

Biochemical aspects of a whey fraction capable of promoting hybridoma proliferation. Comparison with fetal calf serum

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Summary — Human and bovine milks were used as serum substitute for cell cultures. Two whey fractions termed LBS65 and LELm prepared by different steps were routinely used for culturing hybridomas in our laboratory. They stimulated ³H-thymidine incorporation into hybridoma, but a minimum of 1% of fetal calf serum (FCS) was required when the cells were propagated in the long-term. In this investigation, the chemical composition of LBS65 and LELm was studied in parallel to FCS composition. LBS65 and FCS had a total protein level of about 40 g/l, while LELm contained them at a concentration of 11 g/l. The amino acid content of FCS and LBS65 was very similar (about 4 000 μmol/l), whereas it was lower in LELm (about 300 μmol/l). Although the values of nitrogen compounds were different in LBS65 and LELm, these 2 whey fractions behaved similarly to a substitute of FCS. Whey fractions were low in sodium, and high in calcium compared to FCS. The cholesterol level was lower and triglycerides were higher in whey than in FCS. The ratio of unsaturated/saturated fatty acids varied from 1 whey fraction to another. It differed from the FCS ratio. FCS and whey fractions contained small amounts of steroid or peptide hormones. However, progesterone, 17-OH-progesterone, ACTH and cAMP concentrations were higher in the whey fraction than in FCS. Whey fractions are a complex medium containing various compounds able to promote cell growth. However, some unknown compounds present in FCS and absent in milk fractions are required in long-term cell culturing.

bovine milk / whey / whey hormone / whey amino acid

Résumé — Composants biochimiques du lactosérum pouvant induire la prolifération cellulaire d'hybridomes. Comparaison avec le sérum de veau fœtal. Les laits humains ou bovins ont été utilisés comme substitut du sérum dans les cultures cellulaires. Deux fractions de lactosérum, appelées LBS65 et LELm, préparées de façon différente, sont couramment utilisées au Laboratoire pour la culture d'hybridomes. Ils stimulent l'incorporation de ³H-thymidine dans des hybridomes, mais si les cultures doivent être faites à long terme, 0,5 ou 1% de sérum de veau fœtal (FCS) reste indispensable. Dans ce travail, est étudiée la composition chimique de LBS65 et LELm parallèlement à celle du sérum de veau fœtal. LBS65 et FCS ont une concentration en protéines totales voisine (environ 40 g/l), alors que LELm n'en contient que 11 g/l. De même, les acides aminés sont au même taux (environ 4 000 μmol/l) dans FCS et LBS65 et à des taux faibles dans le LELm (environ

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300 $\mu\text{mol/l}$). Bien que très différentes par leur contenu azoté, LBS65 et LELm se comportent sensiblement de la même façon comme substitut de FCS. Les lactosérums ont, par rapport au FCS, une teneur faible en sodium et élevée en calcium. Le taux de cholestérol est plus bas dans les lactosérums et les triglycérides sont plus élevés. Le rapport (acides gras (AG) insaturés/AG saturés) est très variable d'un lactosérum à l'autre et différent de celui du FCS. FCS et lactosérums contiennent des quantités faibles d'hormones stéroïdes ou peptidiques. Il faut cependant noter que les taux de progestérone, 17-OH-progestérone, ACTH et surtout AMPc sont supérieurs dans les lactosérums. Le lactosérum est donc un milieu complexe contenant de nombreux composés dont chacun peut avoir une activité de croissance. Cependant, certains facteurs encore inconnus, présents dans le FCS, qui sont nécessaires à la croissance à long terme des hybridomes, manquent dans les lactosérums.

lait bovin / lactosérum / hormone du lactosérum / acide aminé du lactosérum

INTRODUCTION

Human or bovine milk has been used as a serum substitute for promoting cell growth (Serini and Baserga, 1981; Nano *et al*, 1982). We have shown that bovine whey fractions, a dairy by-product, can also replace fetal calf serum (FCS) in eucaryote cell lines cultured in the short or long-term (Damerdjii *et al*, 1988). However, in the latter case 0.5 or 1% of FCS must be added to promote long-term proliferation. Furthermore, hybridoma can be stored at -80°C for 2 years in the same milk fractions (Derouiche *et al*, 1989).

