Demonstration of two pools of albumin-bound fatty acids

C. Soler-Argilaga,[1] R. Infante, and J. Polonovski

Unité de Recherches d'Hépatologie (I.N.S.E.R.M. U-9) et Laboratoire de Biochimie, Faculté de Médecine Saint-Antoine, 75012 Paris, France

Abstract The uptakes of albumin-bound nonesterified fatty acids and of [1-^{14}C]palmitic acid complexed to albumin by the isolated perfused rat liver were compared. During perfusion, the rate of uptake of nonesterified fatty acids decreased and became zero when the fatty acid:albumin molar ratio reached 0.3, but the rate of uptake of radioactive palmitic acid remained constant. This finding suggests the existence of two pools of fatty acids bound to albumin with different fractional turnover rates. This view was supported by the fact that when delipidated albumin complexed in vitro to radioactive and nonradioactive fatty acids was used no difference was observed between the uptakes of nonesterified fatty acids and radioactive fatty acids by perfused liver. Similar results were found with albumin-bound radioactive fatty acid in vivo (obtained from rats fed radioactive palmitic acid), showing a homogeneous distribution of the label in both pools. The existence of two nonesterified fatty acid pools in plasma would arise from the differences in the nature of bonds between fatty acid and albumin molecules, which could determine the rate of exchange of fatty acids between the albumin-bound and soluble forms preceding their uptake by the cells.

It is generally assumed that the plasma nonesterified fatty acids (NEFA) constitute a single metabolic pool in which the different fatty acids have similar fractional turnover rates. In previously published studies (1, 2), the fractional turnover rate of NEFA in humans was calculated from the plasma radioactivity decay curve after the intravenous injection of albumin-complexed labeled fatty acids. From data obtained with palmitic, oleic, or linoleic acid it was assumed that the turnover rates of the different plasma fatty acids were similar.

However, some facts seem to contradict this interpretation. The rate of uptake of the plasma free fatty acids by the isolated perfused rat liver is not the same when it is calculated from measurement of portal and vena cava concentrations as when it is calculated by difference in radioactivity after perfusion of a labeled fatty acid complexed to albumin (3). Thus, the fractional turnover rates calculated in vivo from injected labeled fatty acids cannot be extrapolated to the total mass of plasma NEFA.

The data presented in this paper strongly suggest the existence of at least two pools of plasma NEFA that equilibrate with each other slowly: one with a high and one with a very low turnover rate. Some findings in previously published studies (4–13), in which plasma NEFA is assumed to be a homogeneous, single pool, should thus probably be recalculated considering the existence of two different pools.

MATERIAL AND METHODS

Animals

Wistar rats were used as liver donors. They were maintained on a standard commercial diet and weighed an average of 250 g. They were fasted more than 18–20 hr and were allowed to drink a 20% (w/v) glucose solution.

In the in vivo experiments, younger rats weighing 80 ± 5 g were used.

Liver perfusion

The standard technique used for perfusion of the liver, with perfusate recycling, has been described elsewhere (14). Open circuit perfusion without recycling was performed as follows. Before starting the experiment, the isolated liver was perfused for 20 min in a thermostatically controlled cabinet with the conventional recycling system (14) in order to stabilize liver blood flow and oxygenation. The portal catheter was then connected to a reservoir containing oxygenated fresh perfusate, which, after flowing through the liver, was collected for periods of 30 sec into graduated plastic tubes, thus allowing measurement of the transhepatic blood flow.

This open circuit system was used for the measurement of fatty acid uptake. Five samples from each representative period were taken for FFA titration and radioactivity estimation.

Address requests for reprints to: Dr. R. Infante, I.N.S.E.R.M., 184, Fbg. Saint-Antoine, 75012 Paris, France.

Abbreviations: NEFA, nonesterified fatty acid(s); FFA, free fatty acid(s); $\bar{v}$, nonesterified fatty acid:albumin molar ratio; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

[1] Present address: Department of Pharmacology, School of Medicine, University of Missouri, Columbia, Mo.

