Infusion of Long-Chain Fatty Acid Anions by Continuous-Flow Centrifugation

WILLIAM B. GREENOUGH III, STEPHEN R. CRESPIN, and DANIEL STEINBERG

From the Molecular Disease Branch, National Heart Institute, Bethesda, Maryland 20014

ABSTRACT We have developed a method for the rapid infusion into plasma of large amounts of long-chain free fatty acids (FFA). Unanesthetized dogs were connected by a peripheral artery to a closed, continuous-flow centrifuge from which cells and plasma emerged in separate lines. Sodium oleate was infused directly into the plasma line before cells and plasma were recombined and returned to the animal through a peripheral vein.

The centrifugation procedure itself produced only small changes in circulating levels of glucose, FFA, and electrolytes. Plasma flow rates as high as 100 ml/min could be maintained, and centrifugations of 12 hr were accomplished without complications.

During centrifugation, sodium oleate was infused at rates up to 80 μEq/kg per min for 2.5 hr; the maximum molar ratio of FFA to albumin without hemolysis was 10:1. Plasma FFA levels rose rapidly after infusions were started and reached constant elevated levels within 15–20 min. Oleate infusion at 10–50 μEq/kg per min produced a rise in plasma FFA proportional to the infusion rate. The maximum increment in plasma FFA above control values was 1.66 μEq/ml. When infusions ended, plasma FFA declined rapidly to control levels. Oleate infusion at rates below 30 μEq/kg per min did not reduce levels of other plasma FFA. Infusion at high rates was accompanied by a marked fall in blood glucose.

This method permits administration of long-chain fatty acids in sufficient quantities to study their individual metabolic effects, and provides a new way to supply lipid calories parenterally.

This work was presented in part at The American Federation for Clinical Research, National Meeting, Atlantic City, N. J., 30 April 1967 (1). Preliminary reports of this work have appeared (1, 2).

Dr. Steinberg's present address is Department of Medicine, School of Medicine, University of California, San Diego, La Jolla, Calif. 92037.

Received for publication 4 February 1969 and in revised form 22 May 1969.

INTRODUCTION

It has not previously been possible to infuse large quantities of long-chain fatty acids directly into experimental animals without causing hemolysis, thrombosis, or even sudden death (3, 4). Hence, current knowledge of fatty acid metabolism itself, and its interrelationships with lipid, carbohydrate, and protein metabolism, is based on studies using trace amounts of radioactively labeled fatty acids or those employing hormones, pharmacologic agents, or dietary manipulation (5–8). In several recent studies, plasma FFA levels have been raised by fat feeding or infusion of lipid emulsions combined with heparin administration (9–12). In this type of experiment, however, the effects of chylomicrons or emulsified lipid in the blood or metabolic changes accompanying digestion, absorption, and lipolysis introduce additional variables which complicate evaluation of the role of fatty acids per se in the observed responses.

Since fatty acids bound to albumin do not cause cellular damage, one might try to raise FFA levels by infusing them in the bound state. However, the half-life of albumin is measured in days while that of fatty acids is measured in minutes. Even if the albumin infused were loaded to maximum capacity, about 6 moles fatty acid per mole of albumin (13), infusion at a rate sufficient to raise the plasma FFA level by 1.0 μEq/ml would double the intravascular albumin pool in about 3 min.[1] Clearly this is impractical for any but the shortest experiments.

[1] As shown in the Results section, FFA infusions of 40 μEq/kg per min were needed to raise plasma FFA levels by 1 μEq/ml. Using albumin containing 6 moles of FFA per mole, one would have to infuse 400 mg of albumin-FFA complex/kg per min. Assuming a plasma volume of 40 ml/kg and a plasma albumin concentration of 30 mg/ml, the initial plasma albumin pool would be 1200 mg/kg. Hence, the pool of albumin would be doubled in 3 min, transport of albumin to extravascular spaces being negligible over such a short time interval.

We chose to infuse fatty acids into cell-free plasma derived from the experimental subject by continuous centrifugation. The process of continuous-flow centrifugation has been widely applied in the dairy industry since its invention in 1878 (14). With the advent of World War II, the same technology was first applied to separating plasma from blood cells (15), and subsequently modified further for blood fractionation (16). As a result of the development of a closed, continuous-flow centrifuge (National Cancer Institute and International Business Machines Experimental Blood Cell Separator, hereafter referred to as the "Cell Separator"), a means to infuse FFA into plasma was developed. Our paper describes this process and reports some of its effects on fatty acid metabolism in conscious dogs.

METHODS

Experimental animals. Male and female mongrel dogs weighing 10 24 kg were maintained on a Purina Chow diet composed of 7% fat, 24% protein, and 69% carbohydrate.

