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Synthesis of lipoprotein lipase in the liver of newborn rats and localization of the enzyme by immunofluorescence

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In newborn rats, lipoprotein lipase (LPL) activity was higher in the liver than in several other tissues, such as heart, diaphragm or lungs, and accounted for about 3% of total LPL activity in the body. There was no significant correlation between LPL activity in liver and in plasma. Thus transport of the enzyme from extrahepatic tissues was probably not the major source of LPL in liver. To study LPL biosynthesis directly, newborn rats were injected intraperitoneally with [^{35}S]methionine, and LPL was isolated by immunoprecipitation and separation by SDS/polyacrylamide-gel electrophoresis. Radioactivity in LPL increased with a similar time course in all tissues studied, including the liver. Substantial synthesis of LPL was also demonstrated in isolated perfused livers from newborn rats, whereas synthesis was low in livers from adult rats. There was strong LPL immunofluorescence in livers from newborn rats, mainly within sinusoids and along the walls of larger vessels. This labelling disappeared after perfusion with heparin, which indicates that much of the enzyme is in contact with blood and can take part in lipoprotein metabolism.

INTRODUCTION

Lipoprotein lipase (LPL, EC 3.1.1.34) is the enzyme responsible for hydrolysis of triacylglycerols in plasma lipoproteins (Robinson, 1970). This reaction makes the fatty acids available for use in cellular metabolic processes, and is generally thought to take place in extrahepatic tissues where LPL displays high activity, e.g. heart and skeletal muscle, adipose tissue, lungs (Cryer, 1981; Nilsson-Ehle *et al.*, 1980). LPL is thus considered to be an extrahepatic enzyme, but significant amounts of LPL activity are also present in the liver (Chajek *et al.*, 1977; Llobera *et al.*, 1979; Grinberg *et al.*, 1985; Peterson *et al.*, 1985; Olivecrona *et al.*, 1986; Vilaró *et al.*, 1986; Semb *et al.*, 1987). Evidence suggests that in adult rats this LPL is not made in the liver, but originates in extrahepatic tissues. Evidence for this is that when labelled LPL is injected intravenously it is taken up by the liver (Wallinder *et al.*, 1979, 1984), and that LPL activity increases in both plasma and liver during starvation (Testar *et al.*, 1985; Peterson *et al.*, 1985), on treatment with cholera toxin (Knobler *et al.*, 1984) or with tumour necrosis factor (cachectin) (Semb *et al.*, 1987), and on infusion of a fat emulsion (Peterson *et al.*, 1985; Vilaró *et al.*, 1986) or of heparin (Peterson *et al.*, 1985). These observations have led to the view that the liver is a catabolic site for LPL and that avid uptake by the liver serves to keep the LPL concentration low in the circulating blood.

The possibility that LPL is synthesized in the liver has not been much considered. In a study on mice with the *cld/cld* mutation (for combined lipase deficiency), Olivecrona *et al.* (1985) reported incorporation of [^{35}S]methionine into immunoprecipitable LPL in liver. In these experiments *in vivo*, samples were obtained after labelling for 1 h. Thus newly synthesized LPL could have been transported to the liver, even though this appears less likely in the defective mice, since the defect severely impairs release of LPL from cells (Scow & Chernick, 1987).

The aim of the present study was to investigate more directly whether synthesis of LPL can take place in the liver, and to study the localization of the enzyme in the tissue. We used newborn rats for our study, since they have high LPL activity in their livers (Llobera *et al.*, 1979). For comparison we also studied rats at 5 and 15 days of age and adult rats.

MATERIALS AND METHODS

Animals

Sprague–Dawley rats (A-lab, Stockholm, Sweden) were used. At 2 h after delivery, newborns from each litter were divided into two groups. One group of rats were allowed to suckle for the first 8 h of life and were then separated from their mothers and kept at 37 °C for the following 16 h. At this time no milk remained in the stomach or intestine. These rats are designated 'fasted'. The other group of newborns (7–8 per litter) remained with their mother for the first 24 h of life, and are designated 'fed'. Other rats were kept with their mothers until killed at the age of 5 or 15 days. Adult rats weighed 180–200 g and were either fed, fasted for 48 h, or given Intralipid as previously described (Vilaró *et al.*, 1986).

Materials

Hypnorm Vet was from AB Leo, Helsingborg, Sweden. Heparin was from AB Lövens, Malmö, Sweden. Intralipid was from AB Kabi-Vitrum, Stockholm, Sweden. [^{35}S]Methionine, Enlighting and ^{14}C-labelled M_r markers were from NEN Products, Stockholm, Sweden. Tri-[9,10-^{3}H]oleoylglycerol was prepared by Dr. L. Krabisch, Department of Physiological Chemistry, Lund, Sweden.

Abbreviations used: FITC, fluorescein isothiocyanate; LPL, lipoprotein lipase; PAGE, polyacrylamide-gel electrophoresis; PBS, phosphate-buffered saline (0.02 M-sodium phosphate/0.15 M-NaCl, pH 7.4).