Whole bovine milk contains various compounds able to stimulate cell growth: growth factors (Shing and Klagsbrun, 1984; Campbell and Baumrucker, 1989), vitamins and trace elements (Blanc, 1981; Alais, 1984), hormones (Koldovsky and Thornburg, 1987), peptides with various activities (Maubois and Léonil, 1989). This paper reports the cell growth promoting activity and composition of 2 whey fractions prepared differently, attempts to correlate the composition with their activity and compare them with the composition of FCS generally employed to supplement culture media.

MATERIALS AND METHODS

Preparation of whey fractions

The first fraction (termed LBS65) was prepared by Bel (Vendome, France) according to an industrial process. The fats and cell debris were removed by centrifugation (4 500 g) at 5°C for 20 min. The skim milk was heated at 67°C for 15–20 s and the proteins (mostly casein) were precipitated at 34°C with 40 ml of diluted 1/1 000 enzyme mixture (chymosin 80% and pepsin 20%) for 100 l of skim milk. The supernatant was heated at 65°C for 30 min and concentrated 18 times by ultrafiltration (mineral membrane cut-off 10 000). The remaining substance was washed with distilled water and again concentrated 18 times by ultrafiltration at 55°C . It was then centrifuged twice at 30 100 g for 1 h, then 30 min after removal of the pellet the supernatant was heated at 50°C , and sterilized by filtration through a $0.22\ \mu\text{m}$ membrane. The fraction LBS65 was stored at -20°C .

The second fraction (termed LELm) was recovered during cheese-making (Sodial, Metz, France). The milk was heated at 65°C for 30 s, and ultrafiltered through mineral membrane (cut-off 10 000). The fat content of the remaining substance was adjusted and biological fermentation was carried out for 18 h and the substance was then pasteurized at 72°C for 30 s. Penicillium and Cl_2Ca were added and biological maturation pursued for 1 h 35. The exudate

was recovered and concentrated 2.5 times by ultrafiltration at 40 °C (mineral membrane cut-off 10 000). The substance was centrifuged twice for 1 h, then 30 min, sterilized by filtration at 50 °C through a 0.22 µm membrane. The LELm fraction was stored at -20 °C in aliquots.

Cell culture

The hybridomas used in this study were hybridoma F₃₄ secreting an IgG₁ monoclonal antibody against gonadotropin (gift from Sanofi, Montpellier, France) and hybridoma A₄₉ secreting an IgM monoclonal antibody against blood group A (gift from INSERM, Unité 284, Nancy, France). The cells were routinely cultured in RPMI 1 640 medium (Boehringer Mannheim, FRG) supplemented with 10% FCS (Flow Laboratories, Puteaux, France) heated at 56 °C for 30 min and 4 mmol/l of glutamine.

Short-term cell culture and measurement of DNA synthesis

Hybridoma cell suspension (100 µl) were seeded at the concentration of 7 × 10⁴ cells/ml in 96 well microtiter plates (Falcon, Grenoble, France). 5, 10% and 20% of LBS65 or LELm or FCS were added in 100 µl of RPMI 1 640 and the cells were incubated for 24 h. ³H-thymidine (50 µl, 1 µCi/ml specific activity 4.5 Ci/mmol) were added for the last 18 h. The cells were collected with Harvester titertek skarton, washed and the radioactivity was counted.

Long-term cell proliferation

Hybridomas were subcultured in 25 cm² Falcon flasks every 3 d (F₃₄) or every 2 d (A₄₉) in medium containing 1% FCS and 9% whey fractions (LBS65 or LELm). For each passage, viable cells were counted using a hemocytometer after the cells were stained with 0.5% trypan blue and the cell suspension was then brought to 2 × 10⁵ cells/ml and seeded again in a new flask in fresh medium containing the same whey fractions and 1% FCS. This experiment was stopped at 7 or 9 d since our experience has shown that this amount of time is enough to evaluate the ability of the medium to maintain a long-term prolifera-

tion. However, we also used the same medium in perfused bioreactors for 3 months (data not shown).

