THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 259, No. 7, Issue of April 10, pp. 4295–4300, 1984
Printed in U.S.A.

Diurnal Variation of Cytosolic Fatty Acid-binding Protein Content and of Palmitate Oxidation in Rat Liver and Heart*

(Received for publication, August 31, 1983)

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Delipidated proteins from albumin-free liver and heart cytosol obtained from rats sacrificed at the mid-dark or the mid-light phase of the light cycle were assayed for their palmitate-binding capacity. In both tissues a marked variation of this binding capacity was observed from about 3–4 nmol/mg of protein in the mid-light phase of the cycle to about 7–8 nmol/mg of protein in the mid-dark phase. Sephadex G-75 chromatography of the cytosolic proteins revealed that the palmitate binding could in all cases almost entirely be attributed to proteins of M_r = 12,000–14,000, suggesting that the observed diurnal variations are related to differences in the content of fatty acid-binding protein (FABP). In both rat liver and heart FABP represents about 4 (mid-light) to 8% (mid-dark) of the total soluble proteins. Cholestyramine feeding increased the FABP content of liver cytosol from rats sacrificed at the mid-light phase, but not in those sacrificed at the mid-dark phase, in such a way that the diurnal variation of the FABP content virtually disappeared. The palmitate oxidation capacity and citrate synthase activity also exhibited a concomitant diurnal periodicity in rat liver and, to a lesser extent, in rat heart. The results provide additional evidence for an important role of FABP in cellular fatty acid metabolism in both liver and heart and for the similarity of FABP with sterol carrier protein.

FABP,[1] which is found in the cytosol of many eucaryotic cells, exhibits a high affinity for long chain fatty acids and their corresponding coenzyme A thioesters and, therefore, is considered to function in the intracellular fatty acid utilization (1, 2). The rather high concentration of this M_r = 12,000 protein in liver (3) suggests that it might also serve for storage of fatty acids and their derivatives. In liver and intestine, modulations of the rates of fatty acid uptake or utilization, in response to nutritional, hormonal, and pharmacological manipulations, correlated with changes in the cytosolic content of FABP (4–9). There is a growing amount of evidence that FABP is similar, if not identical, to SCP_1 in physical and molecular properties and in tissue distribution (10, 11). SCP_1 is required for activation of membrane-bound enzymes catalyzing cholesterol synthesis and metabolism to bile acids and steroid hormones (10, 12–14). Recently Dempsey *et al.* (15) reported that in liver SCP_1 undergoes a significant diurnal variation corresponding in time to that of major enzymes in lipid metabolism, such as hydroxymethylglutaryl-CoA reductase (16, 17) and methyl sterol oxidase (18). The hepatic SCP_1 content varied from 1 to 10% of the total cytosolic protein during a 24-h light-dark cycle and is probably regulated at the level of translation of SCP_1-mRNA (19).

In the present report we show that rat heart cytosol contains a previously unrecognized fatty acid-binding capacity, which is similar to that of liver cytosol, and, furthermore, that this capacity exhibits a diurnal rhythm in both heart and liver, being about 2-fold higher at the mid-dark than at the mid-light phase of the light cycle. In concert, the palmitate oxidation capacity and the citrate synthase activity were significantly elevated during the dark period in both tissues. The results provide further evidence for a potential role of FABP in cellular fatty acid utilization and for its similarity with SCP_1. For the assay of fatty acid binding by cytosolic proteins, we applied a recently developed radiochemical procedure in which after equilibration unbound and protein-bound fatty acids are effectively separated with the aid of Lipidex 1000 at 0 °C (20). This assay appears to be more useful and sensitive than measurement of coelution of labeled fatty acids with specific protein fractions from a gel filtration column (20).

EXPERIMENTAL PROCEDURES

Materials—Lipidex 1000 was purchased from Packard Instrument Co.; [1-^{14}C]palmitic acid and $^{63}NiCl_2$ from Amersham International, U.K.; silicic acid from Mallinckrodt Chemical Works; and bovine serum albumin (fraction V) and calibration proteins from Sigma. Albumin was freed of fatty acids by Lipidex chromatography at 37 °C (21), dialyzed, and lyophilized at neutral pH. Protein A-Sepharose CL-4B was supplied by Pharmacia Fine Chemicals, Uppsala, Sweden, and rabbit anti-rat albumin antiserum by Nordic Immunology, Tilburg, The Netherlands. L-Carnitine was a gift from Sigma Tau, Rome, Italy. All other chemicals were of the purest grade available.