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Leupeptin and pepstatin were from The Peptide Institute Inc., Osaka, Japan. DEAE-Sephadex A-50 and CNBr-activated Sepharose were from Pharmacia, Uppsala, Sweden. Trasylol was from Bayer, Leverkusen, Germany. Lipoluma and Lumasolve were from LKB, Stockholm, Sweden. Phenylmethanesulphonyl fluoride was from Serva Feinbiochimica, Heidelberg, Germany. From Sigma Chemical Co., St. Louis, MO, U.S.A., were Tris, Hepes, bovine serum albumin (fatty acid-free, fraction V), bovine insulin, methionine, glutamine, glycine, glutaraldehyde, paraformaldehyde and Coomassie Blue. FITC-conjugated rabbit anti-chicken IgG was from Nordic, Tilburg, The Netherlands. All other chemicals were of the highest commercially available quality.

Antisera

For immunoprecipitation, immunofluorescence and immunoinhibition we used an antiserum against bovine milk LPL raised in a chicken as previously described (Olivecrona & Bengtsson, 1983). Antiserum against hepatic lipase was raised in a rabbit by using hepatic lipase purified from heparin perfusates of rat liver (Wallinder *et al.*, 1984) as antigen. This antiserum at 1:1 dilution fully inhibited the endogenous hepatic lipase activity present in liver or plasma samples. Antiserum against chicken IgG was raised in a goat. IgG was purified from this antiserum by precipitation with Na_2SO_4, followed by chromatography on DEAE-Sepharose A-50 (Garvey *et al.*, 1977) and was coupled to CNBr-activated Sepharose according to the manufacturer's instructions.

LPL assay

Fed or fasted newborn rats were killed by decapitation at 24 h of life. Blood (30–50 μl) was collected immediately from the neck into small tubes containing heparin, and centrifuged. Plasma was stored at 4 °C until it was used for assay, always within 2 h. Tissues were homogenized in 9 vol. of ice-cold solution containing heparin (5 i.u./ml), leupeptin (10 μg/ml), pepstatin (1 μg/ml), Trasylol (25 kallikrein units/ml), Triton X-100 (0.8%, w/v), SDS (0.04%, w/v) and EDTA (5 mM), pH 8.2 (buffer A). The homogenates were centrifuged for 10 min at 27000 *g* at 4 °C, and the clear supernatant under the floating fat layer was used for assay of LPL activity. To inhibit the activity of hepatic lipase, plasma and liver samples were first treated with antiserum to hepatic lipase. For this, 1 vol. of sample was mixed with 1 vol. of antiserum (or control serum), and the mixture was kept on ice for 2 h before the assay.

To prepare the substrate emulsion for assay of LPL (Peterson *et al.*, 1985), 200 μl of a benzene solution of tri[9,10-^{3}H]oleoylglycerol (approx. 5×10^8 d.p.m.; 8120 mCi/mmol) was dried under N_2 on the walls of a small glass vessel, and 2 ml of Intralipid was immediately added. The mixture was chilled in ice/water and sonicated with a Branson sonifier (standard tip, setting 4, for 8 min). The emulsion was stored at 4 °C and used during the next 4 days. In order to provide activator protein, 17 μl of heat-inactivated (56 °C for 10 min) rat serum was added to 10 μl of the labelled emulsion. When samples of rat plasma were to be assayed, the amount of serum was decreased correspondingly. Then 100 μl of a mixture containing 12% (w/v) bovine serum albumin, 300 i.u. of heparin/ml, 0.2 M-NaCl and 0.3 M-Tris/HCl (pH 8.5) was added. Then 20 μl samples were added. The total volume was adjusted to 200 μl with 0.9% (w/v) NaCl. Incubations were carried out in a shaking water bath for 30 min at 25 °C. Fatty acids were extracted and counted for radioactivity as described by Peterson *et al.* (1985). A milliunit of activity is defined as 1 nmol of fatty acid released/min.

Incorporation of [^{35}S]methionine *in vivo*

Fasted newborn rats were injected intraperitoneally with 1.25 mCi of [^{35}S]methionine in 10 μl of PBS, and killed by decapitation 15, 30, 45 or 60 min later. Tissues were excised and homogenized individually in 1 ml of cold buffer A. The homogenizer was rinsed out with 0.2 ml of buffer A and the homogenate was centrifuged for 10 min at 27000 *g*, as described above. To determine incorporation of methionine into total proteins, 10 μl portions of the homogenates were applied on Whatman GF/C filters, which were then dropped into ice-cold 10% (w/v) trichloroacetic acid. The filters were washed by boiling in 10% trichloroacetic acid for 10 min. After cooling, the solution was discarded and the filters were washed once with water, twice with methanol and twice with acetone. The filters were then air-dried and the precipitates were dissolved in a scintillation cocktail (Lipoluma/Lumasolve/water) and counted for radioactivity. The remaining homogenate was immunoprecipitated as described below.