Analysis of whey fractions

Protein and non-protein nitrogen compounds

The total proteins were determined by the Gornall's method adapted to SMA II X8 (Technicon, France). Proteins were separated by FPLC according to Andrew's method (Andrews *et al*, 1985). Amino acids were assayed with a Beckman apparatus (Beckman 7 300) equipped with ion-exchange column (Gelot *et al*, 1990).

Creatinine was measured with Jaffe's method, uric acid with the uricase method (Boehringer, ref: 242616). These tests were automatically performed on a Prisma apparatus (Analysinstrument Stockholm).

Carnitine was tested according to the radioenzymoassay described by Cederblard and Lindstedts (1972). Histamine was determined by RIA with Immunotech kit (ref: 1 302). NH₃ was tested with Berthelot's method (Biolyon-Wako, ref: 31 291).

Minerals

Na⁺, K⁺ were determined by flame photometry (SMA II X8 Technicon), Ca²⁺ by an automatic (Prisma) colorimetric method using the α -cresolphthalein complexon (Sarkar and Chauhan, 1967), iron by the automatic (Prisma) colorimetric method using the ferrozine (Ichida, 1968), Cu and Zn, by flame atomic absorption spectrometry with Pye Unicam 1 900, Philips as apparatus (Arnaud *et al*, 1984) and Se by flameless atomic absorption spectrometry (Perkin-Elmer, 2 390) (Dubois *et al*, 1990). Osmolarity was assayed with an osmometer (Osmomat 030, Genotec).

Energetic compounds

Lactose was determined by gas chromatography, using a capillary column OV₁ (250 × 0.3

cm) and silylated derivatives. Other compounds were assayed by enzymatic methods with kits for which the references will be given in the table of results.

Lipids

Cholesterol and triglycerides were measured with enzymatic methods (cholesterol enzymatic endpoint Radox Laboratories. Triglycerides 800/9, Biolabo) with automatic apparatus (Prisma).

Fatty acids (FA) were extracted by Folch's method (Folch *et al*, 1957). Free fatty acids (FFA) were chromatographed (gas chromatograph, Packard 427) on a carbowax column (200 x 0.3 cm) after methylation with methyl 8 (Interchim, Montluçon, France). Total fatty acids (TFA) were measured under the same conditions after alkaline hydrolysis (KOH-ethanol) and methylation with methanol-HCl (Garcia *et al*, 1986).

Vitamins A and E were evaluated by fluorimetric methods (Hansen and Warnick, 1968, 1969) and vitamin D by radioimmunoassay (RIA) (Amersham TKR 558).

Peptide hormones and cAMP were assayed by RIA with polyclonal or monoclonal antibodies prepared against human hormones. The references of kits are given with the results.

RESULTS

Figures 1 and 2 illustrate the hybridoma F₃₄ and A₄₉ growth at short and long-term in medium containing whey fractions or FCS. Without addition of FCS, whey fractions were able to promote ³H-thymidine incorporation into DNA. However, they were less efficient than FCS. When the media were supplemented with 1% FCS and 9% whey fractions, the cell proliferation in the long-term was possible. It was as effective as with 10% FCS. The antibodies secretion was measured on the 7th d (A₄₉) or on the 9th d (F₃₄). Table I presents the results. Antibodies concentra-

