Kinetics of chylomicron triglyceride removal from plasma in rats: a comparison of the anesthetized and the unanesthetized states

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ABSTRACT The kinetics of chylomicron-TG removal were studied using an experimental method which allows measurements to be made under optimal physiological conditions. Chylomicrons, labeled with palmitic acid-^{14}C, were constantly infused at a rate of 0.5 mg total lipid per min into chronically cannulated, unanesthetized, unrestrained rats which had been fasted for 18 hr. Serial blood samples were withdrawn from an arterial cannula during a 20 min infusion period and for 10 min following the infusion. Plasma lipoproteins were separated into two fractions in the ultracentrifuge, and the lipids were extracted. Radioactivity in the low-density fraction ($d<1.006$) was taken to represent chylomicron-TG radioactivity. Using this method we studied the influence of anesthesia on the kinetics of removal of chylomicron-TG. The following three phases of the radioactivity-time curve were plotted: (*a*) the increase in ^{14}C during infusion of chylomicrons, (*b*) the steady-state phase during the infusion, and (*c*) the decay of ^{14}C after chylomicron infusion was stopped. The values for the anesthetized rats failed to reach a steady-state phase during the course of the experiment. From the disappearance of ^{14}C following the end of the infusion, the apparent half time of removal of chylomicron-TG was estimated to be 2.8 ± 0.37 min in unanesthetized rats, 4.5 ± 0.37 min in rats anesthetized with sodium pentobarbital, and 4.4 ± 0.44 min in rats anesthetized with halothane. Thus, two anesthetics with different physical properties markedly slowed the removal of chylomicron-TG from the circulation. The reduced rate may have resulted from alterations in cardiac output or distribution of blood flow induced by the anesthetic agents.

SUPPLEMENTARY KEY WORDS turnover rates · constant infusion · fasted · $t^1/_2$ · pentobarbital · halothane

THE METABOLISM of chylomicron-TG has been extensively studied in recent years (3–15). From these investigations, much has been learned about both the kinetics and mechanism of removal of chylomicron-TG from the circulation. In many of these studies, however, facets of the experimental design make an evaluation of the quantitative aspects of removal difficult. In a number of experiments, the amount of lipid injected has far exceeded the quantity which could be delivered to the circulation during the period of the study, based upon maximum absorption rates from the gut (16–20). In at least one study (21), chylomicrons were collected and stored at low temperatures. Such treatment may alter the kinetics of removal (22). The isolation of chylomicrons by centrifugation has been used by some investigators, and this procedure may produce aggregation or coalescence of the particles (23, 24) which may

A preliminary report of these results was presented at the Federation of American Societies for Experimental Biology in Atlantic City, April 1967 (1).

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Abbreviations: TGFA, triglyceride fatty acids; TG, triglycerides; LPL, lipoprotein lipase; FFA, free fatty acids; VLDL, very low density lipoproteins ($d<1.006$).

alter the kinetics of removal (25, 26). In studies with rats, serial blood sampling techniques have not been employed, and thus it has been necessary to use points obtained from many animals to construct a plasma concentration-time curve (7–9, 27). Anesthetized animals have been employed in some studies, and the effect of anesthesia on the removal of chylomicron-TG has not been clearly evaluated (27).

Because the experimental conditions in many of the aforementioned studies have varied, it is difficult to reach meaningful conclusions about the rates of removal from the circulation of chylomicron-TGFA. We have designed an experimental method which simulates the normal manner in which chylomicrons are delivered to the circulation. This method makes use of a constant intravenous infusion of physiological amounts of chylomicron-TG into unanesthetized, unrestrained rats. Serial blood samples can be withdrawn during the infusion, and thus it is possible to construct a complete plasma concentration-time curve for each animal. In order to establish the rate of chylomicron removal under physiological conditions, we have measured the kinetics of removal in unanesthetized, fasted rats and in fasted rats anesthetized with either sodium pentobarbital or halothane.