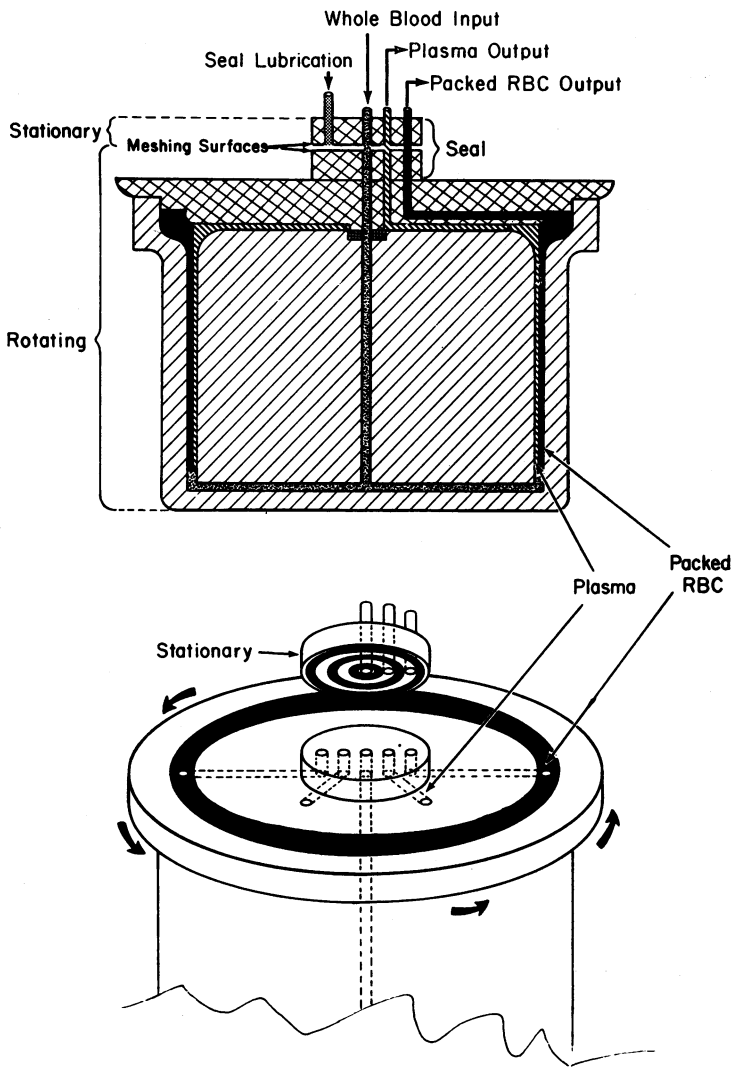

FIGURE 1 Schematic cross section and oblique view of the NCI-IBM Cell Separator centrifuge bowl. The illustrations are modified to show the continuous separation of whole blood into packed red cells (RBC) and plasma. More detailed and less schematic views are available in references (18, 19). Red blood cells separate from plasma and collect in the flared outer lip of the bowl. Leukocytes and platelets accumulate at the plasma-RBC interface. The separation is readily seen through the transparent cover. Ports for collecting leukocytes are not illustrated, RBC ports are at the extreme periphery and plasma ports are central.

Indwelling Teflon-Silastic arteriovenous shunts (Extracorporeal Medical Specialties Inc., Mount Laurel Township, N. J.) were placed between the carotid artery and jugular vein while animals were under sodium pentobarbital anaesthesia (17). All studies were performed 18 hr after the last feeding and from 1 to 10 days after shunt insertion. No anaesthesia or other medication was used in these studies except during several preliminary trials.

Continuous-flow centrifuge. The continuous-flow centrifuge employed in our studies (Cell Separator) has been described in full detail (18, 19). It consists of a rotor and head into which a bowl made of polycarbonate plastic is fitted. Whole blood entering the rotating bowl flows through a solid filler piece (Fig. 1) and is separated into packed red cells, buffy coat, and plasma as it rises up the side of the bowl. Individual ports conduct each component through the seal. The seal maintains separation by means of circular channels with intervening dividers or "lands," which are lubricated by saline under pressure. Whole blood and each fraction are carried to and from the centrifuge in polyvinyl chloride tubing, 0.25 inches O.D., 0.125 inches I.D. (Tygon, The U. S. Stoneware Co., Akron, Ohio). Individual peristaltic pumps withdraw each component at the desired rate (up to 100 ml/min each for plasma and red cells for a maximum total blood flow of 200 ml/min). The speed of the centrifuge motor may be varied continuously between 0 and 2200 rpm (0–350 *g* of centrifugal force at 2.5 inches radius). Fittings and connections were made from platelet recipient sets (HB 182, Fenwal Laboratories, Inc., Morton Grove, Ill.). Infusion catheters (Medium Deseret Intracath, C. R. Bard Inc., Murray Hill, N. J.) were placed in the plasma line with the Intracath needle and sealed with a drop of tetrahydrofuran.

Centrifugation procedure. First, the seal lubrication pump was started to provide saline under pressure at the rate of 0.5 ml/min to the closely contacting surfaces, or lands, which separate the collecting channels of the seal (shown schematically in Fig. 1). The centrifuge itself was then started and the bowl brought to the desired speed. For FFA infusions, 1500 rpm (160 *g*) was used to sediment platelets as well as leukocytes and erythrocytes. The dead space (220 ml), which included the bowl, collecting channels, and all tubing, was then primed with isotonic saline containing 5 U/ml heparin (Upjohn Co., Kalamazoo, Mich.). When all air had been removed from the system, a constant (0.5 ml/min) infusion of heparin (80 U/ml) into the arterial line was started. Then 5000 U of heparin were injected into the jugular vein, and the arterial limb of carotid-jugular shunt was connected to the arterial input line leading to the centrifuge. The return line was then promptly attached to the venous limb of the shunt (Fig. 2).