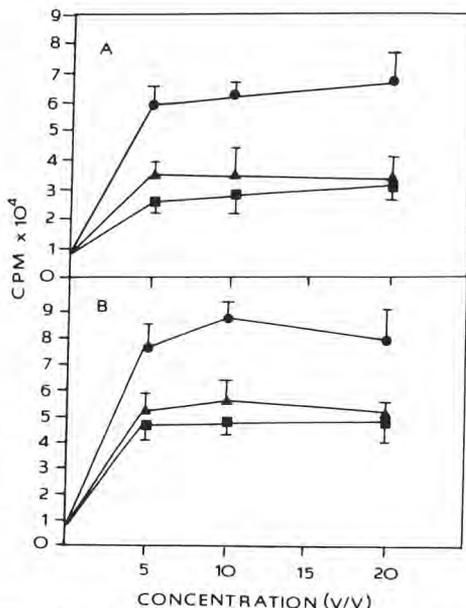


Fig 1. Cell growth measured as ³H-thymidine incorporation into DNA. Cell concentration: 7 x 10⁴ cells/ml in microplate of 96 wells. Each point represents mean and standard deviation of 4 replicates. A: hybridoma F₃₄; B: hybridoma A₄₉; ●—● fetal calf serum (FCS), ▲—▲ LBS65, ■—■ LELm.

Mesure de la croissance cellulaire par l'incorporation de ³H-thymidine dans l'ADN. Concentration cellulaire de départ: 7 x 10⁴ cellules/ml dans des microplaques de 96 puits. Chaque point représente la moyenne et l'écart type de 4 essais. A: hybridomes F₃₄; B: hybridome A₄₉. ●—● sérum de veau foetal, ▲—▲ LBS 65, ■—■ LELm.

tion is as important in medium with whey fraction as in medium supplemented with FCS.

Table II gives the composition in proteins and non-protein nitrogen compounds of whey fractions and FCS. The levels of various compounds were lower in LELm, which has been less concentrated. However, LBS65 and LELm were as effective in promoting growth.

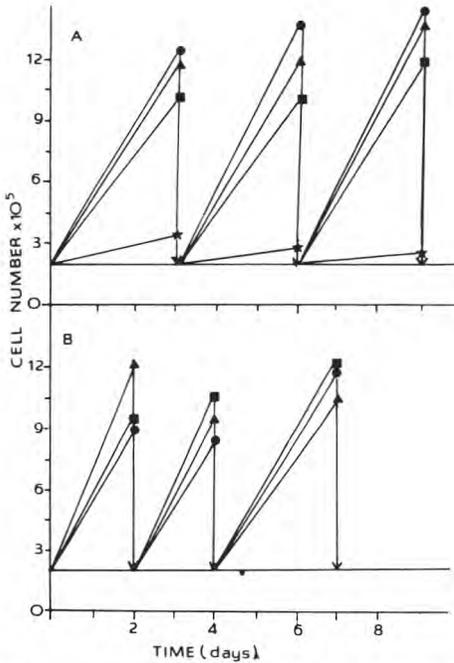


Fig 2. Cell proliferation in the long term. For each passage cell suspension was brought to 2×10^5 cells/ml and seeded in 25 cm² Falcon flasks. Each point is the mean of 3 counts. A: hybridoma F₃₄; B: hybridoma A₄₉; ●—● FCS 10%; ○—○ FCS 1%; ▲—▲ LBS65, ■—■ LELm. *Mesure de la prolifération cellulaire à long terme. La concentration cellulaire de départ est de 2×10^5 cellules/ml, elle est ramenée à cette valeur à chaque passage. Culture en flacons de 25 cm². Chaque point est la moyenne de 3 comptages. A: hybridomes F₃₄, B: hybridome A₄₉. ●—● sérum de veau foetal 10%; ○—○ sérum de veau foetal 1%; ▲—▲ LBS65, ■—■ LELm.*

Amino-acids and nitrogen metabolites such as creatinine, uric acid and NH₃, were still present in LBS65 and LELm although whey fractions were ultrafiltered (cut-off 10 000) and washed.