Preparation of perfusates partly depleted of fatty acids

This system was also used for the preparation of FFA-depleted perfusate used in some experiments (see Fig. 1 and Tables 3–5). For this, the perfusate, after one or more circulations through the organ, was recovered in a second reservoir that was connected to the portal catheter when the first reservoir was empty. Other technical details are indicated in the figure and table legends.

Chemicals

Reagent grade chemicals were obtained from Prolabo R.P. (France). Isooctane was from E. Merck (West Germany), Nile blue was from Edward Gurr Ltd. (England), and human albumin (fraction V) was from the Centre National de Transfusion Sanguine (France). [1-^{14}C]Palmitic acid (sp act, 48 mCi/mmole) and [9,10-^{3}H]palmitic acid (sp act, 5 Ci/mmole) were purchased from C.E.A. (Saclay, France); chemical purity was tested by GLC. Radiochromatography on thin-layer plates and gas–liquid radiochromatography showed that the radioactive fatty acids were 98–99% pure. Chemicals used for radioactivity measurements were purchased from Packard Instrument Co. (USA) and E. Merck.

Binding of fatty acid to albumin

Albumin (fraction V) was first delipidated as described by Chen (15). The method of Tinker and Hanahan (16) was routinely used to bind fatty acids to albumin. When a relatively large amount of nonradioactive fatty acid was complexed, the technique of Spector and Hoak (17) was preferred. 95–97% of the radioactive fatty acid was recovered in albumin, as shown by electrophoresis on Cellogel (Chemetron; Italy). In some experiments, ^{14}C- and ^{3}H-labeled palmitic acid was complexed to albumin as follows. A buffered albumin solution with a fatty acid:albumin molar ratio of 0.8 was prepared. [1-^{14}C]Palmitic acid was complexed, and then nonradioactive palmitic acid was added to raise the ratio ($\bar{v}$) to 1.8. Finally, [^{3}H]palmitic acid was complexed to the albumin, giving a final $\bar{v}$ of 1.9–2.0. Binding of ^{14}C- and ^{3}H-labeled fatty acids was tested by electrophoresis.

Labeling of plasma NEFA in vivo

Blood-donor rats were fasted for 48 hr and then fed a standard commercial diet containing [9,10-^{3}H]palmitic acid for 1 wk. The average daily intake of radioactivity was calculated to be 500 μCi/rat. After this prelabeling period, the animals were fasted for 24 hr before exsanguination. Blood (citrated) was collected and diluted with Krebs bicarbonate buffer for perfusion (perfusate A, Table 5). In other experiments, citrated blood from animals fed radioactive palmitic acid was diluted with buffer, and the perfusate was recirculated four times through an isolated liver; this latter perfusate (perfusate B, Table 5), partly depleted of FFA, was used for uptake studies in other isolated liver perfusions.

In addition, samples of perfusates A and B were centrifuged and the plasma was used for intravenous injection into rats in the experiments on fatty acid clearance in vivo reported in Table 6.

Analytical methods

Nonesterified fatty acids were extracted in isooctane and titrated as previously described (3). Albumin was isolated by the method of Schwert (18), and protein was estimated by the technique of Lowry et al. (19). The molar ratio of fatty acids to albumin ($\bar{v}$) was calculated on the assumption that all the free fatty acids were complexed to the albumin. The fact that a minor fraction of fatty acid exists in soluble form not complexed to the albumin (20) was not taken into consideration. Average molecular weights of 280 for fatty acids and 69,000 for albumin were used for the calculations.

Measurement of radioactivity

FFA were isolated from the isooctane extract by TLC on silica gel G (E. Merck), using petroleum ether–diethyl ether–acetic acid 90:30:1 (v/v) as the developing solvent. The fatty acid spot was located after exposing the plate to iodine vapor, and the silica gel was quantitatively scraped off into counting vials. Iodine was removed by sublimation under vacuum before addition of 15 ml of scintillation solution (2,5-diphenyloxazole, 5 g; 1,4-di-2-[4-methyl-5-phenyloxazolyl]-benzene, 0.3 g; toluene, 1 l). Radioactivity was measured in a Mark II spectrometer (Nuclear-Chicago) after the vials had stood overnight in order to ensure complete solution of fatty acids in toluene. Preliminary assays using pure radioactive fatty acid indicated that recovery of radioactivity was 90–98%. In in vivo experiments, the radioactivity in lipids was measured as described by Spector and Steinberg (20).

