Volume 301, number 2, 202–206 FEBS 10941 April 1992

The expression of lactase enzymatic activity and mRNA in human fetal jejunum

Effect of organ culture and of treatment with hydrocortisone

Marco Villa[a], Daniel Ménard[b], Giorgio Semenza[a] and Ned Mantei[a]

[a]*Department of Biochemistry, Swiss Federal Institute of Technology, ETH Zentrum, CH-8092 Zürich, Switzerland* and [b]*Groupe de Recherche en Biologie du Développement, Département d'Anatomie et de Biologie Cellulaire, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Qué. J1H 5N4, Canada*

Received 27 February 1992

Very sensitive procedures were developed for the parallel determination of intestinal lactase (LPH) activity and the cognate mRNA. Between 14 and 20 weeks of gestation, lactase activity is low and varies only slightly; at 37 weeks, a relatively high level of activity is observed. The amounts of LPH mRNA correlates with the enzymatic activity ($r = 0.64$). Culture of fetal jejunal explants for 5 days induces by itself a 2-fold increase in LPH mRNA, without any significant change in lactase enzymatic activity. This increase may reflect the loss of a negative transcriptional regulation operative in vivo, and suggests an additional post-transcriptional regulatory component. The addition of hydrocortisone (50 ng/ml) during culture induces a doubling of lactase activity without variation in LHP mRNA, indicating a post-transcriptional modulation by hydrocortisone. The intestinal lysosomal acid β-galactosidase activity was shown to be unaffected by hydrocortisone treatment. This observation clearly illustrates that the two intestinal β-galactosidases are regulated differently. Our results suggests a complex developmental regulation of human intestinal lactase and that the perinatal increase in lactase activity could be modulated at a post-transcriptional level by hydrocortisone.

Lactase-phlorizin hydrolase; Intestine; Gene regulation; Development; Transcription

1. INTRODUCTION

Lactase-phlorizin hydrolase (LPH) (EC 3.2.1.23- 62) is a major integral glycoprotein of the small intestinal brush border membrane of most mammals, including man. The complete primary structure of human, rabbit, and rat pre-pro-LPH, and hence of brush border LPH, has been elucidated via cDNA cloning [1,2]. The structure of the human chromosomal gene [3] and its chromosomal location [4] has also been reported. The LPH enzymatic complex consists of a single type of polypeptide chain carrying two separate catalytic sites, one splitting lactose (the 'lactase' proper) and the other splitting aryl- and alkyl-β-glycosides such as phlorizin and β-glycosylceramides ('phlorizin hydrolase') (for reviews see [5,6]). The ontogenetic profile of intestinal lactase is now well documented in mammals such as rat [7] and rabbit [8,9]. LPH activity is detectable in late fetal life, attains maximal activity during the first 2 weeks after birth, and then declines 3–20-fold, reaching adult levels after about 4 weeks. In human, LPH activity is already present at 10 weeks of gestation. However, contrary to the other disaccharidases, lactase remains very low until the last 4–6 weeks of gestation. At this time lactase activity increases markedly, leading to levels at term which are 2–4 times higher than those found in normal infants 2–11 months of age [10]. Although a number of studies have explored the complex biosynthesis and regulation of intestinal LPH [5,6,11,12], especially the mechanism(s) involved in the ontogeny of human LPH remain incompletely understood.

The fact that in the human fetus the serum level of hydrocortisone doubles during the last 4–6 weeks of gestation has often been interpreted as an indication of a possible specific modulatory influence of glucocorticoid hormones on human fetal intestine, specially in relation to the perinatal increase of lactase activity [10,13]. The addition of hydrocortisone to cultured human fetal jejunum (12–14 weeks of gestation), at a concentration comparable to that found in vivo at 35–40 weeks of gestation, leads selectively to an increase in lactase activity [14]. These data represent the first experimental evidence directly implicating glucocorticoid hormones in the regulation of human lactase expression.

In many human populations, lactase activity decreases during childhood leading to adult-type hypolactasia, the most frequent genetically based syndrome in man (for review see [15]). Hypolactasic subjects can

Correspondence address: N. Mantei, Department of Biochemistry, Swiss Federal Institute of Technology, ETH Zentrum, CH-8092, Zürich, Switzerland.

 Published by Elsevier Science Publishers B.V.

code for LPH identical to that of subjects with persistence of lactase [3]. Measurements of the rate of LPH biosynthesis in organ culture of intestinal biopsies suggests there are at least two phenotypes, one in which very little pro-LPH is produced and one in which slow processing of the precursor is the only defect [16,17]. Subjects with the low levels of pro-LPH have been found to produce very little LPH mRNA [18], and a single subject with the slow processing phenotype had a reduced amount of messenger. In another study, however, no clear difference was found at the mRNA level between adults with hypolactasia and those with persistent high-lactase activity, therefore suggesting a post-transcriptional control of lactase expression [9].

Taking advantage of the availability of human lactase cDNA and the possibility of culturing human fetal small intestine in serum-free medium in the presence of hormones [14,19], we investigated the mechanism(s) controlling lactase gene expression in man. In the present work we have applied very sensitive procedures for the precise determination of lactase enzymatic activity and the cognate mRNA levels between 14 and 37 weeks of gestation. We have also investigated and correlated the mRNA levels with those of lactase enzymatic activity in cultured fetal jejunum with and without hydrocortisone in order to take the first step towards elucidating at what level the primary control of lactase gene expression is established.