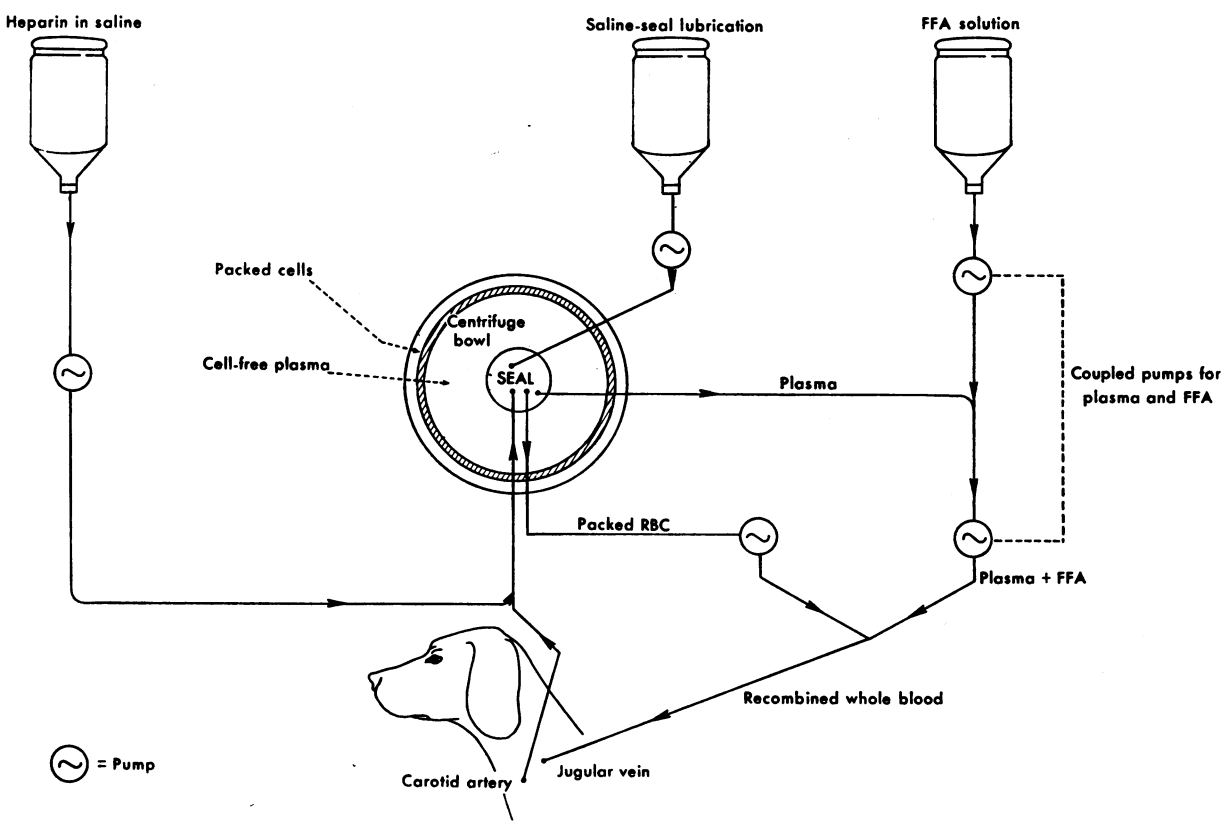

FIGURE 2 Flow diagram for sodium oleate infusion via a continuous-flow centrifuge. Heparinized carotid artery blood is continuously separated into packed red cells and plasma, which are pumped out of the centrifuge bowl in separate lines. FFA levels are raised by infusing sodium oleate directly into the plasma line before the cells and plasma are recombined and returned to the dog.

Studies of up to 12 hr were carried out on animals confined in cages which were large enough to allow the animal to stand or lie down but narrow enough to discourage turning around.

During FFA infusions all formed elements and plasma were recombined and returned to the animal except in those instances when intermittent collections of small volumes of buffy coat were made for white cell studies.

Fatty acid infusion. Oleic acid (Hormel Institute, Austin, Minn.) was 99.9% pure by gas-liquid chromatography. To insure complete conversion to sodium oleate, slightly more than an equimolar amount of 2.5 N sodium hydroxide was added directly to the liquid fatty acid. The oleate was taken up in 0.082 M sodium chloride solution to yield a final concentration of 3 g/100 ml by weight of the original oleic acid (106 mEq/liter). The final total osmolarity of the solution was 376 mOsm/liter. Heating to 70°–80°C for 2–5 hr with mixing resulted in an optically clear solution. This treatment did not result in detectable peroxides (procedure of Taffel and Rivis [20]) or significant amounts of azelaic or pelargonic acids measured by gas-liquid chromatography. The pH of the final solution was adjusted with 0.1 N HCl to the lowest pH that could be attained without inducing turbidity (about pH 9.4). This value agrees closely with that observed by Mattson and Volpenhein (21). The clear oleate solution was then introduced into the plasma line leading from the centrifuge (Fig. 2). It was pumped with the same pump used for the plasma itself, by means of a second deck with identically positioned compressing rollers, producing a fixed ratio of FFA to plasma. The rollers on the second deck accepted Technicon AutoAnalyzer Tubing (Technicon Co., Ardsley, N. Y.). A ratio of tubing I.D. of 0.030 inches for oleate to 0.25 inches for plasma achieved the maximum ratio of FFA to plasma possible without hemolysis. At a plasma flow of 100 ml/min, this gave an infusion rate of 7.3 ml/min ±1.2 (SD) for oleate.

Different concentrations of oleate were tried initially. When oleate solutions of more than 3 g/100 ml were mixed with plasma, precipitate formed which proved to be primarily a calcium soap. Increasing the osmolarity of the infusate to 376 mOsm/liter with sodium chloride reduced precipitation such that a 3 g/100 ml solution of oleate could be infused without significant precipitation.