Preparation of Cytosol—Male albino Wistar rats, weighing 180–220 g, were maintained on a cycle of alternating 12-h periods of light and darkness and were sacrificed at the midpoint of the light or of the dark period. Their feeding conditions and the preparation of the cholestyramine-containing diet were exact as described previously (22). Rats were anesthesized with diethyl ether and after severing the vena cava inferior, the liver was perfused *in situ* through a catheter in the vena portae for 2 min with ice-cold buffer, consisting of 100 mM sucrose, 50 mM KCl, 30 mM EDTA, and 50 mM K phosphate (pH 7.2). Thereafter, a 4-g piece of the left major liver lobe was removed. Rat hearts were similarly perfused through a catheter inserted directly into the right ventricle with the perfusate emerging through the cut cerebral arteries and subsequently entirely removed. After excision the tissues were immediately cooled in the buffer given above or in a buffer consisting of 0.25 M sucrose, 2 mM EDTA, and 10 mM Tris-HCl (pH 7.4). A 25% (w/v) homogenate was prepared in

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[1] The abbreviations used are: FABP, fatty acid-binding protein; SCP, sterol carrier protein.

the same buffer by hand homogenization at 0 °C using a Teflon-glass Potter-Elvehjem tissue homogenizer. The homogenate was centrifuged for 10 min at 600 × *g* and 4 °C, and the resulting supernatant was centrifuged for 90 min at 105,000 × *g* and 4 °C in a Beckman L2-65 B ultracentrifuge, using rotor 50 Ti. The clear 105,000 × *g* supernatant (cytosol) was aspirated so as to minimize contamination by floating fat and stored at −20 °C. Protein content was determined with the method of Lowry *et al.* (23), using bovine serum albumin as standard.

The amount of residual albumin present in the liver cytosol preparations was found to be negligible, as judged by crossover electrophoresis against rabbit anti-rat albumin antiserum. The heart cytosol preparations, however, initially still contained some albumin, which was removed by passage at 4 °C through an affinity column of the immunoglobulin G fraction of rabbit anti-rat albumin antiserum bound to protein A-Sepharose CL-4B. The complete removal of albumin after this treatment was confirmed by both crossover electrophoresis and immunoelectrophoresis.

Delipidation of Protein Samples and Assay of Fatty Acid Binding—For both delipidation and assay of fatty acid-binding activity of cytosolic proteins we used Lipidex 1000 (a 10% (w/v) substituted hydroxyalkoxypropyl derivative of Sephadex G-25), which effectively removes unbound as well as protein-bound fatty acids from aqueous solutions in a temperature-dependent manner according to the protein-lipid interaction kinetics (20) (Fig. 1). For delipidation, portions of about 30 mg of protein were subjected to chromatography on a column of Lipidex (1 × 15 cm), equilibrated with 10 mM K phosphate buffer (pH 7.4) at 37 °C. Elution was performed with the same buffer (flow rate, 20 ml/h). All protein was present in the void volume.

For assay of fatty acid binding, protein samples were incubated in 1.5-ml polyethylene vials in 10 mM K phosphate buffer (pH 7.4) with various concentrations of [1-^{14}C]palmitate. The final volume was 0.45 ml. After incubation for 10 min at 37 °C the vials were cooled in ice. Unbound fatty acids were removed from the solution by adding 0.05 ml of an ice-cold Lipidex/buffer suspension (1:1, v/v) and incubation for 10 min at 0 °C. Fatty acid binding was calculated from the amount of radioactivity present in the supernatant after centrifugation of the vials and is expressed as picomoles/μg of protein. Further details of these procedures and properties of the assay are described elsewhere (20).

Fatty Acid Content Determination—For determination of the endogenous free fatty acid content, samples of 20–40 mg of cytosolic protein were subjected to lipid extraction according to Dole and Meinertz (24). Phospholipids were removed from the free fatty acids by binding to silicic acid. Thereafter, fatty acids were assayed radiochemically using ^{63}Ni as tracer (25). With standard solutions of palmitic acid in heptane we found a fatty acid to Ni ratio of 1.95 ± 0.07 (mean ± S.D. of 4 determinations), which corresponds to the formation of a Ni (fatty acid)$_2$ complex (25). The recovery of fatty acids from the extraction procedure was examined by adding a known amount of palmitic acid in heptane solution to the extraction mixture containing the protein sample and was found to be 85–95%.

Assay of Palmitate Oxidation and Citrate Synthase Activity—Rats were sacrificed by cervical dislocation. Liver and heart were rapidly excised and homogenized in 19 volumes of an ice-cold buffer, consisting of 0.25 M sucrose, 2 mM EDTA, and 10 mM Tris-HCl (pH 7.4). The palmitate oxidation rate was measured in a final volume of 0.5 ml of medium, containing 25 (heart) or 50 μl (liver) whole homogenate and 25 mM sucrose, 30 mM KCl, 10 mM potassium phosphate, 5 mM $MgCl_2$, 1 mM EDTA, 75 mM Tris-HCl (pH 7.4) supplemented with 5 mM ATP, 1 mM NAD^+, 25 μM cytochrome *c*, 0.1 mM coenzyme A, 0.5 mM L-malate, and 0.5 mM L-carnitine. Incubations were started by the addition of 120 μM [1-^{14}C]palmitate bound to fatty acid-free albumin (molar ratio 5:1). The oxidation proceeded for 30 min at 37 °C and was stopped by the addition of 0.2 ml of 3 M perchloric acid. Measurement of radioactivity of trapped CO_2 and of acid-soluble products and further details of the assay procedure were previously described (26).