Incorporation of [^{35}S]methionine during liver perfusion

Rats were anaesthetized with Hypnorm, and their livers were cannulated at the level of the superior cava vein in newborn and 5-day-old rats, and at the level of the portal vein in 15-day-old and adult rats. Immediately after cannulation, perfusion was started with buffer B [containing 136.8 mM-NaCl, 5.4 mM-KCl, 0.81 mM-$MgSO_4$, 0.98 mM-$MgCl_2$, 0.44 mM-KH_2PO_4, 1.33 mM-Na_2HPO_4, 1.3 mM-$CaCl_2$, 5.5 mM-glucose, 10 mM-Hepes and 1% albumin, pH 7.4, gassed with O_2/CO_2 (19:1)] at a flow rate of 3–4 ml/min per g of liver. The fluid was allowed to escape through a cut in the inferior cava vein at the level of the renal vein. While the liver was being washed out of residual blood in this open perfusion system, it was carefully excised, separated from non-hepatic tissue and placed in the perfusion chamber kept at 37 °C. For adult rats, the mass of liver perfused was decreased 3-fold by placing a ligature around the base of some of the lobules. The flow rate was immediately adjusted to the decreased weight. The whole process from laparotomy to the placing of the liver in the perfusion chamber did not take longer than 4 min. Within 10 min after the start of perfusion, all residual blood had been washed out. Then the perfusion medium was changed to buffer C (5 ml of Eagle's minimal essential medium modified with Earle's salts and 2 g of $NaHCO_3$/l without glutamine and methionine, and supplemented with 5% bovine serum albumin, 5 mM-glucose, 1 mM-glutamine, 100 nM-bovine insulin, 75 μg of methionine/ml and 1.25 mM-Hepes, pH 7.4). Then, 5–10 min later, 2 mCi of [^{35}S]methionine was added to the recirculating medium, and the perfusion was continued for 30–60 min. At the end of the experiment livers were weighed, homogenized in 9 vol. of ice-cold buffer A and centrifuged at 27000 *g* for 10 min. Portions of the homogenate and the perfusion medium were taken for determination of the incorporation of ^{35}S into total

proteins, as described above. The remaining homogenate was immunoprecipitated as detailed below.

Immunoprecipitation

SDS was added to the supernatant fraction of the liver homogenates to give a final concentration of 1%, and the mixture was heated at 95 °C for 6 min. Then, the samples were diluted with buffer D (containing, per ml: 3.564 mg of Na_2HPO_4,2 H_2O, 55 μg of NaN_3, 8.766 g of NaCl, 10 mg of Triton X-100, 1 mg of SDS, 100 mg of heparin, 745 μg of EDTA and 17.4 μg of phenylmethanesulphonyl fluoride, pH 7.4). To minimize non-specific binding, the samples were first mixed with 20 μl of normal chicken serum and incubated at 4 °C for 15–20 h. Goat anti-chicken IgG coupled to Sepharose was then added and, after incubation at 4 °C for 4–5 h, the resulting precipitates, which did not contain any LPL-like protein on SDS/PAGE, were removed by centrifugation at 3000 *g* for 5 min. Two portions of the supernatant were each mixed with 10 μl of chicken antiserum to bovine LPL, and a third portion was mixed with 10 μl of non-immune chicken serum. After 15–20 h at 4 °C, goat anti-chicken IgG coupled to Sepharose was added; 4–5 h later the immunoprecipitates were collected by centrifugation at 3000 *g* for 5 min, washed twice in buffer D and twice in buffer D without SDS. The precipitates were then dissolved in sample buffer by heating at 95 °C for 6 min, and separated by SDS/PAGE. Gels were stained with Coomassie Blue, destained, impregnated with Enlighting and dried between sheets of cellophane under vacuum, as previously described (Semb & Olivecrona, 1986). Autoradiographs were obtained by exposure of Kodak X-omat films to the gels for periods of 4–15 days at −70 °C. The band corresponding to LPL was identified, cut out, eluted in a scintillation cocktail (Lipoluma/Lumasolve/water) and counted for radioactivity in a liquid-scintillation counter.

To study if the chicken antiserum to bovine LPL was able to immunoprecipitate rat LPL and if this immunoprecipitation was quantitative, several control experiments were carried out. For this, we used homogenates of adult-rat adipose tissue that had been labelled *in vitro* with [^{35}S]methionine, essentially as described for guinea-pig adipose tissue by Semb & Olivecrona (1986). In these experiments, the amounts of homogenate, the amount of antiserum and the time of incubation were systematically varied. The immunoprecipitates formed under each of these conditions were recovered. Then an excess of antiserum was added to each of the supernatants, and a second set of immunoprecipitates was obtained. Both sets of immunoprecipitates were washed, separated by SDS/PAGE, and the radioactivity in LPL was determined. The results showed that, under the conditions used in the present study, 10 μl of chicken antiserum was enough to precipitate the LPL synthesized in 1 h by 100 mg of adipose tissue.