RESULTS

NEFA and [1-^{14}C]palmitic acid uptake by liver after one or more circulations of perfusate

As shown in **Fig. 1,** the percentage of NEFA taken up by the liver decreased in preparations that had been preperfused. Uptake of NEFA from perfusates that had been preperfused four times was in the order of 4% compared with 15–20% for previously uncirculated perfusate. Conversely, the uptake of [1-^{14}C]palmitic acid was essentially the same for all preparations, about 22–25%. This difference substantiates two facts: (*a*) the fatty acids remaining in the perfusate after circulation through the liver become less and less available for uptake by the cells, and (*b*) the

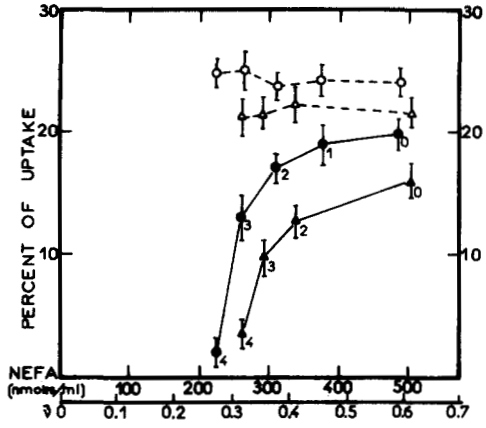

Fig. 1. Percentage of uptake of NEFA and [1-^{14}C]palmitic acid by isolated rat liver. In each experiment the liver was successively perfused with five fractions of 50 ml of perfusate (blood cells suspended in buffered solution of albumin). The fatty acid concentration of the first fraction was that of the native albumin; the other fractions were previously perfused through a different liver one to four times (as indicated on the figure) in order to decrease NEFA levels. [1-^{14}C]Palmitic acid was complexed to albumin (16) before the preparation of the five perfusate fractions. Hepatic blood flow (ml/g of liver): 1.62–1.74 (expt. 1) and 1.80–1.88 (expt. 2). Liver weight (g): 6.7 (expt. 1) and 7.2 (expt. 2). NEFA uptake: ● (expt. 1) and ▲ (expt. 2). [1-^{14}C]Palmitic acid uptake: ○ (expt. 1) and △ (expt. 2). Each point is the mean ± SD of five determinations.

NEFA mass and the [^{14}C]palmitic acid complexed to albumin do not seem to act as a single pool with regard to uptake by the liver.

Experiments were carried out in order to obtain further information about (*a*) the existence of a single, homogeneous pool or, alternatively, of several pools of albumin-bound fatty acids, and (*b*) the rate of exchange of fatty acids between the two pools of NEFA suggested by the above experiment.

If a single NEFA pool existed, it would be expected that [1-^{14}C]palmitic acid added in vitro to the perfusate 24 hr before the liver perfusion would enter the NEFA pool and, thus, the differences observed in Fig. 1 would not have appeared. However, **Table 1** shows that when the perfusate was used after 0.5, 5, or 24 hr of equilibration time, the liver took up a significantly higher percentage of [1-^{14}C]palmitic acid than of NEFA.

The question arose as to whether different molar ratios could modify the equilibrium between the in vitro complexed radioactive fatty acids and the in vivo complexed fatty acids preexisting in the albumin. In order to test this, the experiments summarized in **Table 2** were undertaken. It was found that the [1-^{14}C]palmitic acid complexed to nondelipidated albumin at an NEFA:albumin molar ratio of 0.7–0.8 equilibrated with the [9,10-^{3}H]palmitic acid added to the same albumin solution after the molar ratio was raised to 1.8–1.9 by addition of nonradioactive palmitic acid. Nevertheless, the percentage of the NEFA mass uptake was lower than the radioactivity uptake, again suggesting poor mixing of fatty acids complexed in vitro with those bound in vivo to the native albumin.