Citrate synthase activity was assayed in the 10,000 × *g* supernatant of sonicated homogenates according to Shepherd and Garland (27). One unit of enzyme is defined as that amount which, under assay conditions, catalyzes the liberation of 1 μmol of coenzyme A per min at 25 °C.

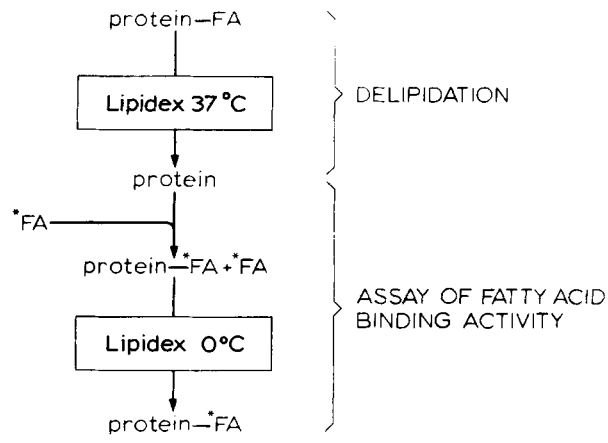

FIG. 1. **Schematic presentation of the use of Lipidex 1000 at 37 °C for the delipidation of protein samples and at 0 °C for the separation of protein-bound and unbound fatty acids.** *FA*, endogenous fatty acid; **FA*, labeled fatty acid.

RESULTS

Palmitate Binding by Cytosolic Protein—The binding of palmitate increased proportional to the amount of protein at both 0.2 and 1 μM [1-^{14}C]palmitate (up to the binding of about 50–60% of the available palmitate), only when delipidated protein samples were used (Fig. 2). With cytosol preparations that had not been delipidated a nonlinear relationship was observed, and at a higher amount of protein the palmitate binding was underestimated to an increasing extent. This underestimation is due to label dilution by endogenous fatty acids and possibly also to competition by endogenous ligands, other than fatty acids, which may be associated with cytosolic FABP (1, 28, 29). This competition is more pronounced at higher protein and lower palmitate concentrations (Fig. 2). Palmitate binding did not decrease after storage of the protein samples at −20 °C (up to 6 months).

With the delipidated cytosol of liver and heart, obtained from rats sacrificed at the mid-dark or at the mid-light phase of the light cycle, we studied the binding of palmitate by cytosolic protein as a function of the total palmitate concen-

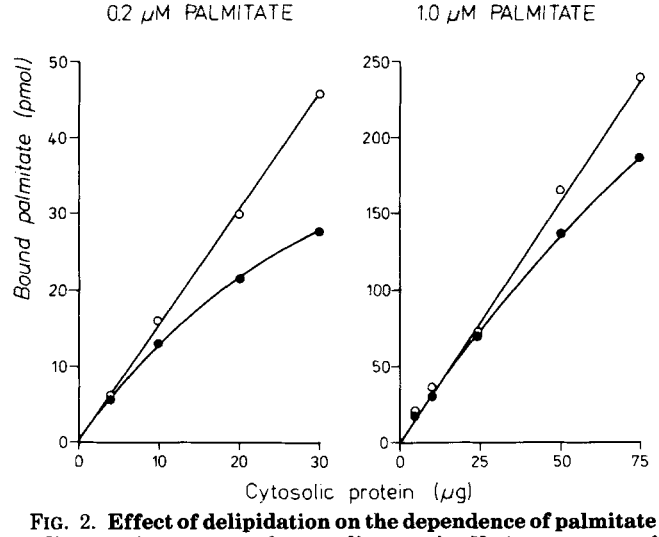

FIG. 2. **Effect of delipidation on the dependence of palmitate binding on the amount of cytosolic protein.** Various amounts of nondelipidated (●) and of delipidated (○) liver cytosolic protein were assayed for palmitate binding in the presence of 0.2 (*left*) and 1.0 μM (*right*) [1-^{14}C]palmitate, as described under "Experimental Procedures." The results are shown of one representative experiment out of three, using the liver cytosol obtained from rats sacrificed at the mid-dark phase of the light cycle. With cytosol preparations from rat heart similar results were obtained (data not shown).