Immunofluorescence

Livers from fasted newborn rats were perfused as indicated above. After wash-out, heparin (5 i.u./ml) was added (or not), and the perfusion continued for 10 min. Then the livers were washed by perfusion with 15 ml of PBS at a flow rate of 3–4 ml/min per g. The buffer was then changed to PBS containing 0.1% glutaraldehyde and 2% paraformaldehyde, and the perfusion continued for 10 min. Then small tissue blocks were obtained and

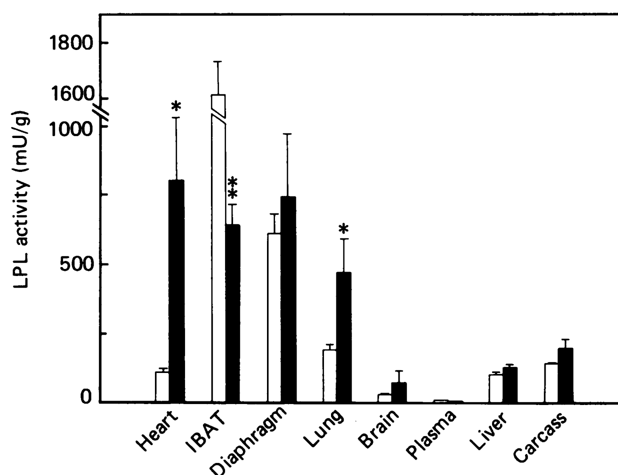

Fig. 1. LPL activity in tissues from fed or fasted newborn rats

Values are means ± S.E.M. for tissues from five to seven rats. Statistical comparison was by Student's *t* test. *$P < 0.05$; **$P < 0.001$. □, Fed; ■, fasted. Abbreviation: IBAT, interscapular brown adipose tissue.

kept in the fixative solution at 4 °C for 1 h. Next, the blocks were first incubated with 0.2 M-NH_4Cl in PBS for 15 min at 4 °C to neutralize free aldehyde groups, and then extensively washed with 1 M-sucrose in PBS and finally frozen in liquid nitrogen. Sections (5–7 μm thick) were obtained in a cryostat (−25 °C), mounted on gelatine-treated slides, dried at room temperature and processed for immunofluorescence as follows. Sections were washed with buffer E (0.1 M-glycine in PBS), permeabilized with 0.1% Triton X-100 in buffer E, incubated for 2 h with chicken antiserum (or control serum) diluted 1:50 in buffer E, and finally washed and incubated for 1 h with affinity-purified FITC-conjugated rabbit anti-chicken IgG. After three additional washes, sections were mounted, viewed and photographed on Kodak Tri-X film by using epifluorescence, with an Olympus IMT2-RFL microscope.

RESULTS

Fig. 1 illustrates LPL activities in tissues of 24 h-old rats. For plasma and liver, the LPL activity was determined by using specific antisera to hepatic lipase and LPL as described in the Materials and methods section. For other tissues and for the carcass, the activities in Fig. 1 represent total activities in the LPL assay. Control experiments showed that, for these tissues, more than 98% of the activity was inhibited by antiserum to LPL. The highest activity per g was in the interscapular brown adipose tissue of fed rats. In several tissues there were marked differences between the fed and the fasted states. The activity per g of tissue increased significantly on fasting in heart (8-fold) and lungs (3.5-fold), but decreased (60%) in the brown adipose tissue. There were significant amounts of LPL activity in liver and in plasma, but these activities did not differ between the fed and the fasted states. The activity in plasma was about twice as high, and that in the liver was about 10 times as high, in fed newborn as in fed adult rats.

From the data in Fig. 1 and the weight of the organs,

Table 1. LPL activity in whole organs of newborn rats

Data are calculated from values in Fig. 1 and the weights of the organs, and are means ± S.E.M. for five to seven animals per group. Statistical comparison was by Student's t test: *$P < 0.05$; **$P < 0.001$.

	LPL activity (munits/whole organ)	
Tissue	Fed	Fasted
Heart	4.5±0.4	24.6±8.4*
Brown adipose	81.8±7.8	25.6±3.6**
Diaphragm	10.4±2.2	19.6±6.0
Lung	19.8±2.4	49.2±12.8*
Brain	6.8±1.6	15.0±7.0
Plasma	2.5±0.2	1.9±0.3
Liver	27.0±2.8	23.4±1.6
Carcass	788±53	944±177

we calculated the total LPL activity per whole organ (Table 1). Owing to the high percentage of carcass, its LPL activity accounted for more than 80% of total body LPL activity. The LPL activity in the liver accounted for about 3% in fed rats; this percentage was significantly higher than those of heart, diaphragm, lung or brain.

The presence of LPL activity in the liver might be due to importation of LPL from other tissues, as has been proposed for adult rats (Peterson *et al.*, 1985). To study this we determined the LPL activity in plasma and liver of newborn rats. Unlike what has been found in adult rats (Semb *et al.*, 1987), there was no significant correlation between the two activities. In fact, some animals had high LPL activity in their livers in spite of low plasma activity, and vice versa. This suggests that, in newborn rats, transport through plasma is not a major source of liver LPL.