The low fatty acid uptake by the liver after successive circulations (see Fig. 1) could be the consequence of the lower fatty acid concentration in the perfusate or of the decreasing NEFA:albumin molar ratio. To clarify this point, a sample of standard perfusate was partly depleted of fatty acids by circulations through a preparative isolated liver; as the albumin concentration remained constant, the $\bar{\nu}$ fell from 0.70–0.73 to 0.31–0.35 (perfusate B). A second sample of the original perfusate was diluted with buffer in order to adjust the fatty acid concentration to a level close to that of perfusate B, but the original $\bar{\nu}$ was unchanged. The three perfusates were circulated successively through the same liver, and the total NEFA and radioactive fatty acid uptake was measured as described. The results (**Table 3**) clearly show that a 50% decrease in fatty acid concentration does not affect the percentage of uptake, provided that the molar ratio of NEFA to albumin is the same (C vs. A). In contrast, a decreased $\bar{\nu}$ sharply diminished the percentage of NEFA uptake (B vs. C), so the slope of the NEFA uptake curve in Fig. 1 is mainly the

TABLE 1. Uptake of NEFA and [1-^{14}C]palmitic acid by isolated perfused rat liver

Pre-equilibration Time	Percentage Uptake	
	NEFA	[1-^{14}C]Palmitic Acid
hr		
0.5	18.3 ± 1.2	24.7 ± 1.4
5	17.9 ± 0.8	25.1 ± 0.9
24	18.0 ± 1.3	24.3 ± 1.5

[1-^{14}C]Palmitic acid was complexed (16) with albumin in buffered solution and allowed to equilibrate with the NEFA for 0.5, 5, or 24 hr before the perfusion. The perfusate was made by suspending washed red blood cells in Krebs bicarbonate buffer, pH 7.4, containing the albumin–fatty acid complex. NEFA concentration, 502 nmoles/ml ($\bar{\nu}$ = 0.7); transhepatic blood flow, 1.4 ml/g of liver/min; liver weight, 7.9 g. Values are means of five determinations ± SD.

TABLE 2. Uptake of total NEFA and radioactive palmitic acid by isolated perfused rat liver

Determination of Uptake	Expt. 1		Expt. 2	
	$\bar{\nu}$	Percent of Uptake	$\bar{\nu}$	Percent of Uptake
NEFA	0.8	22.4 ± 1.3	0.7	25.0 ± 2.0
[1-^{14}C]Palmitic acid	0.9	32.8 ± 2.2	1.7	34.2 ± 2.7
[9,10-^{3}H]Palmitic acid	2.0	33.1 ± 1.4	1.8	35.4 ± 1.9

Albumin dissolved (30 mg/ml) in Krebs bicarbonate buffer, pH 7.4, was successively complexed (17) with [1-^{14}C]palmitic acid, nonradioactive palmitic acid, and [9,10-^{3}H]palmitic acid. Blood flow, 1.3 (expt. 1) and 1.1 (expt. 2) ml/g of liver/min; liver weight, 6.5 (expt. 1) and 6.8 (expt. 2) g. Values are means of five determinations ± SD.

TABLE 3. Comparative uptake of NEFA mass and [1-^{14}C]palmitic acid by isolated perfused rat liver

Expt. No.	Perfusate	NEFA Concentration	$\bar{v}$	Percentage of Uptake: NEFA	Percentage of Uptake: [1-^{14}C]-Palmitic Acid
		nmoles/ml			
1	A	510	0.70	18.1 ± 1.8	24.3 ± 2.1
	B	226	0.31	4.0 ± 1.3	20.9 ± 1.9
	C	232	0.70	16.7 ± 1.5	22.6 ± 2.3
2	A	545	0.73	17.0 ± 0.9	24.0 ± 1.6
	B	268	0.35	5.3 ± 1.1	25.3 ± 1.2
	C	280	0.73	19.0 ± 1.8	26.2 ± 1.8

The liver was successively perfused with (A) a native perfusate sample made by suspending red blood cells in Krebs bicarbonate buffer containing nondelipidated albumin complexed (16) with [1-^{14}C]palmitic acid, (B) a sample of the same perfusate recirculated through a "preparative" liver four times, and (C) a sample of the native perfusate A diluted with Krebs bicarbonate buffer to adjust NEFA to levels similar to those in sample B. Blood flow, 1.55–1.63 (expt. 1) and 1.47–1.52 (expt. 2) ml/g of liver/min; liver weight, 6.9 (expt. 1) and 7.1 (expt. 2) g. Values are means of five determinations ± SD.