tration. Scatchard plot analysis of the binding isotherms revealed the presence of a single class of saturable binding sites on the cytosolic proteins from both tissues (Figs. 3 and 4). At the mid-dark phase both the liver and heart cytosol contain a significantly higher capacity for palmitate binding per μg of protein than at the mid-light period (Figs. 3 and 4; Table I). With both types of cytosol preparations, however, half-maximal saturation is reached at the same palmitate concentration (1 and 0.8 μM for rat liver and heart, respectively). These findings suggest that at the mid-dark phase the liver and heart cytosol contain 1.8- and 2.3-fold more binding sites, respectively, but on the same molecular species. Since at the mid-dark phase of the light cycle the rat liver weight is about 1.5-fold higher than at the mid-light phase (30), the total amount of fatty acid-binding sites expressed per whole liver even shows a 2.7-fold diurnal variation.

When the cytosolic proteins were fractionated by chromatography on Sephadex G-75, the major fatty acid-binding fraction eluted at the same position with all preparations examined (Fig. 5). This fraction corresponds to M_r = 12,000–14,000 and contains FABP (3, 31). The specific palmitate

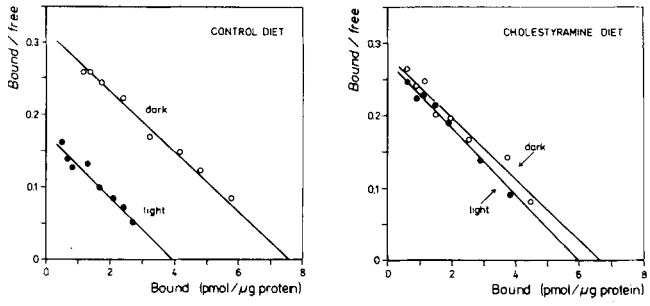

FIG. 3. **Scatchard analysis of the binding of palmitate by the delipidated liver cytosol from control (*left*) and cholestyramine-fed (*right*) rats sacrificed at the mid-dark (○) or at the mid-light (●) phase of the light cycle.** Cytosolic protein samples were incubated with 0.1–3 μM [1-^{14}C]palmitate and after equilibration protein-bound and unbound palmitate were separated by the use of Lipidex at 0 °C, as described under "Experimental Procedures." The results are shown of representative experiments, in which 20 (control diet) and 18 μg (cholestyramine diet) of protein were used per assay.

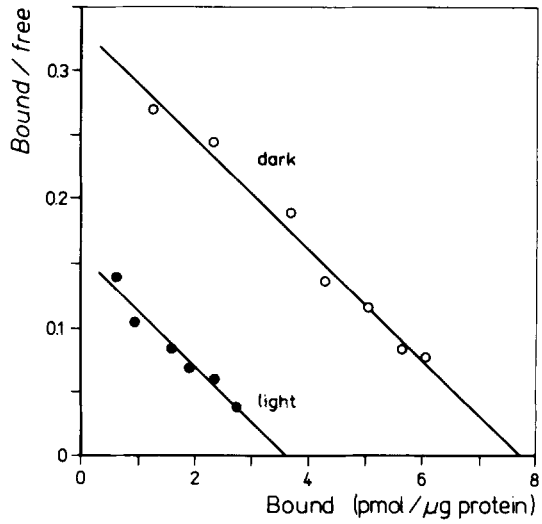

FIG. 4. **Scatchard analysis of the binding of palmitate by the dealbuminized and delipidated heart cytosol from rats sacrificed at the mid-dark (○) or at the mid-light (●) phase of the light cycle.** The results are shown of representative experiments, using 18 μg of protein per assay. For further experimental details, see the legend of Fig. 3.

TABLE I

Diurnal variation of palmitate binding by cytosolic protein and of endogenous fatty acid content of the cytosol in rat liver and heart

Data of palmitate binding are derived from Scatchard analysis of individual binding isotherms, as shown in Figs. 3 and 4. The amounts of delipidated protein used per assay were 15–25 μg. For determination of the endogenous fatty acid content, fatty acids were extracted from 20–40 mg of the cytosolic protein samples and assayed radiochemically with the use of ^{63}Ni as tracer, as described under "Experimental Procedures." For comparison the fatty acid content is expressed as picomoles/μg of protein. All values represent means ± S.D. of the number of separate preparations given within parentheses. Values are compared by the unpaired *t* test.