FPLC analysis of proteins (fig 3) showed that α lactalbumin, and β lactoglobulin variants A and B were the princi-

pal proteins of whey fractions. α lactalbumin represents 23.75% (LBS65) or 32% (LELm) of total protein, β lactoglobulin variant A 28% (LBS65) or 10% (LELm) variant B 39% or 21% respectively. Serum albumin and immunoglobulin were found in small amounts. LELm was found to be rich

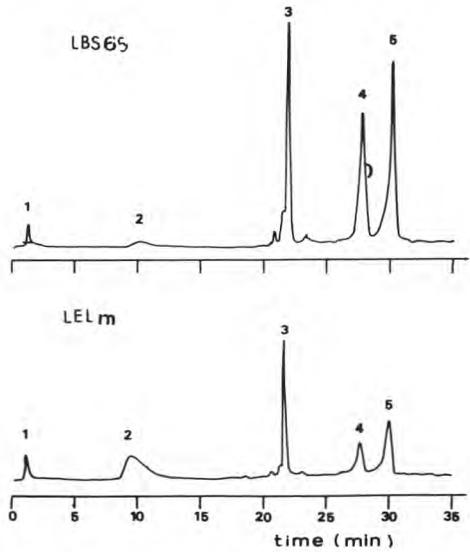


Fig 3. Chromatograms of LBS65 and LELm obtained with FPLC. Experimental conditions: Column Mono Q HP 5/5 (Pharmacia). Elution buffer: buffer A: Tris-HCl 20 mmol/l pH 7, buffer B: Tris-HCl 20 mmol/l pH 7 + NaCl 0.35 mol/l. Elution: buffer A (3 min) linear gradient from 0 to 100% of buffer B (20 min). Buffer B (12 min). Solution of peptides: 10 mg/ml. Injection: 200 μl. 1: immunoglobulins, 2: lactoferrin, 3: α lactalbumin, 4: β lactoglobulin A, 5: β lactoglobulin B. *Analyse par FPLC des protéines contenues dans LBS65 et LELm. Conditions expérimentales: Colonne Mono Q HP 5/5 (Pharmacia). Tampon d'éluion: A: Tris-HCl 20 mmol/l pH 7; B: Tris-HCl 20 mmol/l pH 7 + NaCl 0.35 mol/l. Elution: tampon A (3 min), gradient linéaire de 0 à 100% de tampon B (20 min). Tampon B: 12 min. Injection: 200 μl d'une solution à 10 mg/ml de peptides. 1: immunoglobulines, 2: lactoferrine, 3: α lactalbumine, 4: β lactoglobuline A, 5: β lactoglobuline B.*

Table I. Antibody concentration measured in medium of A₄₉ and F₃₄ cultures. Medium was supplemented either with FCS (10% and 1%), or with whey fractions (9% plus 1% FCS).

Concentration en anticorps mesurée dans le milieu des cultures A₄₉ et F₃₄. Le milieu était supplémenté soit en FCS (10% et 1%), soit en fractions de lactosérum (9% + 1% FCS).

RPMI 1 640 supplemented with:	Antibody concentration (mg/ml)	
	A ₄₉ IgM	F ₃₄ IgG 1
FCS 10%	6.6	4.5
LBS65 9% + FCS 1%	8	6.2
LELM 9% + FCS 1%	6.8	4.7
FCS 1%	—	< 1

in lactoferrin (28.5%) while LBS65 only contained it in a small quantity (3.5%).

Table III comprises data on the mineral composition. The high concentrations of calcium in whey fractions is explained by the addition of calcium during their preparation, and a proportion of this calcium will remain bound to the proteins.

The lipid composition of whey fractions is shown in table IV. Although the milk has been skimmed, whey fractions still contain triglycerides. The ratio of unsaturated/saturated fatty acids differs from 1 fraction to the other. The liposoluble vitamins present in milk have disappeared during whey fraction preparation except for vitamin E which is found in small amounts.

The levels of energetic compound are summarized in table V. Whey fractions were characterized by a very high content of lactose, which was not removed by ultrafiltration at cut-off 10 000.

Data on steroid and peptide hormones is given in table VI. Whey fractions also contain cAMP not found in FCS.

DISCUSSION

Like whole milk, whey fractions were able to promote cell growth. They are less effective than FCS at the same concentration and cell cultures at long-term require at least 0.5–1% of FCS. The hybridoma were stable in medium supplemented with whey fractions, they continued to secrete antibodies as in medium supplemented with 10% FCS.