To study biosynthesis of LPL, rats were given [^{35}S]-methionine intraperitoneally, and 1 h later tissues were removed and homogenized, and the enzyme was immunoprecipitated. When the precipitates from extrahepatic tissues were separated by SDS/PAGE, we obtained a single band of radiolabelled protein in the same position as for bovine LPL. This is illustrated in Fig. 2 for lungs. As previously observed in guinea pigs (Semb & Olivecrona, 1986), the position of this band was the same for all extrahepatic tissues (results not shown).

Fig. 3 shows the time course for incorporation of [^{35}S]methionine into immunoprecipitable LPL in tissues of newborn rats. Radioactivity in LPL increased progressively with time in all tissues studied, including the liver. From these data we calculated LPL synthesis as percentage of total protein synthesis. These data are presented in Table 2. The highest value was for brown adipose tissue, where LPL accounted for 580 p.p.m. (0.06%) of total protein synthesis. In lungs, diaphragm, and heart LPL accounted for 120–130 p.p.m. These values are 5–10 times lower than what was previously found in a study with adult guinea pigs (Semb & Olivecrona, 1986). This is not unexpected, since tissues of newborn animals synthesize other proteins more actively than do adult animals. Synthesis of LPL in the liver was a very low fraction of total protein synthesis (33 p.p.m.),

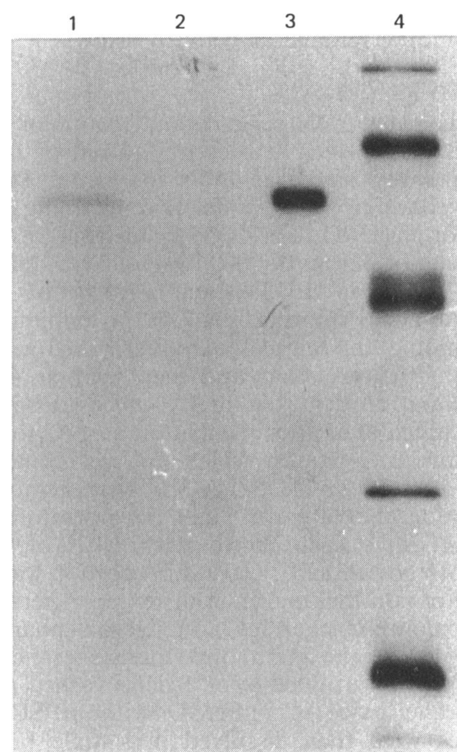

Fig. 2. Immunoprecipitation of ^{35}S-labelled LPL from lungs

Fasted newborn rats were injected intraperitoneally with 1.25 mCi of [^{35}S]methionine and killed 60 min later. The lungs were homogenized and immunoprecipitated as described in the Materials and methods section. Immune complexes were dissolved and separated by SDS/PAGE. The gel was dried and exposed to Kodak X-Omat film. Lanes: 1, immunoprecipitate with antiserum to LPL; 2, precipitate with a corresponding non-immune serum; 3, ^{125}I-labelled bovine LPL; 4, M_r markers [albumin (69000), ovalbumin (46000), carbonic anhydrase (30000), lactoglubulin A (18400)].

but, since hepatic protein synthesis was much higher than in other tissues, LPL radioactivity in liver was as high as in heart, lung and brown adipose tissue (Fig. 3), in accord with the observation that LPL activity was also high in the liver (Table 1).

The observation that there was significant radioactivity in LPL in liver already at 15 min argues strongly against the possibility that the labelled enzyme originated in other tissues, and hence suggests that the liver synthesizes LPL in newborn rats. To study this further, isolated livers from newborn rats were perfused with [^{35}S]-methionine for 1 h. Fig. 4 shows SDS/PAGE of immunoprecipitates from these livers. Several bands were seen both for precipitates obtained with the antiserum and for precipitates with control sera. There was a distinct band running in the same position as bovine LPL. This band was seen with antiserum, but not with control serum. Furthermore, it was competed out by excess bovine LPL. The other bands seen in the precipitate with antiserum were also seen in varying amounts with control serum and after competition. Whether any of them are related to LPL is not known.

Previous studies have shown that the LPL activity in liver remains high during suckling, but is low in adult