METHODS

Male Long–Events rats maintained on a stock diet (Berkeley Diet; Feedstuffs Processing Company, San Francisco, Calif.) were used for all experiments. The animals used for production of chylomicrons weighed approximately 350 g, and the recipient animals weighed 210–250 g.

Preparation of Labeled Chyle

Rats were fasted for 18 hr, and cannulation of the thoracic duct was performed by a modification of the technique of Bollman, Cain, and Grindlay (28). 30–60 min before surgery, the animal was intubated and given 1–2 ml of evaporated milk. The rat was anesthetized with ether, and a midline abdominal incision was made. The abdominal thoracic duct was cannulated with polyethylene tubing (PE 90, Intramedic; Clay–Adams, Inc, New York), the cannula was exteriorized, and the incision was closed. A heptane solution containing 50 μc palmitic-1-^{14}C acid (New England Nuclear Corp., Boston, Mass.) was evaporated to dryness. The sodium salt of the palmitic acid was formed by the dropwise addition of 0.1 N NaOH to the acid while it was gently heated. To this solution was added 2 ml of evaporated milk, and this mixture was administered to the rat by intubation. The rat was placed in a restraining cage and allowed free access to 0.15 M NaCl. The ^{14}C-labeled chyle was collected at room temperature overnight. The clots which formed were removed before use. A sample of the whole chyle was extracted with alcohol–ether 1:1, and total lipids were estimated by a dichromate oxidation method (29). The chyle was then diluted with 0.15 M NaCl so that the final concentration of total lipid was 10 mg/ml. Chyle prepared in this manner was used within 8 hr. The time from cannulation of the thoracic duct to the end of the experiments with recipient animals never exceeded 24 hr. No detectable bacterial growth occurs in the chyle during this period.[1]

The distribution of ^{14}C in the labeled whole chyle was determined after separation of the lipids on silicic acid columns (30) and further separation of the TG from FFA by a modification of the method of Borgström (31). The distribution of ^{14}C was as follows: TG, 96.7%; FFA, 1.7%; phospholipid fatty acids, 1.6%.

Recipient Animals

The right carotid artery and the left external jugular vein were cannulated under ether anesthesia by a modification of the method of Popovic and Popovic (32). Both cannulas were 30-cm lengths of polyethylene tubing (PE 50, Intramedic; Clay–Adams, Inc.). A 3 cm length of silicone rubber tubing, I.D. 0.020 inches, O.D. 0.037 inches (Silastic, Dow Corning Corp., Midland, Mich.) was attached to the arterial cannula by stretching it over the polyethylene for a distance of 3–5 mm. The cannulas were filled with 0.15 M NaCl, and the ends were clamped with rubber-tipped hemostats. The venous cannula was inserted 2.5 cm and secured with surgical silk. The silicone rubber end of the arterial cannula was inserted into the carotid artery and secured at the junction of the polyethylene and silicone rubber tubes. The cannulas were passed under the skin and through a small incision on the back between the scapulae. They were flushed with 0.15 M NaCl and sealed at the distal ends by pressure after they had been heated with a flame. A "backpack" (see description below) was centered above the scapulae and sutured at the four corners to the back of the rat. The cannulas were drawn through the center hole, coiled, and affixed with adhesive tape to the backpack so that the rat was unable to damage them.

The animals were caged separately and allowed to recover for 3–4 days following surgery before the experiments were performed. It was not found necessary to flush the cannulas during this period. The use of heparin in this preparation was carefully avoided.

Preparation of the Backpack

The backpack described here is a modification[2] of a preparation by Weeks (33). Shim brass (0.010 inches)

[1] Felts, J. M. Unpublished observations.