The whey fractions used in these experiments have different protein contents. The optimal protein concentration to stimulate cell growth seems to be 15–20 g/l (data not shown). Milk fractions contain, as FCS, binding proteins: β lactoglobulin which binds hydrophobic molecules such as free fatty acids, triglycerides, vitamin A or metals (Zn, Fe, Cu) (Spieker-Polet and Polet, 1981). Lactoferrin, which is present in the whey fraction LELM, accounts for a great part in the mitogenic activity of milk (Nichols *et al*, 1989). It is an essential growth factor for human lymphocytic cell lines and it is used to manufacture defined serum-free media which promote growth comparable to media containing serum for various cell lines (Hashizume *et al*, 1983). Lactoferrin is able to bind 2 iron atoms per protein molecule (Masson *et al*, 1966). It is noteworthy that the level of iron in LELM is higher than in LBS65.

LELM has an amino-acid content of 291 μ mol/l while LBS65 contains 3 828 μ mol/l and FCS 4 640 μ mol/l. Levels of amino-acids may not play a great role in the mitogenic activity of whey fractions because the basal medium RPMI 1 640 already contains an excess of amino-acids. The NH₃ content is smaller in whey fractions. This compound is toxic for cells in culture.

Whey fractions, like FCS, include carnitine. Carnitine is essential for the intramitochondrial translocation of fatty acids and branched chain keto-acids and for the

Table II. Concentration of nitrogen compounds contained in whey fraction LBS65 and LELm compared to FCS. Detection threshold of each method is also given. Ind: not detectable; ND: not determined.

Concentration en composés azotés des fractions de lactosérum LBS65 et LELm comparée au FCS. Le seuil de détection de chaque méthode est également indiqué; Ind : non détectable; ND : non déterminé.

Component	Detection threshold	FCS	LBS65	LELm
Total proteins (g/l)	5	38	42	11
Free amino acids ($\mu\text{mol/l}$) :	1			
Ala	—	938	509	16
Amino-butyric acid	—	73	75	—
Arg	—	21	48	10
Asn + Asp	—	86	388	43
Citrulline	—	81	116	5
Cys	—	—	—	—
Glx	—	662	445	64
Gly	—	693	459	32
His	—	10	80	10
Hydroxyproline	—	133	Ind	Ind
Ile	—	110	61	Ind
Leu	—	246	97	2
Lys	—	215	114	14
Met	—	33	28	Ind
Orn	—	216	257	15
Phe	—	121	45	Ind
Pro	—	226	74	Ind
Ser	—	168	564	28
Tau	—	165	127	11
Thr	—	118	151	9
Tyr	—	59	133	11
Val	—	266	107	20
Total A A ($\mu\text{mol/l}$)	—	4 640	3 828	291
NH ₃ (mg/l)	0.100	38.1	24.4	9.2
Creatinine (mg/l)	2	31	13	8
Uric acid (mg/l)	10	26	ND	15
L Carnitine ($\mu\text{mol/l}$)	—	61	180	30
Histamine (nmol/l)	1	Ind	26	ND

elimination of toxic metabolites of acyl CoA excess (Borum, 1988). Carnitine is normally derived from dietary intake or endogenous synthesis *in vivo*. Synthesis occurs to a significant extent, but only in the liver and kidney in the human being (Cederblard *et al*, 1979). Carnitine could

be a limiting factor for energy supply from fatty acids *in vitro*.

The mineral content of whey fractions differs from one to the other and it is very different from the FCS mineral content. LELm and particularly LBS65 have a high calcium concentration. A major proportion

Table III. Osmolarity, pH, and mineral levels in whey fractions LBS65 and LELm compared to FCS. *Osmolarité, pH et concentration en minéraux des fractions de lactosérum LBS65 et LELm comparés au FCS.*