Tissue	Diet	Condition	Palmitate binding		Endogenous free fatty acid content
			B_{max}	Apparent K_d	
			pmol/μg protein	*μM*	*pmol/μg protein*
Liver	Control	Light (3)	3.92 ± 0.38	1.04 ± 0.15	1.47 ± 0.25
		Dark (3)	7.20 ± 0.39[a]	0.98 ± 0.14	1.68 ± 0.53
	Cholestyramine	Light (7)	6.03 ± 0.67[b]	1.06 ± 0.22	1.81 ± 0.23
		Dark (4)	6.79 ± 0.48	0.90 ± 0.10	1.37 ± 0.53
Heart	Control	Light (4)	3.49 ± 0.39	0.83 ± 0.11	1.91 ± 0.22
		Dark (3)	8.02 ± 0.70[c]	0.80 ± 0.05	2.87 ± 0.18[a]

[a] $p < 0.01$, dark *versus* light.
[b] $p < 0.001$, cholestyramine diet *versus* control diet (light period).
[c] $p < 0.001$, dark *versus* light.

binding activity of this fraction was also 1.5- to 2-fold higher in cytosol preparations from rats sacrificed at the mid-dark compared to the mid-light phase, amounting to 28–36 and 20–23 pmol/μg of protein, respectively, for the liver cytosol and to 10–12 and 6–8 pmol/μg of protein, respectively, for the heart cytosol. The specific binding capacities are under both conditions lower in heart than in liver, due to the relatively higher content of low M_r proteins (*e.g.* myoglobin) of heart muscle (Fig. 5). The small fatty acid-binding fraction of M_r = 1500–2000, which contains a fatty acid-binding peptide (32–34), did not show a significant diurnal rhythm (Fig. 5). The presence of such a peptide in heart cytosol was not earlier reported. The absence of albumin in our preparations is also confirmed by a very low palmitate binding activity of the fractions that correspond to its elution position (Fig. 5). These results show that the higher palmitate binding capacity per μg of protein of both the rat liver and heart cytosol during the dark relative to the light period can be attributed to a higher content of FABP or to an increased availability of the fatty acid-binding site(s) on this protein. The former possibility is most likely since modulation of the hepatic content of FABP is known to occur in several conditions involving nutritional, hormonal, and pharmacological manipulations (4–9), whereas no evidence is available on the regulation of the availability of the fatty acid-binding site(s).

By comparison of the maximal fatty acid binding by rat liver cytosolic proteins with the cytosolic FABP concentration as found by Ockner *et al.* (3) on the basis of quantitative radial immunodiffusion studies, we established earlier (20) that FABP possesses only one fatty acid-binding site per protein molecule. As FABP accounts for 75–85% of the fatty acid binding of our cytosol preparations (Fig. 5) and has a M_r of 12,000 (3), the cytosolic FABP concentration can be estimated from the maximal fatty acid binding (Table I). It appears that in liver the cytosolic FABP concentration amounts to about 40 and 70 μg/mg of protein at the mid-light and the mid-dark phase of the light cycle, respectively, and in heart to about 35 and 80 μg/mg of protein, respectively.

Effect of Cholestyramine Feeding—In a previous study (35)