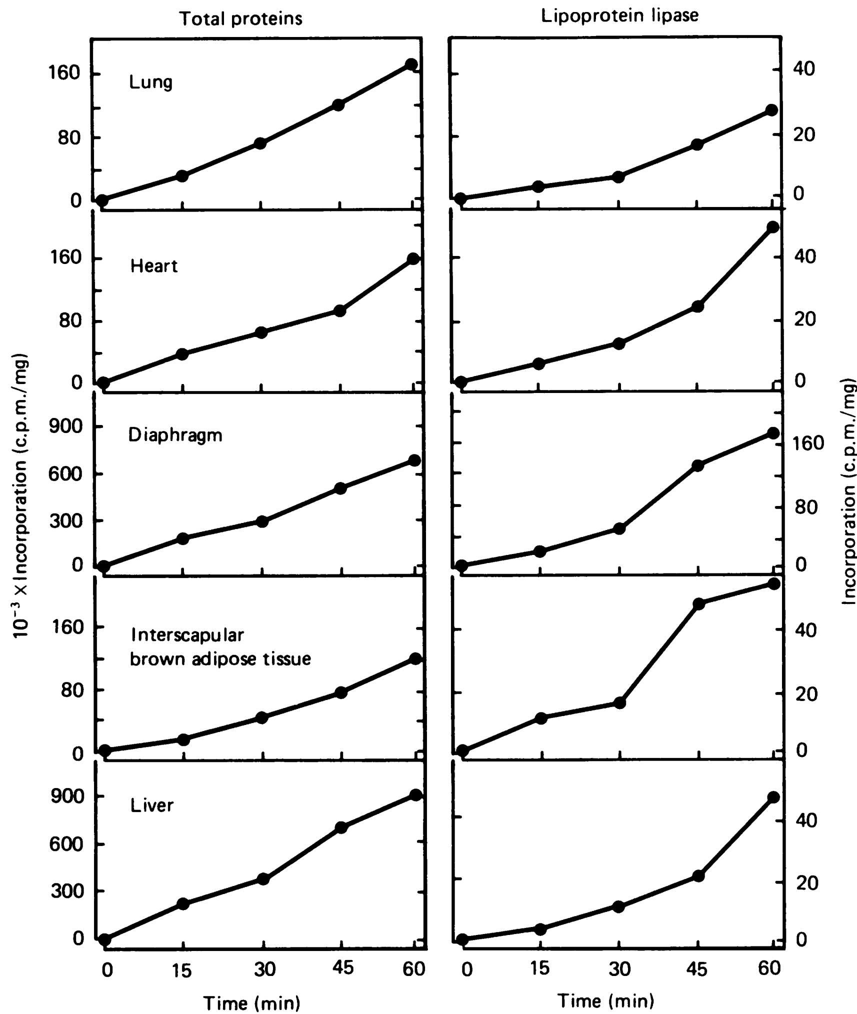

Fig. 3. Time course for incorporation *in vivo* of [^{35}S]methionine into total proteins and immunoprecipitable LPL in newborn rats

Fasted newborn rats were injected intraperitoneally with 1.25 mCi of [^{35}S]methionine and killed 15, 30, 45 or 60 min later. Total proteins were precipitated with trichloroacetic acid from homogenates of the tissues, and radioactivity was determined. Other samples of the homogenates were used for immunoprecipitation. The immune complexes were dissolved and separated by SDS/PAGE. The band corresponding to LPL was identified, cut out and counted for radioactivity. Values are expressed in radioactivity per mg wet wt. of tissue.

Table 2. Relative synthesis of LPL in tissues of newborn rats

Values are radioactivity in LPL divided by radioactivity in total proteins from data in Fig. 4, expressed as parts per million, and are means ± S.E.M. of the data obtained at 15, 30, 45 and 60 min.

Tissue	LPL synthesis (p.p.m.)
Lung	120 ± 18
Heart	213 ± 37
Diaphragm	195 ± 34
Brown adipose	580 ± 118
Liver	33 ± 06

rats (Reina & Vilaró, 1985). Table 3 demonstrates that livers from adult rats synthesized less LPL than do livers from newborn rats. The value obtained was 3 p.p.m. of total protein synthesis, about 10-fold lower than in the newborns. About 200 c.p.m. were recovered by immunoprecipitation of homogenate from 100 mg of liver and subsequent separation of the immunoprecipitate by SDS/PAGE. By comparison, total protein radioactivity in the sample was 50×10^6 c.p.m.

We have previously reported that intravenous administration of Intralipid to adult rats increases the LPL activity in liver (Peterson *et al.*, 1985; Vilaró *et al.*, 1986). Therefore we studied the incorporation of [^{35}S]-methionine into immunoprecipitable LPL in the liver of a rat given Intralipid. The percentage incorporation of label into LPL was similar in this rat to that in control rats, in contrast with the marked increase in hepatic LPL activity (results not shown).

To localize the enzyme within the liver, we used immunofluorescence. Unfortunately, the antisera available to us had only moderate affinity for rat LPL. Hence, we had to do our studies under conditions where substantial fluorescence was obtained also with non-immune sera, and the contrast between immune reaction and background was insufficient to resolve fine details of

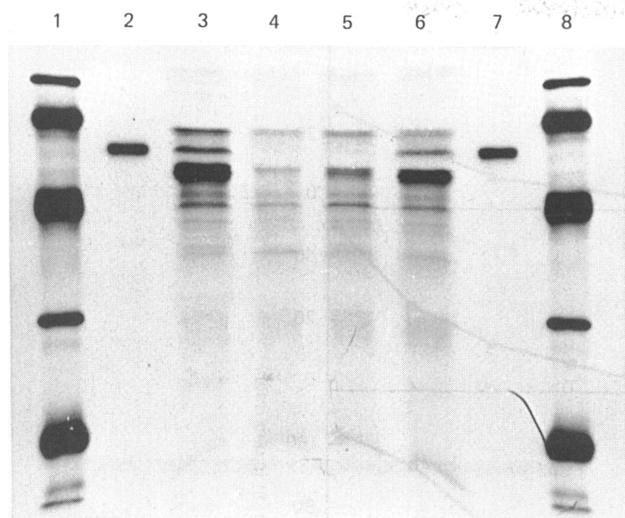

Fig. 4. Incorporation of [^{35}S]methionine into LPL by isolated perfused livers from newborn rats