<i>Component</i>	<i>Detection threshold</i>	<i>FCS</i>	<i>LBS65</i>	<i>LELm</i>
Osmolarity (mOsm/l)		304	138	57
pH		8.02	6.75	7.21
Na (g/l)	0.230	3.22	0.437	< 0.230
K (g/l)	0.011	0.39	0.39	0.39
Cl (g/l)	0.355	3.51	0.96	0.39
Ca (mg/l)	5	128	800	207
P (mg/l)	5	81	205	71
Mg (mg/l)	5	ND	82	73
Fe (mg/l)	0.2	1.63	0.49	1.58
Cu (mg/l)	0.104	< 0.104	< 0.104	0.100
Zn (mg/l)	0.130	ND	0.5	0.150
Se (µg/l)	5	< 5	< 5	< 5

Table IV. Lipids, fatty acids, liposoluble vitamins contained in whey fractions LBS65 and LELm compared to FCS. Ind: not detectable. *Concentrations en lipides, acides gras, vitamines liposolubles des fractions de lactosérum LBS65 et LELm comparées à celles du FCS. Ind = non détectable.*

<i>Component</i>	<i>Detection threshold</i>	<i>FCS</i>		<i>LBS65</i>		<i>LELm</i>	
Cholesterol (mg/l)	50	360		190		60	
Triglycerides (mg/l)	200	900		1 740		1 410	
Fatty acids (mg/l)	1	Free	Total	Free	Total	Free	Total
Myristic	–	Ind	–	25	–	4	–
Palmitic	–	34	84	46	263	12	224
Palmitoleic	–	Ind	17	2	14	5	16
Phytanic	–	Ind	Ind	Ind	4	Ind	Ind
Stearic	–	39	39	32	95	7	202
Oleic	–	48	113	23	226	7	150
Linoleic	–	16	16	1	42	15	23
Linolenic	–	Ind	–	Ind	–	–	–
Eicosatrienoic	–	–	Ind	–	7	–	Ind
Arachidonic	–	–	22	–	7	–	Ind
Total	–	137	291	129	658	50	615
Ratio unsat/sat	–	0.87	1.20	0.25	0.81	1.17	0.44
Vitamin D (µg/l)	4	< 4		< 4		< 4	
Vitamin E (mg/l)	0.2	1.1		0.6		0.4	
Vitamin A (µg/l)	90	< 90		< 90		< 90	

Table V. Energetic component concentration in whey fractions compared to FCS.
Concentration en composants énergétiques des fractions de lactosérum comparée à celle du FCS.

Component	Reference kits	Detection threshold	FCS	LBS65	LELm
Glucose (mg/l)	Biotrol ref A02463	70	860	90	50
Galactose (mg/l)	Boeringher ref 124273	50	< 50	< 50	56.5
Lactose (g/l)	CPG	0.100	—	120	16
Pyruvate (mg/l)	Boeringher ref 124982	0.8	1.3	< 0.8	< 0.8
Lactate (mg/l)	Boeringher ref 256773	13	190	1 481	73

of this calcium could be bound to proteins (Harzer *et al*, 1986), which may be the reason for the nontoxicity of calcium. Iron and zinc are found in whey fractions. The iron concentration is higher than that reported by Blanc (1981) in whole bovine milk. It is known to stimulate cell growth (Perez-Infante and Mather, 1982).

Although the milk was skimmed before use, whey fractions still contain cholesterol and triglycerides. It is well established that cholesterol is essential for the growth of mammalian cells in culture (Chen, 1984). It plays a role in maintaining the integrity of the cells and regulating the fluidity of cellular membrane lipids. Most cultured cells are able to synthesize cholesterol, but they also efficiently utilize cholesterol from the medium. The cholesterol supplementation was variable with the cell lines: 7.5 µg/ml for human fibroblasts (Ostlund and Yang, 1985), 40 µg/ml for human renal cells (Gonzalez *et al*, 1974), 1.9 µg/ml for BSC1 and human heart cells. However, auto-oxidation of cholesterol occurs during handling of culture media and oxysterols formed inhibit sterol synthesis and cell

growth (Kandutsch *et al*, 1978). The whey fractions added to medium provide 17.1 µg/ml of cholesterol (LBS65) or 5.4 µg/ml (LELm) while FCS supplies 36 µg/ml. However, cholesterol must be transported by specific proteins to be biologically active; perhaps β lactoglobulin plays this role ?