[2] Mayerle, J. A., K. L. Harris, and M. Brown. Unpublished observations.

was cut in 3-cm squares, and the corners were rounded. Five holes ($^5/_{32}$ inch) were punched in the square, one at each corner and one in the center. Polyethylene grommets (PE 330 tubing) were molded with heat around each hole. Closed pore sponge rubber sheeting ($^1/_4$ inch) was cut in squares slightly larger than the shim brass and attached to it with Pliobond cement (Goodyear Tire and Rubber Co., Inc., Chemical Div., Akron, Ohio). A hole was made in the rubber through the center hole in the shim brass to allow the cannulas to pass.

Determination of Plasma Volume

In some experiments plasma volume was determined with albumin-^{131}I (34). Approximately 1 μc of albumin-^{131}I (RISA; Abbott Laboratories, North Chicago, Ill.) in 0.15 M NaCl was injected intravenously into each rat 3–5 min before the start of the chylomicron infusion. ^{131}I radioactivity in plasma samples taken 2–6 min after start of infusion was measured in a crystal scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The volume calculated from the dilution of ^{131}I radioactivity in the plasma was assumed to represent the plasma volume.

Infusion of Chylomicrons and Sampling

Rats, cannulated as described, were fasted for 18 hr but had free access to water. Just before the experiment, the adhesive tape was removed from the backpack, and the cannulas were uncoiled. The cannula ends were cut, and the venous cannula was attached to a needle on the infusion syringe. The arterial cannula was kept closed by clamping it with a rubber-tipped hemostat. The rat was placed in a small cage, and the cannulas were led out through a wire cover. The animal was not restrained in any way.

For each experiment, the animals were divided into unanesthetized and anesthetized groups and were infused with a single preparation of chyle. Anesthesia was induced by intraperitoneal injection of sodium pentobarbital, 60 mg/kg (Diabutal; Diamond Laboratories, Inc., Des Moines, Iowa), or sodium pentobarbital, 50 mg/kg (Nembutal; Abbott Laboratories). Experiments were begun 10 min after the animals appeared to be anesthetized. In other animals, halothane, 3% in oxygen, was administered to induce anesthesia, which was then maintained with 1.25% halothane. The experiments were begun 20 min after the animals appeared to be anesthetized.

The chylomicron preparations were infused through the indwelling venous cannula at a rate of 0.05 ml/min (0.5 mg/min of lipid) with a constant infusion pump Havard) Apparatus Co., Inc., Dover, Mass.). After 20 min the infusion was terminated. 12 serial blood samples of 0.25 ml each were collected from the indwelling arterial cannula during the 20 min of infusion and during an additional 10 min after the infusion was stopped. The saline in the arterial cannula (hold-up volume of 0.1 ml) was withdrawn before each sample, and the cannula was flushed with approximately 0.3 ml physiological saline following each sample.

Sample Handling and Analysis

The blood samples were immediately transferred to heparinized microcentrifuge tubes (Beckman Instruments, Inc., Fullerton, Calif.) and were stored in ice; they were centrifuged at the end of each experiment.

100 μl of plasma were layered under 0.15 M NaCl in 2 ml cellulose nitrate tubes (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.) and centrifuged for 15 hr at 40,000 rpm in the 40.3 rotor of a Spinco Model L preparative ultracentrifuge. The tubes were sliced in a tube slicer, and the low-density fraction ($d<1.006$) was recovered and extracted with chloroform–methanol 2:1. The extracts were washed with 0.03 N HCl, and the lower chloroform phase was transferred to counting vials. The solvent was evaporated, and the residue was redissolved in 18 ml of toluene containing 0.27% 2,5-diphenyloxazole (PPO) and 0.0133% 1,4-bis[2-(5-phenyloxazolyl)]-benzene (POPOP) (Packard Instrument Company, Inc.). The samples were assayed for ^{14}C radioactivity in a liquid scintillation spectrometer (Packard Instrument Company, Inc.) equipped with a punch-card read-out (IBM, Model 526). The degree of quenching was estimated by means of an external standard, and appropriate corrections were made. Computations were carried out with a program written for a digital computer (35).