Below a molar ratio of FFA to plasma albumin of 10:1, hemolysis rarely occurred. This ratio, however, is well above the range of saturation of the strong binding sites of human albumin for fatty acids and probably above that for dog albumin (13). Thus other binding sites for fatty acid appear to exist in plasma, probably on lipoproteins (22, 23), that have sufficient affinity for FFA to prevent cell damage even when the major classes of binding sites of albumin are saturated. In vitro studies were done in which several ratios of 3% sodium oleate were mixed with plasma and then, within less than 1 min, combined with packed red cells. Above a ratio of 10 moles FFA to 1 mole albumin, hemolysis occurred promptly. Prolonging the mixing time of FFA with plasma did not change the level above which hemolysis occurred.

The presence of unusual aggregates of FFA was sought by lipoprotein electrophoresis of the plasma taken from the carotid artery during FFA infusion. No such aggregates were found by this method nor was turbidity of the plasma noted on any occasion. In pilot studies, when oleate was dispersed in distilled water and administered as a 5% solution at a low rate, precipitated material (calcium oleate) entered the circulation in large quantity. No effect was observed on plasma FFA or glucose levels in these experiments.

All FFA infusions were preceded and followed by control periods during which isotonic saline was infused at a rate identical to that used for fatty acid infusion. All other variables were common to both control and experimental periods. Rectal temperature was not significantly affected by the extracorporeal circulation of blood during centrifugation.

Measurements. Blood was taken at 10- or 15-min intervals from the arterial line, unless otherwise noted. All samples were chilled in ice and separated promptly after each experiment. Hematocrits were measured in a microhematocrit centrifuge. Albumin was measured by a dye (2-(4'-hydroxyphenylazo)-benzoic acid) binding method (24) which was corrected for the different affinity of dog albumin for the dye (25) by use of Cohn fraction V of dog plasma (Pentex, Inc., Kankakee, Ill.). Blood urea nitrogen, serum sodium, potassium, chloride, and carbon dioxide content determinations were all carried out on a Technicon AutoAnalyzer. Blood pH, P_{CO_2} and P_{O_2} were measured on arterial blood samples with an analyzer (Instrumentation Laboratory, Inc., Watertown, Mass.). Plasma lipoprotein electrophoresis was done according to Lees and Hatch (26). Plasma glucose determinations were done by the glucose oxidase method ("Glucostat," Worthington Biochemical Corp., Freehold, N. J.). Plasma FFA were extracted with a mixture of 40 parts isopropanol, 10 parts 2,2,4-trimethylpentane, and 1 part 1 N H_2SO_4, a modification of the original method of Dole (27), and then measured by titration of phenol red dye (28) on a Technicon AutoAnalyzer (29). Standards of palmitic acid (Hormel Institute, Austin, Minn.) were extracted exactly as was plasma. Samples were run in duplicate in reverse order in the sample holder to correct for evaporation. 40 estimations per hour could be done with replicates agreeing to within ±0.035 μEq/ml (95% confidence limits on the differences between 41 duplicates assayed on different days). Separation of plasma fatty acids was done by gas-liquid chromatography on a Barber-Coleman Company (Rockford, Ill.) Series 5000 Chromatograph. A 6 ft column packed with polyethyleneglycol succinate adsorbed on Chromosorb P was used at 191°C. The percentage composition of the fatty acid mixture was calculated from the areas under individual peaks as determined by triangulation. Absolute quantities were calculated by multiplying the total value for plasma fatty acid obtained by titration, by the fraction of each component fatty acid. Fatty acids were prepared for chromatography by treating the 2,2,4-trimethylpentane phase from plasma FFA extraction according to Borgström (30). Methylation was carried out by heating for 5 min with boron trifluoride–methanol (31). The methyl esters were extracted into hexane, and aliquots were taken for chromatography.

RESULTS

The dogs withstood shunt insertion well. Activity and appetite returned to normal within 24 hr of surgery. Shunts remained patent for an average of 5 days and sometimes could be used for 8 days. Initial plasma FFA levels, measured immediately after the animals had been transported from animal quarters to the laboratory were usually high. These high levels returned to a stable baseline in 1–3 hr as the animal became accustomed to his surroundings. For this reason, experimental procedures were generally not begun until 2 hr after the animal arrived; baseline FFA levels were measured after the

TABLE I

Effects of Continuous-Flow Centrifugation on Arterial Blood Chemistries in Two Dogs*

Dog No.	Measurement	Control period, 0	Minutes after start of centrifugation 30	60	120	180	240	300	Postcentrifuge‡ +60
1, No oleate infusion	Albumin, *g/100 ml*	2.6	2.3	2.4	2.4	2.6	—	—	2.4
	Urea nitrogen, *mg/100 ml*	10	9	9	8	8	—	—	7
	Glucose, *mg/100 ml*	103	97	100	97	86	—	—	86
	Free fatty acids, *μEq/ml*	0.81	0.82	0.57	0.32	0.44	—	—	0.47
	Sodium, *mEq/liter*	141	141	142	141	143	—	—	141
	Potassium, *mEq/liter*	4.3	4.0	3.9	3.6	3.5	—	—	3.5
	Chloride, *mEq/liter*	119	121	122	122	122	—	—	122
	Carbon dioxide content, *mEq/liter*	22	21	20	18	18	—	—	18
2, Oleate infused§	Albumin, *g/100 ml*	2.6	2.2	2.2	1.9	1.9	1.9	1.9	—
	Glucose, *mg/100 ml*	131	140	126	100	100	120	112	—
	Free fatty acids, *μEq/ml*	0.46	0.41	0.44	1.17	1.11	0.23	0.22	—
	Sodium, *mEq/liter*	143	144	144	144	146	146	147	—
	Potassium, *mEq/liter*	4.1	3.9	3.8	3.3	3.0	3.4	3.4	—
	Chloride, *mEq/liter*	114	116	116	114	113	117	118	—
	Carbon dioxide content, *mEq/liter*	19	18	17	18	23	20	19	—
	Blood pH	7.49	—	7.46	7.47	7.55	7.57	7.58	—
	Blood P_{O_2}, *mmHg*	60	—	71	65	70	72	74	—

* Except for pH and P_{O_2}, all determinations were made on plasma.

