Effect of fasting on the lipid composition and enzyme activity of rat liver plasma membranes

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Summary. After 24-h fasting, when the recovery of plasma membrane protein isolated from rat liver was unchanged, the enrichment in 5'-nucleotidase was decreased by 16%. Modifications of the lipid composition were also observed and resulted in a 27% decrease of the cholesterol/phospholipid molar ratio.

The assay of specific marker enzyme activity and the determination of lipid composition are the 2 most widespread criteria used to characterize the subcellular fractions, and especially the plasma membranes, isolated from rat liver. Nevertheless, the results published in the literature reveal much variation in the 5'-nucleotidase specific activity in plasma membrane fractions isolated from rat liver by similar methods[1–5]. This observation applies also to their lipid composition and particularly their cholesterol/phospholipid molar ratio[2–4,6]. In most of these studies, the lack of information concerning the nutritional state of the experimental animals at the time of sacrifice prevents any valuable comparison of the published data. The present study is an attempt to establish the contribution, if any, of the nutritional state of the animals to the aforementioned discrepancies.

Materials and methods. Plasma membrane fractions were isolated from fed (adult diet UAR, France) or from 24 h-fasted, male Sprague-Dawley rats[5]. Their protein content[7] and enzymes activities[5] (5'-nucleotidase: EC 3.1.3.5.; glucose-6-phosphatase: EC 3.1.3.9.) of total homogenate and of plasma membranes were determined. Determinations of cholesterol[8] and of total phospholipids[9] were carried out on aliquots of the total lipid extract of the plasma membrane fractions[10]. After separation of the main phospholipids by thin layer chromatography[11], their phosphorus content was determined by the method of Fiske and Subbarow[12] modified by Delsal and Manhouri[9].

Table 1. Effect of 24-h fasting on plasma membranes enzymes activity.

	5'-nucleotidase (EC 3.1.3.5.)		Glucose-6-phosphatase (EC 3.1.3.9.)	
Rats	RSA[1]	Recovery[2]	RSA[1]	Recovery[2]
Fed	14.2 ± 1.7	10.4 ± 0.8	0.6 ± 0.1	0.4 ± 0.1
Fasted	9.2 ± 0.9*	6.2 ± 1.3**	0.5 ± 0.2(NS)	0.3 ± 0.1 (NS)

Rats were fed ad libitum a standard laboratory chow or, conversely, fasted with free access to tap water during 24 hours before sacrifice. Plasma membranes were isolated from liver homogenate and enzyme activity was determined[5]. 1: RSA: relative specific activity represents the ratio of the specific activity of the enzyme in the plasma membrane fraction to the specific activity of the enzyme in the total homogenate. 2: recovery represents the percentage of enzyme activity present in 1 g of liver found in the corresponding plasma membrane fraction. Data are the means ± SEM from 6 preparations per group. Comparison between groups were made according to the Mann-Whitney U-Test; * $p<0.05$; ** $p<0.01$; NS: not significant.

Table 2. Effect of 24 hours-fasting on the lipid composition of plasma membranes.

	Cholesterol (CS)	Phospholipids (PL)	CS/PL
Rats	nmoles/mg prot.	nmoles/mg prot.	Molar ratio
Fed	247 ± 20	472 ± 40	0.513 ± 0.042
Fasted	208 ± 19 (NS)	558 ± 49 (NS)	0.373 ± 0.020*

Data are the means ± SEM from 6 preparations of plasma membranes from fed or 24-h fasted rats. Comparison between groups were made according to the Mann-Whitney U-Test; *: $p<0.05$; NS: not significant.

Results and discussion. After 24-h fasting, the liver weight decreases by 34% (fed rats: 9.9 ± 0.2 g; fasted rats: 6.5 ± 0.2 g; mean ± SEM from 6 preparations; $p < 0.01$) but the body weight decreases only by 9% (fed rats: 231.5 ± 5.3 g; fasted rats: 210.4 ± 4.2 g; mean ± from 6 preparations).

Despite these modifications, the total protein recovery of the plasma membrane fraction is very similar in the two groups; fed rats: 1.24 ± 0.11 mg/g liver; fasted rats: 1.30 ± 0.26 mg/g liver; mean ± SEM from 6 preparations.

Contrasting with this stability, the relative specific activity (RSA) of the 5'-nucleotidase (eC 3.1.3.5.), a well known plasma membrane marker enzyme[13] is strikingly decreased in the fasted rats (table 1). This decrease is even more pronounced when expressed for the total liver since the liver weight decreases after fasting, whereas the plasma membrane recovery (expressed per g liver) remains unchanged after fasting.

The phospholipid composition was not affected by fasting: phosphatidylcholine: 44.9 ± 3.1%; 46.4 ± 2.2%; sphingomyelin: 21.2 ± 1.6%; 25.4 ± 2.5%; phosphatidyl-serine, -ethanolamine, -inositol: 33.1 ± 2.1%; 30.3 ± 2.1%; fed and fasted rats respectively. (mean ± SEM from 6 preparations).