result of the successive fall of $\bar{v}$. It should be noticed that, as in previous experiments, the percentage of uptake of radioactive fatty acid remains fairly constant, showing the metabolic heterogeneity of in vivo and in vitro complexed fatty acids. This assumption is supported by the following experiment (**Table 4**). A mixture of plasma NEFA was obtained by delipidating albumin (fraction V). A tracer amount of [1-^{14}C]palmitic acid was added to these fatty acids dissolved in hexane. This mixture of fatty acids was then complexed in vitro to totally delipidated albumin. As in the experiments depicted in Fig. 1 and Table 3, the percentage of NEFA uptake fell after successive circulations through the liver; however, in this experiment the percentage of uptake of both the mass and the radioactivity fell as all the radioactive and nonradioactive fatty acids were bound in vitro to the albumin.

Uptake of fatty acids complexed in vivo to plasma albumin

In order to investigate the uptake by the isolated liver of NEFA bound in vivo to circulating albumin, an isolated liver preparation was perfused with blood obtained from rats fed with [9,10-^{3}H]palmitic acid for 3 wk prior to bleeding and then diluted. It was assumed that after prolonged administration of the label, the different fatty acid pools in both blood and adipose tissue should be in isotopic equilibrium, and, thus, if slow and fast turnover pools of albumin-bound NEFA existed, they should be uniformly labeled. In this experiment, only Krebs bicarbonate buffer was used for blood dilution, and no heterologous albumin was added to the perfusate.

As summarized in **Table 5,** when $\bar{v}$ was 1.30 similar uptake values were found for total NEFA and fatty acid

TABLE 4. Percentage of uptake of NEFA and [1-^{14}C]palmitic acid by isolated perfused rat liver

Perfusate	NEFA Concentration	$\bar{v}$	Percentage of Uptake: NEFA	Percentage of Uptake: [1-^{14}C]Palmitic Acid
	nmoles/ml			
A	567	0.78	18.3 ± 0.8	21.5 ± 1.6
B	275	0.35	6.7 ± 1.1	8.1 ± 1.4

The isolated liver was perfused with red blood cells suspended in buffered albumin solution (A) then with another sample of this perfusate (B) circulated four times through a different liver. The albumin used in these experiments was completely delipidated (15) and then complexed (17) to a mixture of [1-^{14}C]palmitic acid and nonradioactive fatty acids previously extracted from albumin. Blood flow, 1.73–1.80 ml/g of liver/min; liver weight, 6.2 g. Values are means of five determinations ± SD.

radioactivity. Yet, at a low v (0.36), though the percentage of uptake fell to very low values, similar uptake values for NEFA mass and radioactivity were found. These results are identical with those for experiments (Fig. 1 and Table 3) using a mixture of labeled and nonlabeled fatty acids complexed in vitro with delipidated albumin. The data support the view that in both conditions the label was randomly distributed in the fast and slow turnover pools. Conversely, radioactive fatty acids complexed in vitro to nondelipidated albumin do not enter the slow turnover pool.

Experiments were carried out to check whether the results obtained with isolated perfused liver can be related to the whole animal. Two groups of rats were anesthetized with Nembutal, and their right carotid arteries and left saphenous veins were cannulated. The first group of animals received a fast intravenous injection of 0.4 ml of citrated plasma containing radioactive FFA (42,000 dpm) as well as other labeled lipids. This plasma was obtained from rats fed [^{3}H]palmitic acid as described above (Table 5, perfusate A). The second group received 0.4 ml of the same plasma that had been partly depleted of fatty acids (Table 5, perfusate B) by recirculation through a prepara-

TABLE 5. Percentage of uptake of NEFA and [9,10-^{3}H]palmitic acid

Perfusate	NEFA Concentration	$\bar{v}$	Percentage of Uptake: NEFA	Percentage of Uptake: [9,10-^{3}H]Palmitic Acid
	nmoles/ml			
A	524 ± 27	1.30	45.1 ± 3.8	47.9 ± 4.1
B	150 ± 10	0.36	0.3 ± 0.2	0.5 ± 0.3

The isolated liver was perfused successively with blood taken from rats fed [9,10-^{3}H]palmitic acid and diluted with Krebs bicarbonate buffer, pH 7.35 (perfusate A), and with diluted blood, as above, circulated four times through a different liver (perfusate B). Blood flow, 0.58–0.63 ml/g of liver/min; liver weight, 7.1 g. Values are means of five determinations ± SD.