‡ Determined 60 min after end of the 180 min centrifugation period.

§ Sodium oleate infused at 36.0 μEq/kg per min from 60 to 180 min after start of centrifugation.

animal had been attached to the centrifuge for at least 30 min.

Effects of centrifuge procedure. Initial experiments were carried out to determine the effects of continuous-flow centrifugation per se on the experimental animals. The details of hematologic effects in dogs under comparable conditions on the same machine have been published (18). In two dogs, several common clinical chemistries were done during a control period and at intervals during centrifugation. The small decrease in potassium observed has not been studied further as yet. One of these animals also received an infusion of sodium oleate (Table I). The effect of centrifugation itself (without FFA infusion) on hematocrit, plasma FFA, and glucose was studied in more detail in eleven dogs. Although there was considerable variation among animals, there were no major trends over a 3 hr period in any of these parameters (Table II). There was hemodilution at the beginning of each centrifugation when the 220 ml of priming saline in the centrifuge dead space was infused into the animal.

Animals tolerated centrifugation up to 12 hr very well. Generally they stood quietly, or even slept, after becoming accustomed to the limitations imposed by the cage and the tubing attached to their shunts. There were no deaths or severe reactions in any animals in these studies.

Oleate infusion. Sodium oleate was given on 25 occasions in 12 animals. Low infusion rates were used initially and gradually increased. Fatty acid levels were measured at several sites during preliminary experiments. Table III gives data from two such experiments at the highest infusion rates. FFA levels during infusion were much higher in the superior vena cava than in the carotid artery. The levels in right atrial blood were only slightly higher than in carotid arterial blood. Ultimately a maximal rate of FFA infusion of 80 μEq/kg per min was attained, which raised the plasma FFA from 0.42 to 1.85 μEq/ml, an increment of 1.43 μEq/ml. The rise in FFA to plateau levels within 15–20 min after starting the infusion, and a similarly rapid return to baseline after termination of the infusion, was characteristic, as can be seen in a representative experiment (Fig. 3). Despite heterogeneity of size, age, and sex in the animals used, a fairly good correlation between the rate of infusion of oleate and the increase in plasma fatty acid levels over baseline was apparent (Fig. 4 and Table IV). A poorer correlation was obtained when plasma FFA levels were compared directly to the rate of infusion.

These results suggest that the flux of infused fatty acid was added to that of endogenously released fatty acids and that there was no consistent direct effect on endogenous turnover by the infused oleate. Lack of direct feedback regulation was also indicated by the following ob-

TABLE II

Effect of Continuous-Flow Centrifugation on Hematocrit, Plasma FFA, and Plasma Glucose

Measurement*	Control period, 0	Hours after start of centrifugation 1	2	3	Postcentrifuge control,‡ +1
Hematocrit, %	37.3 ±6.7 (11)	35.6 ±5.8 (11)	35.4 ±4.1 (8)	35.6 ±2.6 (8)	35.7 ±3.7 (9)
Plasma FFA, *μEq/ml*	0.64 ±0.27 (9)	0.64 ±0.26 (9)	0.49 ±0.22 (8)	0.51 ±0.16 (4)	0.54 ±0.26 (8)
Plasma Glucose, *mg/100 ml*	95.6 ±20.5 (11)	104.7 ±14.8 (10)	104.4 ±14.6 (10)	96.8 ±9.8 (5)	98.7 ±12.5 (7)

Values were not significantly different from control levels ($P = 0.1$)

* Mean ±SD. Numbers of experiments are in parentheses.

‡ 1 hr after termination of centrifugation.

servations: first, during infusions of oleate sustained over 2 hr or more, plasma fatty acid levels rose quickly to a plateau value, and this was maintained without rising or falling once the level was established as long as the infusion rate remained constant; second, an equivalent absolute decrease in plasma fatty acid level was elicited by glucose loading whether during oleate infusion or during control periods (Table V); third, by gas-liquid chromatography, levels of individual FFA other than oleate were not diminished from control values during oleate infusion. In fact, the levels of the other fatty acids rose slightly (Table VI).

At the higher rates of oleate administration another striking consequence of FFA infusion was observed. Blood glucose levels decreased consistently in proportion to the rate of FFA administration. An increase in plasma insulin was observed which preceded the fall in blood glucose. Preliminary reports of these observations have been published (1, 2), and a detailed description of the nature the insulin response is reported in the subsequent paper (32).

Finally, it was anticipated and observed that the infusion of oleate, a metabolizable anion, might result in metabolic alkalosis. There was a small rise in blood pH and CO_2 content (Table I). The decrease in potassium in this study may be explained by this change in acid-base status.