Assuming a metabolic continuity between the intracellular membranes and the plasma membranes[16–19], we suggest that the modifications of the lipid composition of the plasma membranes in response to different nutritional conditions result from regulations of the rate of synthesis of the endoplasmic reticulum and Golgi apparatus.

As plasma membrane enzymes are closely associated with lipids[20], the modifications of the plasma membrane enzyme activity could result from the alterations of the lipid surroundings.

These fasting-induced changes draw attention to the necessity of stating the precise nutritional conditions in studies concerning the composition and metabolism of rat liver plasma membranes.

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Evidence for the existence of 2 types of α_1-lipoprotein in amniotic fluid from pregnancies older than 20 weeks

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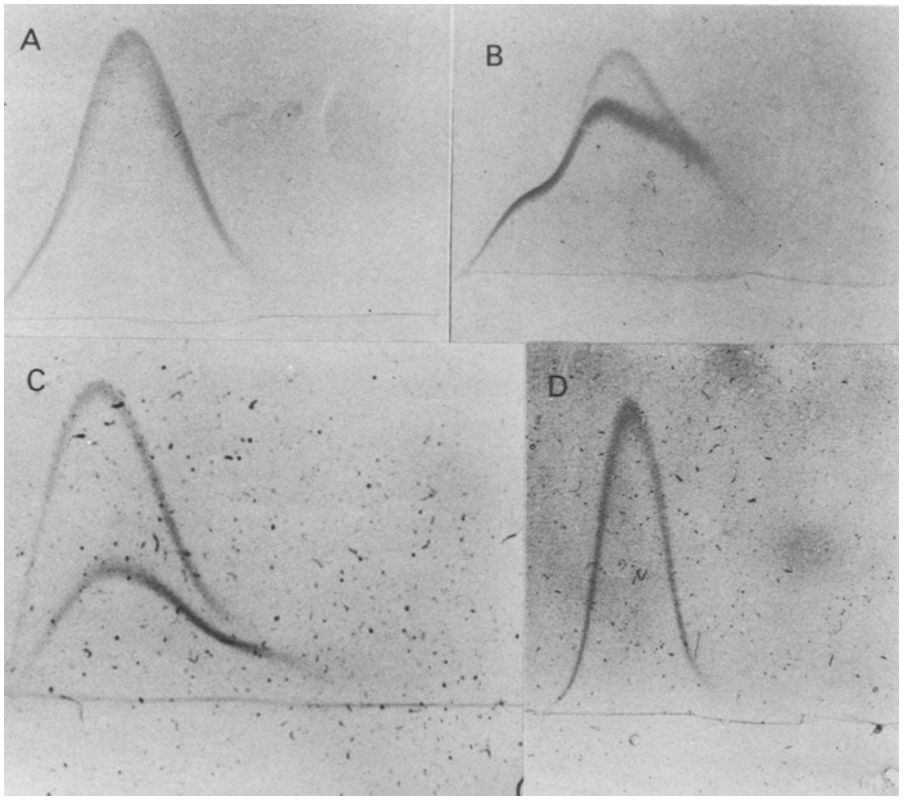
Summary. We have found by means of crossed immunoelectrophoresis that amniotic fluid contains 2 types of α_1-lipoprotein after the 20th week of pregnancy. Before that period the α_1-lipoprotein profile of amniotic fluid resembles that of serum and migrates as one type only.

Various authors[1,2] have suggested that the α_1-lipoprotein (α_1-LP) of serum does not consist of a single macromolecular complex but represents a mixture of several discrete lipoprotein families. There is, however, little evidence for such a hypothesis and it is well known that serum α_1-LP migrates as a single band when subjected to (immuno)electrophoresis. We wish to report that the α_1-LP of amniotic fluid can be separated into 2 components if it is derived from pregnancies older than about 20 weeks.

In our study we have made use of the technique of crossed immunoelectrophoresis in plates containing an antiserum against α_1-LP. The antiserum, which was obtained from Behring AG, Marburg (FRG) was prepared in rabbits immunized against high density lipoprotein (HDL_3). All amniotic fluid samples were concentrated 100 times in an Amicon B-15 concentrator and were then processed as described previously [3]. In the figure the α_1-LP profile of amniotic fluid from a 16-week (A), a 33-week (B) and a 42-week (C) pregnancy and of serum (D) is shown. It is of interest that on addition of a small amount of serum to amniotic fluid with a double peak such as in C, the 2 components combine to form a single α_1-LP peak. The α_1-LP profile of the 16-week amniotic fluid (single peak, A) is similar to that of serum (D), an observation which lends support to the view[4] that in early pregnancy amniotic fluid is a transudate of serum. Single α_1-LP peaks were also found if saline extracts of homogenated placenta, umbilical



The α_1-lipoprotein profile on crossed immunoelectrophoresis of amniotic fluid from *A* a 16-week, *B* a 33-week and *C* a 42-week pregnancy. In *D* the α_1-lipoprotein profile of serum is shown.