TABLE 6. Radioactive NEFA clearance in rat blood in vivo

Injected Solution	Recovery of Radioactivity as NEFA		P
	1 min	4 min	
	dpm/ml of plasma		
Perfusate A (native)	8835 ± 640	6975 ± 395	0.01
Perfusate B (depleted)	2453 ± 300	2520 ± 260	NS

Compositions of perfusates A and B are described in Table 5. Each rat received by intravenous injection 0.4 ml of perfusate A (42,000 dpm of NEFA) or B (10,500 dpm of NEFA). Assuming a total plasma volume of 4.0 ml/100 g body wt, the radioactive plasma injected was diluted about tenfold into the circulation. Values are means ± SD from five rats/group.

tive isolated liver; this plasma contained only 10,500 dpm/0.4 ml. Arterial blood samples were collected in sodium citrate 1 min and 4 min after the injection. FFA were extracted by isooctane and purified by TLC, and their radioactivity was measured. The radioactivity of the other plasma lipids was thus removed. As in the isolated liver experiments (Table 5), the radioactive fatty acid bound to albumin was efficiently cleared from the blood (**Table 6**) when the fatty acid:albumin molar ratio of the injected plasma was 1.3. From 8835 dpm/ml of plasma, or 35,340 dpm/total plasma (80% of the injected NEFA radioactivity), the plasma radioactive fatty acids fell to 6975 dpm/ml, or 27,900 dpm/total plasma (60% of injected NEFA), in 3 min. However, when plasma depleted of fatty acids ($\bar{v}$ = 0.36) was injected, no radioactivity was taken up by the liver. The NEFA radioactivity (2453 dpm/ml or 9800 dpm/total plasma) representing 93% of the injected label remained unchanged at 4 min, showing that the labeled fatty acids in this depleted perfusate were bound to the albumin in a less transferable form.

DISCUSSION

The present experiments offer new information indicating a lack of homogeneity of the nonesterified fatty acids bound to plasma albumin. In the perfused liver experiments, it seems clear that the liver cell can extract most of the circulating FFA. However, when the molar ratio of fatty acid to albumin reaches a value near 0.3, the exchange with the cell membrane virtually disappears and the rate of uptake approaches zero, although the albumin contains about one-third of the physiological amount of NEFA.

It must be emphasized that from a technical point of view a major limitation of the uptake rate measurements in the isolated perfused organs or in vivo experiments using the arteriovenous difference of concentration is the assumption that the metabolite under consideration is taken up by the cell in a unidirectional way, and thus there is no release of the substance from the cell into the circulation. In the liver, such a bidirectional flux occurs for unmodified simple molecules (e.g., glucose) or biotransformed compounds (bilirubin, drugs, etc.). As far as fatty acids are concerned, a bidirectional flux exists in adipocytes, but at present no data are available on an output of nonesterified fatty acids from the liver or other mammalian tissues. The liver cell quickly esterifies fatty acids, and some of them are excreted into the circulation as lipoprotein triglycerides, phospholipids, and, perhaps, cholesteryl esters.