The liver from a fasted newborn rat was isolated and perfused as described in the Materials and methods section; 60 min after the addition of 2 mCi of [^{35}S]methionine to the recirculating perfusion medium, the liver was homogenized. The supernatant fraction of the homogenate was immunoprecipitated, and the washed precipitates were separated by SDS/PAGE. The Figure shows a fluorogram. Lanes: 3, immunoprecipitate with antiserum to LPL; 4, same as lane 3, but with 25 μg of bovine LPL present; 5, same as lane 3, but with non-immune serum; 6, immunoprecipitation of the supernatant from lane 5 with antiserum against LPL; 2 and 7, ^{125}I-labelled bovine LPL; 1 and 8, M_r markers (same as in Fig. 2).

Table 3. Relative synthesis of LPL in isolated perfused rat liver

Livers from 0-, 5- and 15-day-old rats and from adult rats were isolated, and perfused for 1 h with 2 mCi of [^{35}S]-methionine, homogenized and immunoprecipitated as described in the Material and methods section. Results are radioactivity in the LPL band from SDS/PAGE divided by radioactivity in total proteins, expressed as parts per million, and are means ± S.E.M. for the numbers of experiments shown in parenthesis.

Age (days)	LPL synthesis (p.p.m.)
0	22±2 (5)
5	10 (1)
15	18 (1)
Adult	3±1 (2)

localization. There were, however, clear and consistent differences between sections treated with antiserum (Fig. 5*a*) and non-immune serum (Fig. 5*d*). The most striking aspect was reaction at endothelial surfaces, both within sinusoids and along the walls of larger vessels. This reaction disappeared when livers were perfused with heparin (Fig. 5*c*), suggesting that extracellularly located LPL was displaced. Significant immune reaction over cells persisted after the perfusion (cf. Figs. 5*c* and 5*d*). We interpret this as intracellularly localized LPL, but cannot, owing to the high background, resolve whether only certain types of cells within the liver contain immunoreactive LPL.

DISCUSSION

This study demonstrates that in newborn, but not in adult, rats there is substantial synthesis of LPL within the liver, and that some of this LPL is located at the vascular endothelium, suggesting that it takes part in lipoprotein metabolism.

It was previously known that LPL activity in the liver is severalfold higher in newborn than in adult rats (Chajek *et al.*, 1977; Llobera *et al.*, 1979), and that this high activity is maintained during the suckling period (Reina & Vilaró, 1985). The present data reinforce these previous observations, and demonstrate that during the neonatal stage LPL activity is actually higher in the liver than in several of the tissues which are traditionally considered to be sites of high LPL activity, such as the heart, the diaphragm and the lungs. A major reason for the higher hepatic LPL activity was that LPL synthesis in the liver was much higher during the neonatal stage. This was demonstrated both *in vivo* and in isolated perfused livers. Synthesis of LPL was higher for two reasons; LPL accounted for a 5–10-fold higher proportion of total protein synthesis, and total protein synthesis was also more active in the newborns.

Previous studies have indicated that the major source of liver LPL in adult rats is uptake from plasma (see the Introduction). The present observation that LPL synthesis is extremely low in the liver of adult rats is further support for this hypothesis. We cannot deduce from our present data whether any net transport of LPL from extrahepatic tissues to the liver takes place in newborn rats. There was no correlation between the LPL activities in plasma and liver of newborn rats, whereas there is such a correlation in adult rats (Knobler *et al.*, 1984; Peterson *et al.*, 1985; Semb *et al.*, 1987). Hence, it seems likely that in the newborn rats the main source of liver LPL activity was synthesis within the liver.

The histological appearance of the liver is quite different in newborn rats compared with adults (Naito & Wisse, 1977). For instance, in newborn rats the liver is a blood-forming organ, whereas in adult rats it is not. An interesting question is what type of cell synthesizes LPL in the liver. We hope that hybridization studies *in situ* using a recently developed cDNA probe for the enzyme (Enerbäck *et al.*, 1987) will resolve this question.