TABLE III

Free Fatty Acid Levels at Different Sampling Sites in Two Dogs during Control and Oleate Infusion Periods

Dog	Infusion rate	Sample site	Plasma FFA: Control period	Plasma FFA: Oleate infusion
	μEq/kg per min		*μEq/ml*	
SS-1	7.5	Carotid artery	0.40	0.65
		Peripheral vein	0.32	0.61
		Superior Vena Cava	0.44	1.30
SS-2	16.1	Carotid artery	0.43	0.93
		Peripheral vein	0.47	—
		Right atrium	0.49	0.99

DISCUSSION

By means of continuous-flow centrifugation large amounts of long-chain fatty acids may be directly infused into the circulation of unsedated animals without deleterious effects. Sodium oleate has been given at a maximum rate of 16 g or approximately 144 cal/hr, which exceeds the requirement for basal metabolism consid-

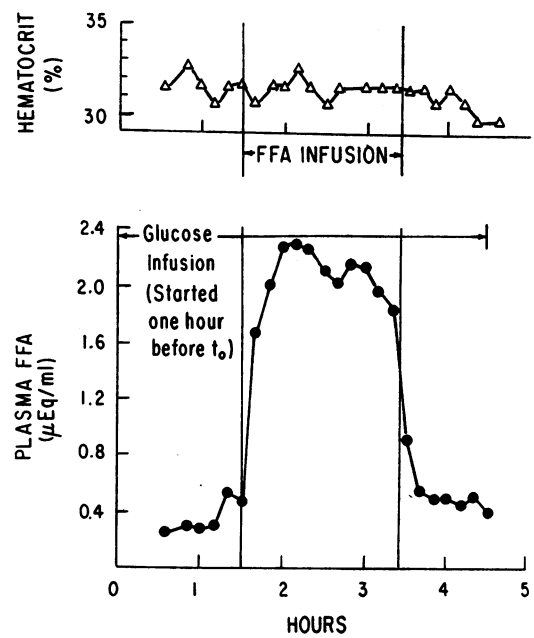

FIGURE 3 Effect of sodium oleate infusion on plasma FFA and blood hematocrit. After a 150 min control period on the centrifuge, 3% sodium oleate was infused at 45.7 μEq/kg per min in a 16.8 kg dog. Glucose was infused at 100 mg/min throughout the study.

TABLE IV

Effect of Infusion of 3% Sodium Oleate on Plasma FFA in 12 Dogs

Expt. No.	Dog	Weight	Rate of infusion	Total infused	Duration of infusion	Mean (±SE) plasma FFA		
						Control*	During infusion‡	ΔFFA
		kg	*μEq/kg per min*	*mEq*	*min*		*μEq/ml*	
1	A	17.3	16.1	40.4	145	0.43 ±0.03	0.93 ±0.03	0.50 ±0.05
2	B	13.2	24.4	40.2	125	0.74 ±0.13	1.66 ±0.08	0.92 ±0.15
3	A	17.3	12.3	10.6	50	0.75 ±0.05	1.25 ±0.02	0.50 ±0.05
4	A	17.3	19.4	48.8	145	0.61 ±0.04	1.07 ±0.05	0.46 ±0.06
5§	C	23.6	32.1	87.2	115	0.34 ±0.02	1.31 ±0.04	0.97 ±0.04
6§	D	18.2	34.8	88.6	140	0.38 ±0.04	1.44 ±0.03	1.06 ±0.05
7§	E	16.8	45.7	88.3	115	0.40 ±0.06	2.06 ±0.06	1.66 ±0.08
8§	F	19.5	43.6	85.0	100	0.42 ±0.02	1.66 ±0.04	1.24 ±0.05
9	G	11.8	80.0	85.0	90	0.42 ±0.01	1.85 ±0.11	1.43 ±0.11
10	H	13.6	64.8	91.7	104	0.61 ±0.07	1.87 ±0.08	1.26 ±0.10
11	H	13.6	43.3	90.6	154	0.54 ±0.03	1.66 ±0.09	1.12 ±0.09
12	I	16.4	58.3	38.2	40	0.59 ±0.03	1.75 ±0.10	1.16 ±0.10
13	J	16.8	59.0	59.5	60	0.86 ±0.05	1.94 ±0.08	1.08 ±0.09
14	J	16.8	36.0	70.1	116	0.41 ±0.02	0.94 ±0.06	0.53 ±0.06
15	J	16.8	40.8	44.6	65	0.48 ±0.05	1.40 ±0.02	0.92 ±0.05
16	K	19.1	9.7	7.4	40	0.62 ±0.05	0.63 ±0.03	0.01 ±0.06
17	K	19.1	18.0	13.8	40	0.31 ±0.01	0.73 ±0.02	0.42 ±0.03
18	K	19.1	22.3	17.0	40	0.31 ±0.03	0.93 ±0.06	0.62 ±0.07
19	K	19.1	26.4	20.2	40	0.28 ±0.06	1.10 ±0.09	0.82 ±0.11
20	K	19.1	33.4	19.2	30	0.48 ±0.07	1.56 ±0.02	1.08 ±0.07
21	L	20.0	24.8	59.6	120	0.60 ±0.08	0.98 ±0.02	0.38 ±0.08
22	L	20.0	37.2	44.6	60	0.57 ±0.07	1.03 ±0.05	0.46 ±0.08
23	L	20.0	23.9	28.7	60	0.54 ±0.04	0.83 ±0.03	0.29 ±0.05
24	L	20.0	15.9	31.9	100	0.37 ±0.05	0.73 ±0.04	0.36 ±0.06
25	L	20.0	20.4	24.4	60	0.30 ±0.05	0.97 ±0.07	0.67 ±0.09

* $n = 3$ (calculated from 3 separate determinations in the 30 min period immediately preceding infusion).

