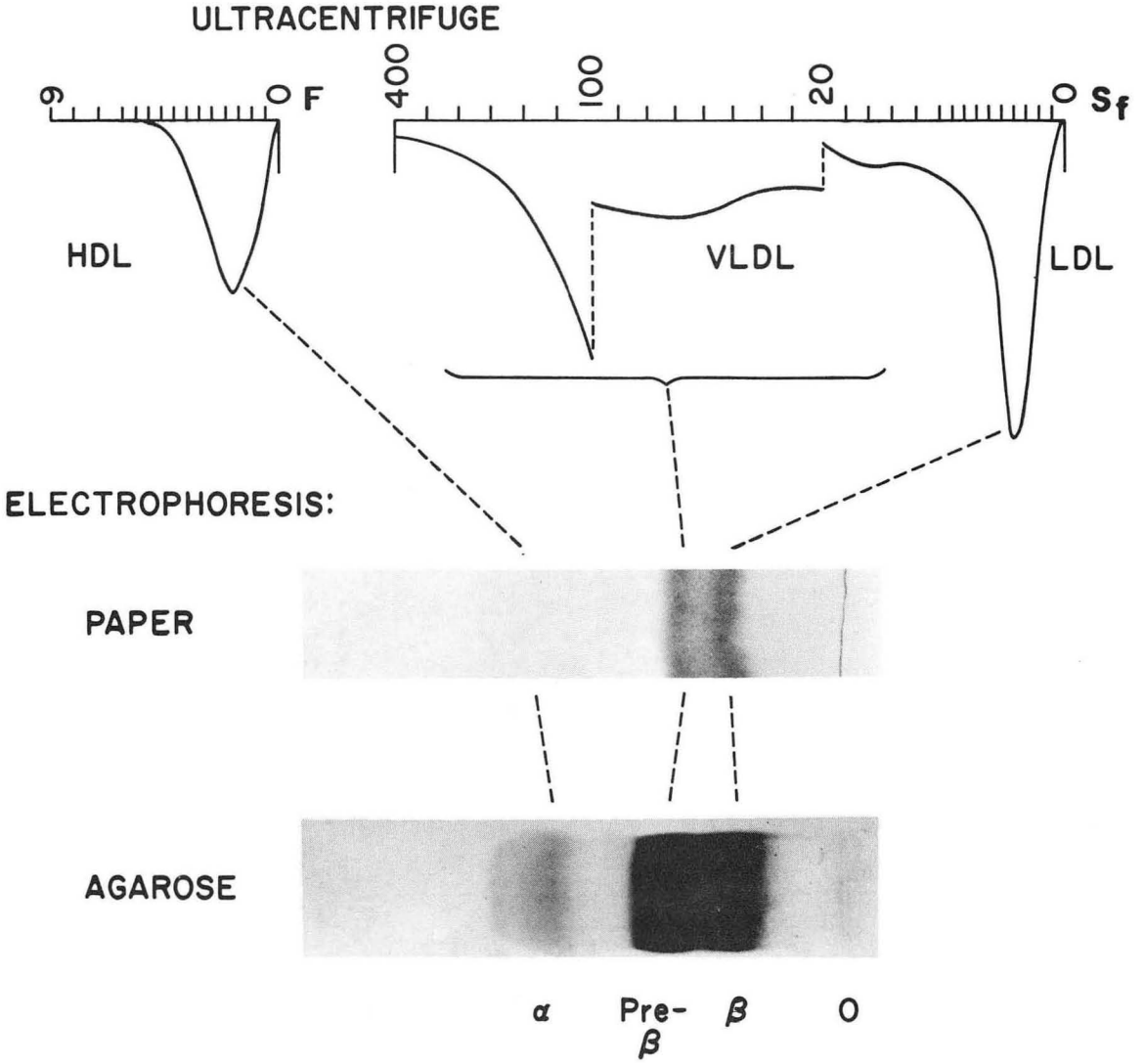

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Figure 23. Correspondence between the electrophoretic and the ultracentrifugal distributions of serum lipoproteins.

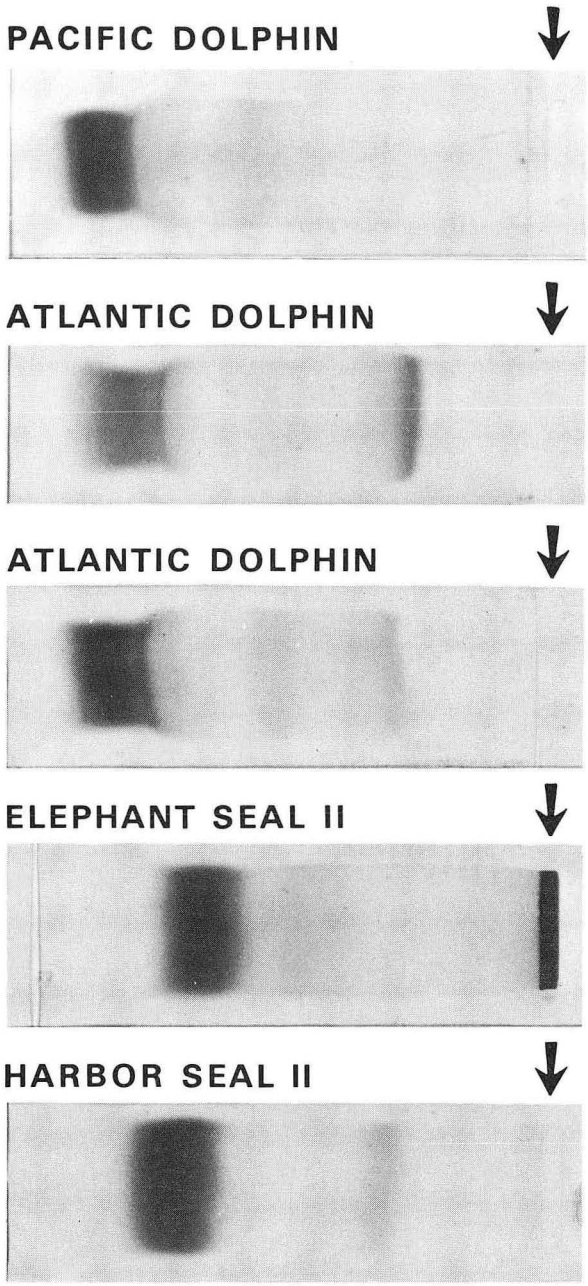

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Figure 24. Electrophoretic (Agarose gel) distribution of serum lipoproteins.

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