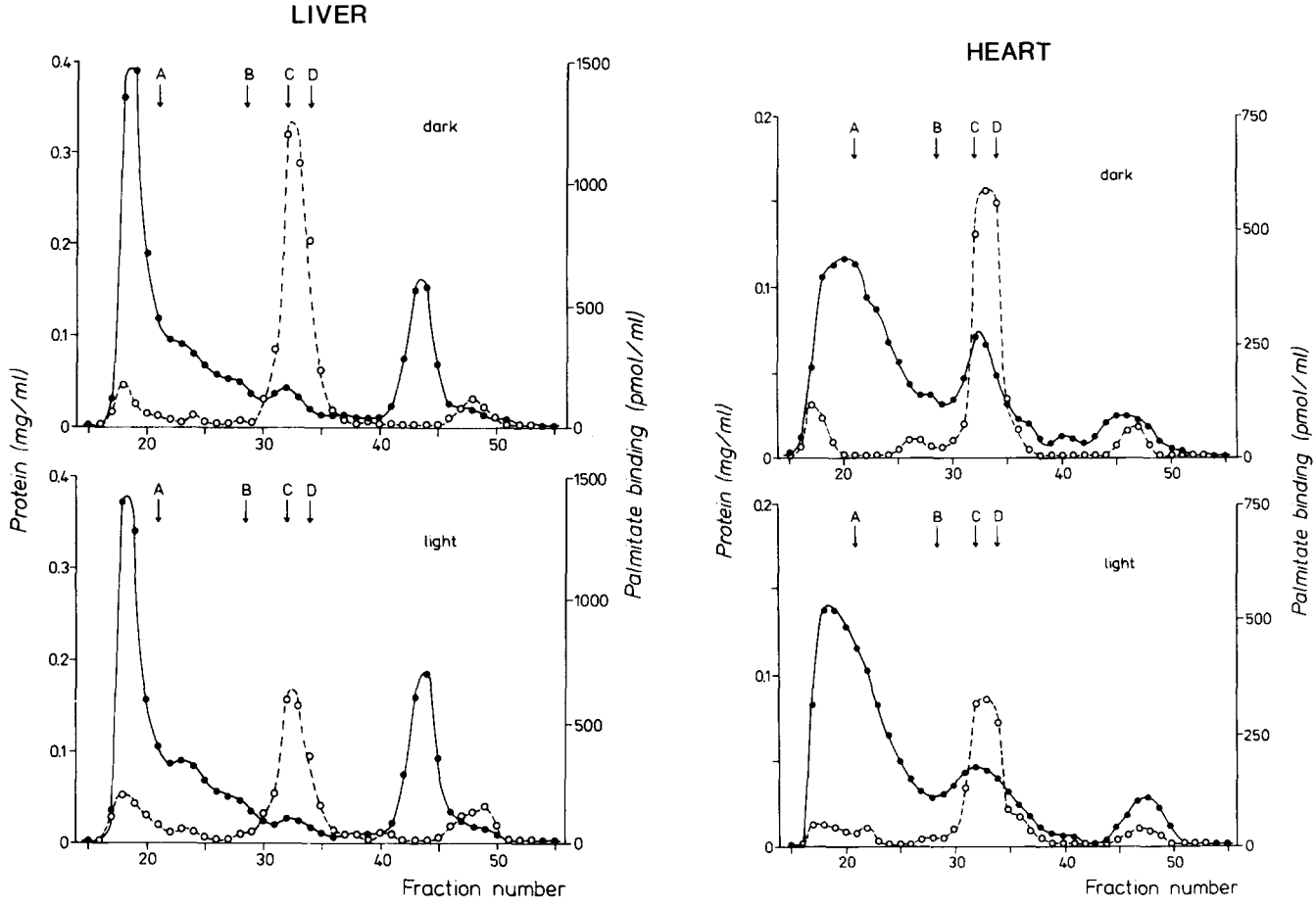

FIG. 5. **Gel filtration on Sephadex G-75 (1.6 × 85 cm) of 10 and 5 mg of delipidated cytosolic protein from liver (*left*) and heart (*right*), respectively, of rats sacrificed at the mid-dark or at the mid-light phase of the light cycle.** Elution was performed at 4 °C with 10 mM K phosphate buffer (pH 7.4). The fraction volume was 3.7 ml. All fractions were assayed for protein (●) and for palmitate binding (○). For the latter 50- to 200-μl samples and 1 μM [1-^{14}C]palmitate were used. *Arrows* indicate the elution positions of calibration proteins: *A*, rat serum albumin; *B*, chymotrypsinogen; *C*, horse skeletal muscle myoglobin; and *D*, horse heart cytochrome *c*. The recoveries of protein as well as of fatty acid-binding activity from the column were 85–95%. The results are shown of a representative experiment out of three.

we observed that feeding rats with a diet containing the bile salt sequestrant cholestyramine is associated with a 1.5-fold increase of fatty acid binding by hepatic cytosolic proteins, which could be attributed to an elevation of the content or binding activity of FABP. For that study rats were sacrificed during the light period. Because of the present evidence for the existence of a diurnal rhythm of the FABP content, it was of interest to investigate if this rhythm is affected by cholestyramine feeding. As shown in Fig. 3 and Table I, cholestyramine feeding did not further increase the palmitate-binding capacity of the liver cytosol at the mid-dark phase, so that with this diet there is no significant diurnal variation of hepatic FABP activity. It is plausible that the levels of palmitate-binding capacity reached during the dark period or during cholestyramine feeding represent a maximum.

Endogenous Fatty Acid Content—Since endogenous free fatty acids are considered to be mainly present associated with FABP, we also examined the possible diurnal variation of the fatty acid content of liver and heart cytosol. In liver the cytosolic fatty acid content did not differ between the dark and the light period nor between cholestyramine-fed and control animals and amounted to 1.4–1.8 nmol/mg protein (Table I). Burnett *et al.* (4) also reported a fatty acid content of 1.4 nmol/mg of protein for liver cytosol of rats killed during the light period. The cytosolic fatty acid content of heart was at the mid-light phase of the cycle comparable to that of liver, but significantly higher at the mid-dark phase (Table I). Comparison of these values with the palmitate-binding capacity of the various preparations (Table I) reveals that under all conditions examined the degree of occupancy

TABLE II

Diurnal variation of palmitate oxidation and citrate synthase activity in rat liver and heart

Oxidation rates were measured with 120 μM [1-^{14}C]palmitate bound to albumin (molar ratio 5:1) and were calculated from the sum of the production of $^{14}CO_2$ and ^{14}C-labeled acid-soluble products. Values represent means ± S.D. of the number of preparations given within parentheses. Values are compared by the unpaired *t* test.