‡ $n = 3$–10 (calculated from all determinations during infusion).

§ Received 10 g/100 ml glucose at 1 ml/min.

erably. Increments in plasma FFA up to 1.66 μEq/ml have been produced, yielding a final constant level of 2.06 μEq/ml. Continuous fatty acid infusions have been maintained without ill effect for 2.5 hr. The only limitation on fatty acid administered in this way thus far encountered is the accompanying fluid volume load. Continuous-flow centrifugation has been carried out for 12 hr without difficulty. The present data provide for the first time unambiguous direct evidence of the enormous capacity of the whole animal to remove exogenously administered FFA from the blood stream.

The final plasma FFA level attained at a given infusion rate is determined by the value of plasma FFA at which the tissue removal rate equals the infusion rate plus the rate of endogenously released FFA. Because of differences in the baseline plasma FFA levels in the animals studied, the final level for FFA attained with infusion did not correlate very closely with the infusion rates. However, when the baseline plasma FFA value was subtracted from the steady-state level achieved during infusion, the increment in plasma FFA due to oleate infusion correlated very well with the rate of administration (Fig. 4). Using this relationship, and assuming that the plasma FFA is distributed only in the plasma volume, the fractional rate of irreversible net transport was found to be 0.83/min.[2] Ono and Fredrickson, using the initial slope of the disappearance curve of ^{14}C-labeled unsaturated FFA from plasma, found half-lives corresponding to fractional transport rates of 0.86–1.39/min in dogs (34). These higher rates would be anticipated for their studies, since isotope methods in which the initial slope of the FFA disappearance curve is measured yield a fractional transport rate that includes both reversible and irreversible transport (35, 36). Calculation of net irreversible transport from isotope data requires assump-

[2] At an infusion rate of 50 μEq/kg per min, there was a mean increment of 1.2 μEq/ml in plasma FFA (Fig. 4). Assuming the plasma volume is 50 ml/kg (33), the fraction of plasma oleate transported per minute is: (50 μEq/kg per min)/(1.2 μEq/ml × 50 ml/kg) = 0.83/min. The half-life = (0.693)/(0.83/min) = 0.83 min.

tions or compartmental analysis to account for the well recognized reversible transport between the plasma FFA pool and extravascular FFA pools that rapidly equilibrate with it. The present studies give values, measured at the steady state, for the true irreversible transport based on direct measurement of the mass of infused FFA being cleared from the plasma.

In the present studies the turnover rate of FFA at a given plasma FFA concentration was found to be greater than values calculated from continuous infusion of palmitate-^{14}C into dogs (37). This finding is perhaps explained by the observations of Ono and Fredrickson that isotopically labeled oleate is removed from the plasma more rapidly than palmitate (34). There is no evidence from the present studies that the fractional turnover rate of FFA increased at low levels of FFA (< 0.8 μEq/ml) as had been observed by Fredrickson and Gordon in man (36). However, since plasma FFA were elevated to at least 0.8 μEq/ml in most experiments

TABLE V

Decrease in Plasma Free Fatty Acid Levels in Response to Glucose Loading During Control and Oleate Infusion‡ Periods*

Dog No.	Plasma FFA					
	Control			Oleate infusion		
	Before glucose	After glucose§	Decrease	Before glucose	After glucose	Decrease
	μEq/ml					
GL 1	0.44	0.24	0.20	0.72	0.52	0.20
GL 2	0.46	0.19	0.27	1.28	0.89	0.39
GL 3	0.84	0.54	0.30	1.00	0.74	0.26
GL 4	0.61	0.20	0.41	0.83	0.67	0.16
Mean	0.59	0.29	0.30	0.96	0.71	0.25
±SEM	0.09	0.08	0.05	0.12	0.08	0.05

* Administered as a bolus of 0.56 g/kg of 50 g/100 ml glucose in water.
‡ Mean rate of oleate infusion: 30 ±4 μEq/kg per min.
§ Measured 30 min after administration of glucose load.