Curves such as that shown in Fig. 1 were constructed for each animal for data obtained in experiments with unanesthetized rats. The graphical representation of plasma ^{14}C levels shows the following three phases: (*a*) a rise in the radioactivity in the plasma (appearance curve), (*b*) a steady state, and (*c*) an exponential fall in the level of radioactivity after cessation of the infusion (disappearance curve). From these three phases, three independent determinations of the fractional turnover rate of chylomicron-TG can be calculated, using the following model:

$$\text{Chylomicrons} \xrightarrow{Ki} \text{Cp} \xrightarrow{k_1} \text{Products}$$

In this model, chylomicrons are infused at a constant rate, K_i, producing a plasma level of chylomicron ^{14}C designated as Cp. The chylomicrons are removed with a fractional turnover rate of k_1. When other factors are constant (i.e. enzyme activity and blood flow) and are not rate limiting, the process may be described by first-order kinetics. This single compartmental model for

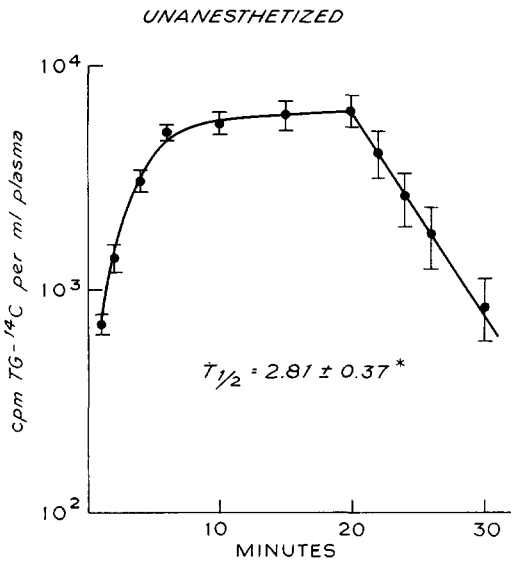

FIG. 1. Averages of radioactivity in d < 1.006 lipoproteins in four unanesthetized rats after a 20 min infusion of labeled chylomicrons (0.5 mg total lipid per min). Radioactivity values were normalized to 20,000 cpm/min infused. *Mean value ±SEM calculated from disappearance curve.

chylomicrons in the plasma is supported by previous reports demonstrating a monophasic exponential decline in radioactivity following an injection of labeled chylomicrons (3, 4, 10, 17). The single compartment of distribution may be approximated by the plasma volume, since chylomicrons are not known to distribute significantly outside the plasma compartment. The following equation may be used to describe the behavior of the chylomicron-TGFA in terms of change of radioactivity in plasma:

$$\frac{\Delta Cp}{\Delta t} = \frac{K_t}{Vc} - k_1\,Cp$$

In this equation, the change in plasma radioactivity with time, $\Delta Cp/\Delta t$ (cpm/ml per min), equals the rate at which the substrate is introduced, K_t (cpm/min), divided by the compartment volume, Vc (ml). From this value is subtracted the rate at which the substrate leaves the compartment, i.e. the fractional turnover rate, k_1 (min^{-1}), times the plasma radioactivity, Cp (cpm/ml).

Using this equation, we calculated (from the first phase of the concentration-time curve) a fractional turnover rate for the removal of chylomicron-TGFA from the plasma. For a representative experiment, we plotted $\Delta Cp/\Delta t$ vs. Cp, and an estimate of the fractional turnover rate, k_1, was obtained from the slope. We also calculated the volume of distribution, Vc, of the chylomicrons from the dose and the intercept on the Cp axis. This volume was compared with the plasma volume as determined by albumin-^{131}I dilution.