FFA are also present in milk fractions. FFA may be required for cell growth as nutrient precursors, structural elements of cell membranes and prostaglandin precursors. Fatty acids can be synthesized by cells, but the cells preferentially use performed FFA from the medium. The FA composition of culture medium greatly influence the FA composition of cells, particularly FA of membrane phospholipids (Delplanque and Jacotot, 1987), FFA of whey fractions may be carried by proteins such as serum albumin and β lactoglobulin (Spieker-Polet and Polet, 1981) and transferred from these proteins to the cells. The percentage of unsaturated FFA is higher in LELm than in LBS65 or in FCS.

Liposoluble vitamins normally present in the whole milk (Blanc, 1981) are largely lost during milk treatment.

Table VI. Steroid and peptidic hormone contents of whey fraction compared to FCS. ND : not determined.*Concentration en hormones stéroïdes et peptidiques des fractions de lactosérum comparée à celle du FCS; ND = non déterminé.*

<i>Component</i>	<i>Reference of kits</i>	<i>Detection threshold</i>	<i>FCS</i>	<i>LBS65</i>	<i>LELm</i>
<i>Steroids</i>					
Dihydrotestosterone (ng/l)	Biomérieux 3H-DHT RIA Kit	50	< 50	50	< 50
Testosterone (ng/l)	CIS SB-Testo	80	< 80	< 80	< 80
Cortisol (µg/l)	Immunotech 1114	4.6	< 4.6	< 4.6	< 4.6
17 OH-progesterone (ng/l)	CIS OHP-H	60	< 60	160	130
Progesterone (ng/l)	CIS SB-PROG	50	< 50	100	53
<i>Peptides hormones</i>					
ACTH (ng/l)	CIS ACTH-PR	10	10	68	< 10
LH (µg/l)	CIS LHK-PR	0.2	< 0.2	0.55	ND
FSH (µg/l)	CIS FSHKPR	0.3	< 0.3	< 0.3	ND
Insulin (mUI/l)	CIS SB-INSI-S	2.5	4	4	< 2.5
Peptide C (nmol/l)	CIS IA-C-PEP	100 (bovine peptide C)	< 100	< 100	< 100
Prolactin (µg/l)	Immunotech 2121	0.5	20	6	ND
HGH (µg/l)	CIS SB-HGH	0.25	< 0.25	< 0.25	< 0.25
Somatomedin C (nmol/l)	CIS IN-SOMC	2	11	3	< 2
Gastrin (ng/l)	CIS GASK-PR	10	56	33	< 10
<i>Second messenger</i>					
cAMP (nmol/l)	CIS cAMP-K	0.2	1	325	592

The presence of steroid hormones such as glucocorticoids, estrogen and progesterone is demonstrated in whole bovine milk (Koldovsky and Thornburg, 1987).

Amongst steroid hormones, only progesterone and 17-OH-progesterone are present in a measurable quantity in whey fractions. Progesterone has sometimes been added

to defined culture media for hybridoma proliferation (Cleveland *et al*, 1983).

The levels of peptide hormones were underestimated because they were determined by RIA using antibodies against human hormones. Only ACTH and gastrin are present in appreciable amounts in the whey fraction (LBS65). ACTH has been described to stimulate hybridoma proliferation (Murakami *et al*, 1982). Prolactin is at a level lower than that in whole milk (Blanc, 1982).

Various growth factors which contribute to the growth promoting properties of milk have been found in human and bovine milks (Shing and Klagsbrun, 1984). A small amount of Sm-C is left behind in milk fractions. Other growth factors were not measured.

It is noteworthy that whey fractions are rich in cAMP, which seems to be assimilable by cells in culture. Perhaps it is bound to other molecules. In fact, its level decreases during cell proliferation and we have established that it is not merely destroyed during culture time.

Although cleared of casein and ultrafiltered (cut-off 10 000) whey fractions are a complex medium containing various components able to promote cell growth. However, some compounds present in FCS and absent in milk fractions are required in long-term cell culturing.

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