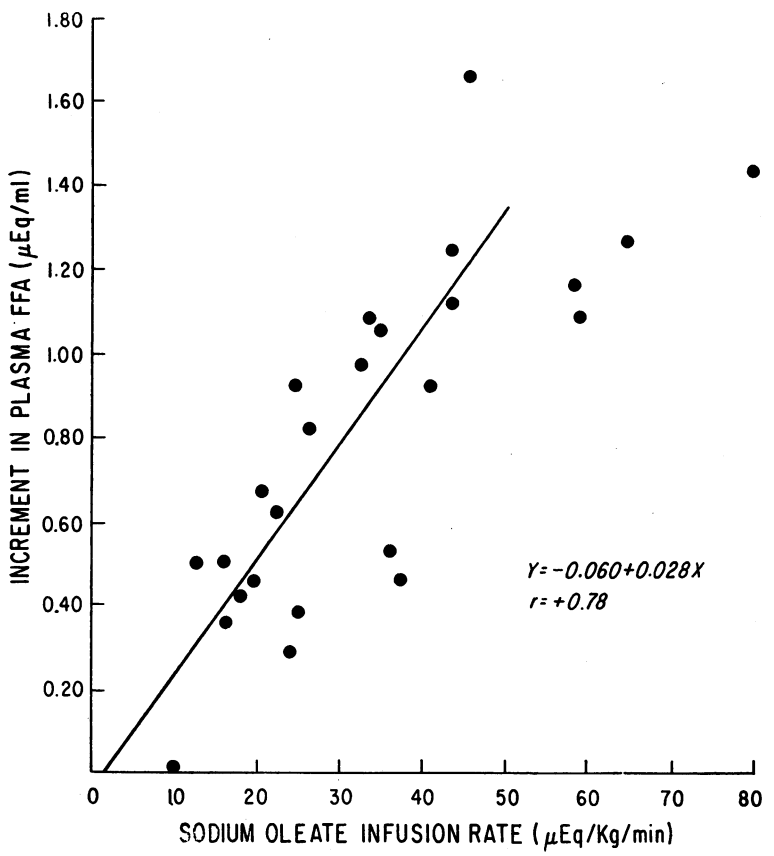

FIGURE 4 Relationship between the mean increment in plasma FFA during oleate infusion and the oleate infusion rate. Increments in plasma FFA were calculated as the differences between the mean of three separate FFA determinations in the 30 min control period just before oleate infusion and the mean of all FFA determinations during infusion. Points above 50 μEq/kg per min were not included in calculating the regression line. For r, $P < 0.01$.

TABLE VI

The Effect of Sodium Oleate Infusion on Plasma Levels of Individual Fatty Acids

Fatty acid	Experiment No. 1 Control	1 During infusion	2 Control	2 During infusion	3 Control	3 During infusion	4 Control	4 During infusion
				μEq/ml				
14:0	0.005	0.007	0.003	0.006	0.005	0.004	0.005	0.006
16:0	0.107	0.175	0.077	0.100	0.085	0.101	0.106	0.141
16:1	0.013	0.016	0.017	0.023	0.011	0.013	0.012	0.012
18:0	0.096	0.159	0.087	0.112	0.088	0.091	0.112	0.138
18:1	0.144	0.363	0.108	0.603	0.090	0.793	0.145	1.000
18:2	0.064	0.108	0.060	0.102	0.093	0.060	0.076	0.074
All other	0.045	0.072	0.048	0.054	0.038	0.204	0.034	0.179
Total	0.474	0.900	0.400	1.000	0.410	1.266	0.490	1.550
Δ-Oleate	—	+0.219	—	+0.495	—	+0.703	—	+0.855
Δ-Nonoleate	—	+0.207	—	+0.105	—	+0.153	—	+0.205

(Table IV), the present data are not sufficient to rule out such an effect. It actually appears that the turnover rate tended to increase at very high FFA levels (Fig. 4), but additional data will be needed to confirm this.

Feedback inhibition by plasma FFA on adipose tissue FFA release could be a reasonable control mechanism of plasma FFA levels. Our data indicate, however, that such a control mechanism does not operate at the FFA levels we have studied. In fact, instead of a decrease in the nonoleate plasma FFA during oleate infusion, a slight increase was observed. This result is somewhat paradoxical in that at high rates of oleate infusion insulin may be released, which normally would inhibit endogenous lipolysis. It is possible that a variety of factors might have operated to enhance endogenous lipolysis slightly during the oleate infusions, but the exact mechanism remains to be clarified. There is additional support for the lack of inhibition of adipose tissue FFA release by FFA infusion. Glucose loads administered either during control or oleate infusion periods resulted in equivalent reduction in plasma FFA levels (Table V).

In 1966, Seyffert, Barker, and Madison described a fall in blood glucose accompanying elevation of FFA levels by infusion of a cottonseed oil emulsion and heparin in anesthetized dogs (38). Early in our studies we also observed a reduction in blood glucose that was roughly proportional to the rate of FFA infusion (1). It occurred either in the presence or absence of concomitant glucose infusions and was subsequently found to be due to stimulation of insulin secretion (2). It is important to note that the rapid reduction in blood glucose and increase in plasma insulin seen at high FFA infusion rates may well cause changes in FFA metabolism not observed at the lower rates of infusion. The nature and mechanism of these responses are the subjects of an accompanying paper (32).

The possibility that some of the effects of FFA infusion might be experimental artifacts must also be considered. In the current studies, we have not been able to demonstrate that oleate exists even transiently in an unusual or unphysiologic state after mixing with plasma. Studies by others have shown that the rate of binding of FFA to albumin is extremely rapid, less than 30 msec (39). It is likely that changes observed in our studies may be attributed to FFA bound to plasma albumin and lipoproteins in the usual manner and circulated to the various tissues.

The possible applications of continuous rapid plasma separation are numerous, and some of these have been discussed previously (18). The method for FFA infusion may be a useful means for providing fat calories in physiologic form. Infusion rates reached in the present studies permit administration of 800 cal in 6 hr. The technique is applicable to smaller or larger animals and might be used in man. The centrifugation procedure itself has already been tested in patients at low flow rates (40). In addition, critical studies of the direct effects of fatty acids on peripheral glucose utilization, oxygen uptake by various tissues, lipoprotein synthesis and secretion, and other important metabolic functions are now possible. The distribution of infused fatty acids, their effect on the respiratory quotient, and effects on oxygen consumption are subjects of further investigations.

ACKNOWLEDGMENTS

The authors are particularly grateful to Dr. Dean Buckner, Mr. Robert Eisel, and Mr. David Boynton for their direct assistance in these studies; to Mr. George Judson and Dr. Robert Kellogg and the staff of IBM Systems Development Division, Endicott, New York for developmental engineering; to Dr. Seymour Perry, of The National Cancer Institute, for allowing us access to the Cell Separator; and to Miss Rose Calini for typing the manuscript.

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