From the steady-state phase where $\Delta Cp/\Delta t = 0$, we made a second calculation of k_1 using the following relationship: $K_t/Vc = k_1\,Cp$

The disappearance curve observed after cessation of the infusion of ^{14}C chyle makes possible a third calculation of the fractional turnover rate. From the disappearance curve plotted on a semilog coordinate (radioactivity vs. time), we obtained a value for the half-life, $t^1/_2$. From the first order rate equation, $t^1/_2 = \ln 2/k_1 = 0.693/k_1$, a value for k_1 was obtained.

RESULTS

Experiments with Unanesthetized Animals

In Fig. 1 are the results obtained from four unanesthetized rats which received infusions of whole chyle. The average half-life of removal of the chylomicrons in these animals, calculated from the disappearance phase of the individual curves, was 2.8 min.

Table 1 shows the values of the fractional turnover rates for each animal in the unanesthetized group calculated from the three phases of the curve. The calculations made from the steady-state phases of the curves using the volume of distribution obtained from the appearance portion of the curves were compared with similar calculations using the plasma volumes obtained from dilution of albumin-^{131}I in animals U-1 and U-2 where such data were available. In these two cases, the rates calculated from steady state were almost identical with those using the volume of distribution obtained from the initial portion of the curves. This supports the hypothesis of a single compartmental model. The average

TABLE 1 FRACTIONAL TURNOVER RATES OF CHYLOMICRON-TGFA IN UNANESTHETIZED RATS

Animal	U-1	U-2	U-3	U-4
		min $^{-1}$		
Calculated from disappearance curve	0.23	0.18	0.28	0.35
Calculated from appearance curve	0.22	0.17	—*	0.31
Calculated from steady-state phase				
a. Using Vc calculated from appearance curve	0.19	0.15	—*	0.27
b. Using Vc calculated from ^{131}I dilution	0.18	0.16	—†	—†
Mean Value ± SEM		0.22 ± 0.018		

* Samples lost during analyses.
† Data not available.

fractional turnover rate calculated from the three phases of the curves for the unanesthetized group of animals was 0.22 min^{-1}. Although calculations of fractional turnover rates and half-lives of disappearance of chylomicron-TGFA-^{14}C from each of the three phases of the curves correlated well with one another in each of these animals, there are certain considerations to be made for each phase. The calculation from the first phase of these curves is probably the least reliable because the first point (1 min) seldom fell upon the line which describes the relationship between $\Delta Cp/\Delta t$ and Cp. Thus, only three points were used to estimate this line. In the second portion of the curve, it was not always clear whether a steady state had been reached since there are only two or three points on this segment of the curve. If a volume for the distribution of chylomicrons cannot be calculated from the initial portion of the curve because of inadequate data, then it must be assumed that the volume of distribution of albumin-^{131}I equals the chylomicron "space." The third portion of the curve is probably the most reliable. There were five points from which to estimate the half-life directly. For these reasons, the half-lives expressed in the figures are calculated from the disappearance portion of the individual curves.

Experiments with Anesthetized Animals

Fig. 2 shows data obtained from rats anesthetized with sodium pentobarbital. Since there seemed to be no effect of the amount of sodium pentobarbital given, the data from all seven animals were combined. The levels of radioactivity in plasma were higher in the anesthetized rats when compared with those in the unanesthetized rats, and a steady state was not attained. The average apparent half-life for removal of ^{14}C in chylomicron-TGFA calculated from the disappearance phase of the individual curves was 4.5 min which was significantly longer than that obtained in the unanesthetized rats ($P<0.01$).

The data from three animals anesthetized with halothane are shown in Fig. 3. The curves were similar to those from experiments in which sodium pentobarbital was used for anesthesia. The average apparent half-life of chylomicron-TGFA removal calculated from the disappearance phase of the individual curves was 4.4 min. This was also significantly longer ($P<0.05$) than that of 2.8 min obtained in the unanesthetized group of rats. It is apparent that in the anesthetized animals a steady state was not reached during the 20 min of infusion, and thus the mechanisms for removal of chylomicron-TGFA may have been saturated. If this were the case, the half-lives estimated from the disappearance curves would not be exact. For this reason, they have been called *apparent* half-lives.