Tissue	Condition	Palmitate oxidation		Citrate synthase activity
		Per g, wet weight	Per unit of citrate synthase	
				unit/g wet weight
Liver	Light (5)	366 ± 28	31.7 ± 3.1	11.6 ± 0.4
	Dark (7)	508 ± 39[a]	32.5 ± 1.9	15.6 ± 1.1[a]
Heart	Light (7)	733 ± 47	10.04 ± 1.03	71.6 ± 4.9
	Dark (7)	824 ± 31[b]	8.88 ± 0.63	93.0 ± 5.9[a]

[a] $p < 0.001$, dark *versus* light.
[b] $p < 0.01$, dark *versus* light.

of the available binding sites did never exceed 50%. In liver, the degree of occupancy drops to about 20% at the mid-dark period of the light cycle.

The yield of cytosolic protein amounted to 25–35 and 15–20 mg/g of tissue, wet weight, for rat liver and heart, respectively, so that at the mid-light phase the endogenous fatty acid content is about 40–55 and 30–40 nmol/g of tissue, wet weight, respectively. For heart this value is of the same order of magnitude as the nonesterified fatty acid acid concentration reported by others (36–38).

Palmitate Oxidation and Citrate Synthase Activity—With homogenates of rat liver and heart palmitate oxidation proceeded proportional with time of incubation and amount of tissue material. The oxidation was measured at concentrations of substrate, coenzymes, and cofactors that gave maximal oxidation rates (39). In both liver and heart the palmitate oxidation capacity, expressed per g of tissue, was significantly increased at the mid-dark compared to the mid-light phase of the light cycle (Table II). The citrate synthase activity showed a similar increase in both tissues, so that when the oxidation rates are expressed relative to this mitochondrial marker enzyme, hardly any diurnal changes are observed (Table II). These results indicate that the diurnal fluctuations of the palmitate oxidation capacity are related to fluctuations of total mitochondrial activity in both tissues.

DISCUSSION

Both immunochemical and nonimmunochemical methods have been applied for the estimation of the total fatty acid-binding capacity of the cytosol or the quantitation of FABP (*cf.* Refs. 3 and 9). The former procedure requires the availability of precipitating antibodies reactive to all forms of FABP that may be present in the cytosol. There is now evidence, however, for molecular heterogeneity of FABP in liver (29, 40–43). The various forms may exhibit a different affinity for long chain fatty acids (*cf.* Refs. 29 and 41) and show differences in antigenicity (41, 42). A nonimmunochemical method, such as the assay used in this study, may be of more physiological interest, since it provides a functional quantitation of the cytosolic fatty acid-binding activity. Several other laboratories recently also presented alternative procedures for measuring fatty acid binding by proteins. Morrow and Martin (42) describe a method based on the use of dextran-gelatin-coated charcoal for the separation of unbound and protein-bound fatty acids. However, since the charcoal also exhibits binding properties for protein, in contrast to Lipidex (20), their assay is less sensitive and accurate than our method. An electrophoretic (44) and a modified equilibrium dialysis (45) technique were also presented, but both procedures appear more complicated and time consuming than our assay.

Few laboratories have to date reported on FABP of heart cytosol (2, 28, 31, 46), although its presence in heart muscle was established already in the first report on FABP (1). Quantitative data on cardiac FABP have only been presented in two papers (2, 28). Mishkin *et al.* (2) reported that the relative amount of oleic acid that coelutes with the FABP-containing fraction upon gel filtration of cytosolic proteins was for rat heart about 30% of that of rat liver. This comparison is, however, of only limited value since the preparations may have contained different amounts of albumin and, as delipidation was omitted, of endogenous ligands, and since the applied assay method is rather inaccurate due to the high affinity of the fatty acids for the gel itself. Rüstow *et al.* (28) used an antibody raised against purified rat liver FABP and estimated that rat heart cytosol contains a 10-fold lower amount of FABP than liver cytosol. The former amount has likely been underestimated since the physical and molecular properties of FABP from rat heart and liver are different[2] and immunochemical cross-reactivity might, therefore, be low. Myoglobin has also been suggested to act as a fatty acid-binding protein in muscle (47), but this appears unlikely because of its low fatty acid-binding affinity (20) and the fact that upon ion exchange chromatography of rat heart cytosol FABP (M_r = 12,000) is completely separated from myoglobin (31).[2] Using albumin-free and delipidated rat heart cytosol preparations we found with our assay a similar palmitate-binding capacity as in liver cytosol in both phases of the light cycle. As the palmitate binding could almost entirely be attributed to proteins of M_r = 12,000–14,000, it was calculated that FABP will make up about 4–8% of the rat heart-soluble proteins. This abundance of FABP as well as its known properties support the view that also in heart muscle this protein plays an important regulatory role in lipid metabolism. In heart FABP may act as a protector for the detrimental effects of high intracellular levels of long chain fatty acids and fatty acyl-CoA thioesters that occur during ischaemia and hypoxia (48).