Our present and previously published data support the view that in the isolated rat liver the outflow of NEFA from the cell is undetectable. If there is a secretion of NEFA into the perfusate, these fatty acids would decrease the arteriovenous difference of NEFA concentration and thus the measured uptake rate. Indeed, one interpretation of the results shown in Fig. 1 could be such a bidirectional flux of FFA. However, when the perfusate is depleted of NEFA by recirculation, the NEFA concentration remains stable after reaching a $\bar{v}$ close to 0.3. As the albumin concentration outside the cell membrane is high enough to accept larger amounts of fatty acids, a secretion of fatty acids would result in a rise of NEFA concentration in the perfusate after several circulations through the liver. However, the existence of two individual and poorly miscible pools of plasma NEFA is sustained by the lack of equilibration of radioactive fatty acid in vitro with the total NEFA pool (Tables 1 and 2) of nondelipidated serum albumin. In addition, when fatty acids are bound to delipidated albumin, a single, metabolically homogeneous pool is obtained (Table 4). In these experiments, the NEFA in the perfusate were randomly labeled with [^{14}C]palmitic acid. The NEFA uptake was identical using NEFA concentration or radioactivity at two different values of $\bar{v}$. Any NEFA secretion by the liver would be reflected by a dissimilarity between the uptake rate calculated by concentration vs. radioactivity arteriovenous differences. These results allow us to consider the existence of two different NEFA pools. A second interpretation of the experiments (Fig. 1) could be that some fatty acids have a dissociation rate from albumin that is much lower than others, and thus they are not exchangeable with the cells.

In fact, individual radioactive fatty acids complexed in vitro to albumin are taken up by the perfused rat liver at different rates (21), which are inversely related to the length of the hydrocarbon chain and directly related to the degree of unsaturation. The rate of uptake of palmitic acid, used in the present experiments, is similar to that of the major NEFA of human or rat plasma. The relatively lower uptake rate of higher ($> C_{20:0}$) saturated fatty acids cannot appreciably account for the nonexchangeable fraction of NEFA demonstrated in the experiments of Fig.

1 and Tables 3–6. One cannot exclude the possibility, however, that the pool having a slow turnover rate is rich in such fatty acids because the reasons for its existence are at present unclear.

It might be assumed that the heterogeneity of the turnover rate of NEFA in our experiments is a characteristic of human albumin vs. the uptake capacity of rat liver. However, the experiments shown in Tables 5 and 6 prove that the fatty acids bound in vivo to the circulating albumin in the rat have an identical behavior in the perfused liver or when injected intravenously into the intact rat. It should be noted that since the plasma injected in these experiments contains other radioactive lipids (associated with lipoprotein), a contribution of radioactive fatty acids from this source to the studied NEFA fraction can be excluded.

On the basis of the present experiments it could be reasonably inferred that the plasma NEFA include two pools of fatty acids with distinct fractional turnover rates. The physicochemical basis of this heterogeneity might be a difference in the type of bond between some of the albumin molecules and the fatty acids. Though no direct evidence for this assumption is available, the heterogeneity of the plasma albumin has been broadly demonstrated by chromatographic techniques (22–24), thermal denaturation (25), and acid isomerization (26). Recently, Spencer and King (27) demonstrated differences in the isoelectric points of albumin molecules, in both human and bovine plasma, but they were unable to find any difference for binding octanoate.

A difference in the binding of some molecules of albumin to fatty acid is supported by the work of Chen (15), who showed that unless the pH is as low as 3.0 a fraction of the albumin-bound NEFA remains after it is delipidated by charcoal. He suggests that at low pH a modification in the structure of the albumin chain makes accessible fatty acid molecules that at physiological pH are masked by the structure of the albumin chains.

The duality of the plasma NEFA pools may explain the difference in uptake of in vitro complexed radioactive fatty acids and fatty acids bound in vivo. The knowledge of the plurality of NEFA pools is important in the interpretation of data obtained in turnover studies using radioactive fatty acids. When the NEFA to albumin molar ratio is lowered, the relative proportion of low turnover rate pool vs. fast turnover rate pool is increased; thus, calculations of fatty acid turnover rates in vivo may be erroneous.

Basso and Havel (28) have reported that, in the dog, the uptakes of NEFA and [1-^{14}C]palmitic acid complexed to albumin administered by continuous intravenous perfusion are identical. These results conflict with our data from rat liver. These authors, however, assume that the hepatic artery contributes 20% of the total blood flow through the liver, and they point out that if the actual contribution of the hepatic artery is 33% their [1-^{14}C]palmitic acid uptake figure would rise by 10%.

As the present work is the first experimental evidence of the existence of a slow metabolic NEFA pool, further studies are necessary in order to elucidate its physicochemical basis and its physiological significance.

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