In adult animals, tissue LPL activities respond rapidly to changes in nutritional state (Robinson, 1970; Nilsson-Ehle *et al.*, 1980; Cryer, 1981). In the present study similar changes were observed in newborn rats. For instance, fasting for 24 h resulted in pronounced increases in the LPL activities in heart and lungs. An interesting new observation was the rather large decrease in LPL activity of brown adipose tissue on fasting. Previously studies have emphasized that the LPL activity in this organ is related to the tissue's thermogenic activity both in the perinatal period (Hemon *et al.*, 1975) and in adult rats (Carneheim *et al.*, 1984), but it has also been reported that the activity decreases with food restriction or starvation (Fried *et al.*, 1983; Carneheim, 1987). In

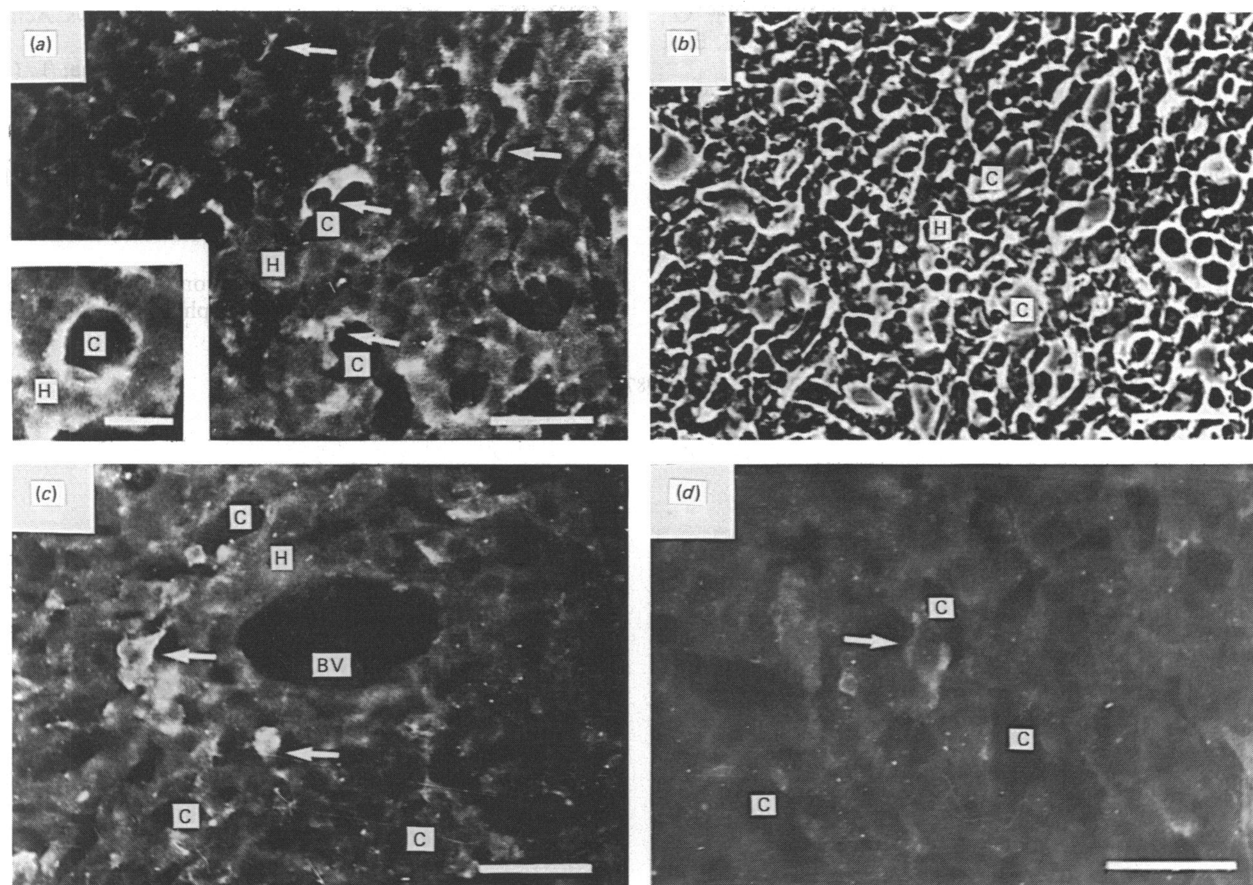

Fig. 5. Localization by immunofluorescence of LPL in livers from newborn rats

Livers from starved newborn rats were cannulated and perfused with buffer B. After 10 min, heparin was added (or not) and the perfusion was continued for 10 min. The livers were washed, fixed and frozen as indicated in the Materials and methods section. Cryosections (5–7 μm) were permeabilized with Triton X-100 (0.1% in 0.1 M-glycine/PBS), incubated first with chicken antiserum against bovine LPL and then with affinity-purified FITC-conjugated rabbit anti-chicken IgG. Panel (*a*) shows the immunofluorescence in livers perfused without heparin; inset shows higher magnification of a capillary lumen from this section. Panel (*b*), phase contrast of the section in panel (*a*). Panel (*c*), section of a liver perfused with heparin. Panel (*d*), liver section incubated with non-immune chicken serum. Abbreviations: BV, blood vessel; C, capillary; H, hepatocyte. Immunofluorescence is indicated by arrows. Bars represent 90 μm in panels (*a*)–(*d*), and 30 μm in the inset of panel (*a*).

contrast with the marked changes in several other tissues, there was no significant change in the activity of LPL in the liver on fasting. This suggests that the function of LPL in the liver of newborn rats is not acutely related to the nutritional state.

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