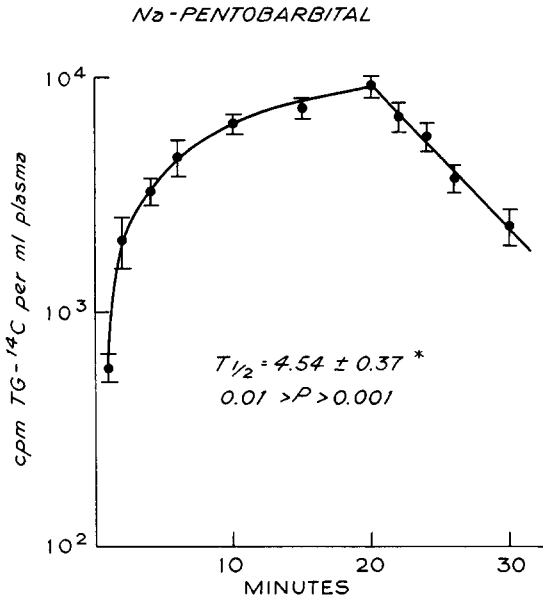

FIG. 2. Averages of radioactivity in d < 1.006 lipoproteins after a 20 min infusion of labeled chylomicrons (0.5 mg total lipid per min) in seven rats anesthetized with sodium pentobarbital. Radioactivity values were normalized to 20,000 cpm/min infused. *P* value was calculated by comparison with results from unanesthetized animals. *Mean value ± SEM calculated from disappearance curve.

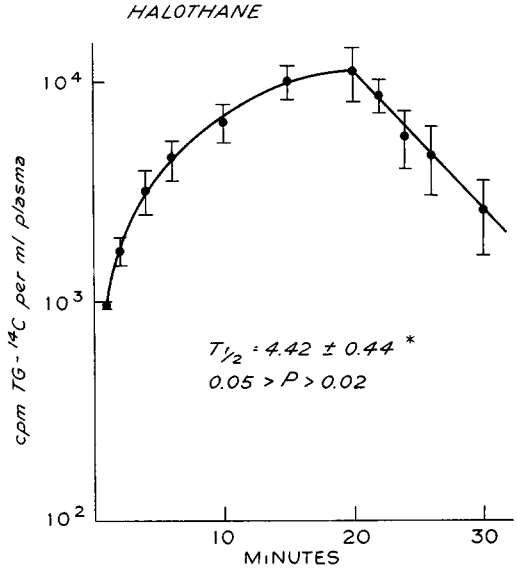

FIG. 3. Averages of radioactivity in d < 1.006 lipoproteins from a 20 min infusion of labeled chylomicrons (0.5 mg total lipid per min) in three rats anesthetized with halothane. Radioactivity values were normalized to 20,000 cpm/min infused. *P* value was calculated by comparison with results from unanesthetized animals. *Mean value ±SEM calculated from disappearance curve.

DISCUSSION

We have attempted to develop a method for studying the removal of chylomicron-TGFA from the circulation under optimum physiological conditions.

Maximal absorption rates of 5 mg/kg per min have been reported for the rat (16, 18–20). This value corresponds with 1.25 mg/min in a 250 g rat. Based upon these values, a constant infusion rate was selected which would deliver to the circulation less than half of that amount, namely, 0.5 mg total lipid per min. The labeled chyle was collected and stored at room temperature for periods not exceeding 24 hr. Whole chyle containing labeled chylomicrons was administered by constant infusion. Greater than 96% of the radioactivity was in the TGFA fraction as determined by separation on silicic acid columns. Serial blood samples were taken from the carotid cannula, so that a single concentration-time curve could be constructed for each animal. 12 blood samples of 0.25 ml each were taken during the 30 min of the experiments. This represents a blood loss of 12–14 ml/kg. It has been reported (36) that *acute* hemorrhages of 10 ml/kg in the anesthetized rat decrease cardiac output and change its distribution as well as reduce blood pressure. In a separate experiment, we prepared rats in the same manner as those used for the chylomicron experiments. It was determined that the removal of serial blood samples (total of 3 ml), over a 30 min period, from unanesthetized rats had no significant effect upon heart rate or blood pressure.