The apparent dissociation constant of palmitate binding by liver cytosolic protein of about 1 μM is identical to that observed with equilibrium dialysis studies (29), but is 3-fold lower than that reported for oleate on the basis of gel filtration experiments (2). With equilibrium dialysis, the total fatty acid-binding capacity of liver cytosol was found to be only 0.1 pmol/μg of protein (29). Purified FABP from pig heart showed an apparent dissociation constant for palmitate of 0.85 μM, as measured by electron spin resonance spectroscopy (46). This value is similar to that for rat heart cytosol (Table I).

The simultaneous rise of the capacities of cytosolic fatty acid binding and of palmitate oxidation during the dark relative to the light period in both liver and heart suggests a correlation between these parameters, as was also found in some other conditions. The FABP concentration in cytosol from livers of female and male rats does not only show a strong correlation with the rate of triacylglycerol biosynthesis (3, 9), but also corresponds to differences in oleate oxidation by hepatocytes obtained from these rats (9). In addition, the 2-fold rise of the hepatic FABP concentration observed after clofibrate administration (6, 7, 49) is also paralleled by a 2.5-fold rise of the palmitate oxidation capacity (50). During diabetes and starvation, however, the markedly increased hepatic fatty acid oxidation (50, 51) is accompanied by a decrease of the hepatic FABP level, but also by an appearance of a fatty acid-binding component of M_r = 400,000 (52). We did, however, not find evidence for the presence of such a component during the dark period. In order to give a better perspective to the functional significance of the diurnal variation in cytosolic FABP content, the possibility of a diurnal rhythm in triacylglycerol biosynthesis should also be explored.

The 2-fold diurnal variations of the cytosolic FABP content in liver and heart (Table I)[1] are smaller than the 10-fold diurnal fluctuation found for SCP_1 in liver (19). However, the maximum levels are of comparable magnitude, amounting to 70–80 (FABP) and about 100 (SCP_1; Ref. 19) μg/mg of protein. Feeding rats a cholestyramine-containing diet results in a comparable increase of the FABP content of liver cytosol as those of SCP_1 and SCP_2 (53), when the rats are sacrificed during the light period. Furthermore, Dempsey *et al.* (15) found an increase of similar size in SCP_1 level induced by partial ileal bypass, a manoeuvre also resulting in breaking the bile salt enterohepatic circulation. The virtual disappearance of the diurnal rhythm of the amount of FABP upon cholestyramine administration may relate to the finding that

[2] J. F. C. Glatz, C. C. F. Baerwaldt, A. M. Janssen, and J. H. Veerkamp, unpublished observations.

the amplitude of the diurnal rhythm of hepatic cholesterogenesis was markedly decreased after cholestyramine feeding (54). All these results are compatible with the idea that FABP and SCP_1 are closely related or identical proteins, as suggested already by others (10, 11).

In heart, the diurnal fluctuation of the cytosolic content of FABP is accompanied by a change in that of endogenous fatty acids. In liver, we did not observe such a combination, as the cytosolic fatty acid content did not differ between the dark and the light period. This may be due to the binding by FABP of other lipidic compounds (28, 29). At present it is still unclear if mono-, di-, and triacylglycerols and cholesterol are also endogenously associated with FABP. Studies of Rüstow *et al.* (28) revealed their presence but others found that FABP does not bind these ligands (1, 2).

Diurnal changes of palmitate oxidation capacity and of citrate synthase activity, as we observed in liver and to a lesser extent also in heart, were to our knowledge not yet earlier reported. A diurnal variation of the triacylglycerol and free fatty acid content of total rat heart has been found (55), but with maximal levels at the very beginning of the dark period and no differences between the mid-dark and mid-light levels. Hepatic fatty acid synthesis is known to show a diurnal rhythm with a peak activity during the dark period (56). Since the nocturnal feeding habit of the rat may be important in relation to the observed diurnal variations, we have started studies to determine the effect of fasting on the diurnal changes of FABP content and palmitate oxidation. The suggested correlation between the fatty acid oxidation capacity and the FABP content and the demonstration that FABP-bound fatty acids can be transferred to the mitochondrial β-oxidative system (31) may indicate that FABP participates in the regulation of fatty acid oxidation. The high fatty acid-binding capacity in both liver and heart suggests that further studies on the role of FABP in these metabolically different tissues are of much interest.

Acknowledgments—We thank Professor Dr. M. E. Dempsey (University of Minnesota, Minneapolis, MN) for stimulating discussions and Maria Van Meijel (Department of Paediatrics, University of Nijmegen; head, Dr. P. J. J. Van Munster) for carrying out the crossover electrophoresis and immunoelectrophoresis experiments.

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J. Biol. Chem. 1984, 259:4295-4300.