Using the experimental design described here, we have shown that anesthesia clearly decreases the rate at which chylomicron-TGFA are removed from the circulation of rats. These results fail to support the observation of Schotz, Arnesjö, and Olivecrona (27) that there are no differences in the removal rates of chylomicrons between anesthetized and unanesthetized rats. However, their observation was based upon the comparison of plasma radioactivity levels at only a *single time interval* following the injection of labeled chyle into rats anesthetized with sodium pentobarbital and into unanesthetized rats.

In an effort to establish that the decreased rate of chylomicron-TGFA removal was due to anesthesia and not to a specific barbiturate effect, another anesthetic agent, halothane, with completely different characteristics was used. The results obtained with halothane were similar to those obtained with sodium pentobarbital suggesting that the effect is not specific to the barbiturate anesthetics.

These results may be helpful in interpreting certain findings reported by other investigators. Boberg and Carlson (37) injected rats with labeled FFA and measured the amount of radioactivity incorporated into TGFA of VLDL. The anesthetized rats incorporated more labeled fatty acid into TG, and the peak values appeared later than in unanesthetized rats. In preliminary experiments, Garfinkel, Baker, and Schotz (38) also obtained data which suggested that the incorporation of labeled FFA into serum TG was increased by ether anesthesia. In view of the findings reported here, it is likely that these observations result in part from a decreased rate of removal of the VLDL-TG from the plasma compartment.

At least two explanations for the reduced rate of removal of chylomicron-TGFA in anesthetized rats are possible. It is well known that anesthesia decreases cardiac output (39) which, in turn, reduces blood flow to certain areas (especially muscle).[3] Lipoprotein lipase, the enzyme to which the role of hydrolysis of chylomicron-TG and VLDL-TG has been ascribed (40, 41) may be located in capillary beds of these areas (42–44). The reduction in cardiac output and blood flow could result in a decreased rate of interaction between enzyme and substrate, thus accounting for the decreased rate of removal of chylomicron-TGFA in the anesthetized rat. It is also possible that because of the redistribution of blood flow which occurs during anesthesia,[3] capillaries rich in this enzyme are bypassed, making the enzyme unavailable for this interaction with the substrate. In support of this explanation are the findings of Hallberg (12) and of Jones and Havel (15). Hallberg observed an increased elimination rate of a fat emulsion from the circulation in postoperative patients. The patients had significantly increased pulse rates and body temperatures and unchanged blood pressures. Hallberg concluded that these patients had increased cardiac outputs which could increase blood flow through sites of removal. Similarly, Jones and Havel (15) observed an increased rate of elimination of chylomicron-TGFA in exercising rats.

The differences which we observed and have attributed to hemodynamic changes in the anesthetized state may also be influenced by changes resulting from loss of blood in these animals. Although we observed no significant change in heart rate or blood pressure when unanesthetized animals were subjected to these blood losses, it is still possible that the circulatory response to this blood loss may be different between the anesthetized and unanesthetized animals.

Another less likely explanation for these observations would be a direct effect of the anesthetic agents on LPL. However, Mallov and Cerra (45) could demonstrate no effect on cardiac LPL by prolonged sodium pentobarbital anesthesia.

In the experiments reported here, the apparent half-lives of chylomicron-TGFA removal in the anesthetized rats were from 20 to 100% greater than those in the unanesthetized rats. These results emphasize the importance of using unanesthetized animals in studies which define rates of chylomicron-TG removal which are physiologically meaningful.

[3] Mayerle, J. A. Personal communication.

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