2. MATERIALS AND METHODS

2.1. *Tissues and organ culture*

Tissues from 10 fetuses varying from 14 to 20 weeks in age (post-fertilization) were obtained after legal abortions. Studies were approved by appropriate Institutional Human Subjects Review Committee. One specimen from a 1 day-old baby born after 37 weeks of gestation was kindly provided by Prof. Stauffer, Kinderspital, Zurich.

The small intestine was cleansed of mesentery, split longitudinally, washed in culture medium and cut into explants (3 × 7 mm). The jejunal explants were cultured in serum-free Leibovitz L-15 medium according to the technique described earlier [19]. After a 3-h period of stabilization for the explants, hydrocortisone (Collaborative Research Inc., MA, USA) was added to the culture medium at a concentration of 50 ng/ml, which has been shown to be the most effective in stimulating lactase activity [14]. Jejunal explants were cultured for 5 days, and the culture medium was renewed after day 1 and then every 2 days. Then, tissues and media were frozen in liquid nitrogen until further processing.

2.2. *Assay of enzyme activities*

The method developed in this work for the precise determination of low LPH activities in crude homogenates is essentially a modification of the procedure first described by Asp and Dahlqvist [20]. Briefly, lactose is used as substrate, and the only other intestinal (enterocytic) activity known to cleave it at an appreciable rate, lysosomal (acid) beta galactosidase, is detected with the specific substrate 2-naphthyl-β-galactoside. The corrected enzymatic activity is then calculated with the aid of the following formula:

$$\text{LPH (at pH 6)} = \text{total lactase (pH 6)} - 0.22 \times (\text{naphthyl-}\beta\text{-galactosidase, pH 4.5})$$

The samples to be analyzed were homogenised (40 s, half maximal speed, with a Polytron mixer, (Brinkmann Instruments Inc., Westbury, NY) at about 20 mg tissue per ml in ice-cold saline (0.9% sodium chloride). Rough protein estimates were first obtained by the method of Bradford [21] using bovin serum albumin (BSA) as standard; the homogenates were then diluted to about 1 mg protein per ml with ice-cold saline and immediately used for the enzymatic asays and for more accurate protein determination by the method of Lowry et al. [22] as modified by Peterson et al. [23], using BSA (0–20 μg) as reference. Enzymatic activities (see below) and protein concentration determinations were both performed in triplicate.

The procedure described below for the total lactase determination represents the spectrophotometric equivalent of the fluorimetric lactase detection assay developed by Dahlqvist and Asp [24]. Crude homogenates were employed for the assay. Heat-inactivated (2 min at 95°C) aliquots were used as sample blanks; calibration references (5 nmol galactose) as well as absolute blanks (water) were incubated and treated in the same way as the normal samples. 20 μl homogenate (or blank or standard) was added to 20 μl lactose solution (56 mM in 0.1 M sodium maleate pH 6.0) and incubated for 60 min at 37°C; the reaction was then terminated by heat inactivation (2 min at 95°C). The galactose detection was performed by the addition of 5 μl NAD solution (10 mg/ml in water), 30 μl 1 M Tris, pH 8.6, and 2 μl *Pseudomonas fluorescens* galactose dehydrogenase suspension (Boehringer, 25 U/ml). The reaction mixture was incubated at room temperature for 30–40 min and then centrifuged for 5 min at 10,000 × *g*; 60 μl supernatant was finally measured at 340 nm against the absolute blank in a spectrophotometer equipped with a 100 μl microcuvette.

The procedure described here for the determination of the acid-β-galactosidase activity is the spectrophotometrical adaptation of the fluorimetric method described by Asp and Dahlqvist [25]. Crude homogenates were also used for the assay. Sample blanks were obtained by separate incubation of the samples in the absence of the substrate; calibration standards (4 nmol 2-naphtol) and absolute blanks (water) were incubated and treated as the normal samples. 20 μl homogenate (or standard or nothing) was added to a freshly prepared mixture of 20 μl 100 mM sodium citrate, pH 4.5, and 5 μl 2-naphthyl-β-galactoside (10 mM in methanol, Aldrich) and incubated for 60 min at 37°C, the reaction was then blocked by the addition of 26 μl 2 M glycine, pH 12. Separately incubated homogenates (without substrate) were added at this point to the appropriate tubes. After centrifugation (15 min at 10,000 × *g*) about 60 μl supernatant was finally read at 345 nm against the absolute blank in a spectrophotometer equipped with a 100 μl microcuvette.

2.3. *RNA analysis*

Total RNA was purified by the urea–lithium chloride method [26] and assayed by an S_1-mapping procedure as described [9]. In most cases it was possible to perform two independent assays with 1 and 3 μg total RNA.

3. RESULTS

Lactase activity and the levels of lactase mRNA are given in Fig. 1 as a function of the gestational age. Lactase activity, as well as lactase mRNA, are present at 14 weeks of gestation and their levels do not significantly vary up to 20 weeks of gestation. However, much higher values are found at 37 weeks of gestation. The developmental profile of lactase activity corresponds to that reported by others [10]. The correlation coefficients between the two parameters is relatively good ($r = 0.64$).

In Fig. 2, jejunal lactase activity (A) and LPH mRNA levels (B) are depicted after 5 days of culture in unsupplemented and hydrocortisone-supplemented medium.

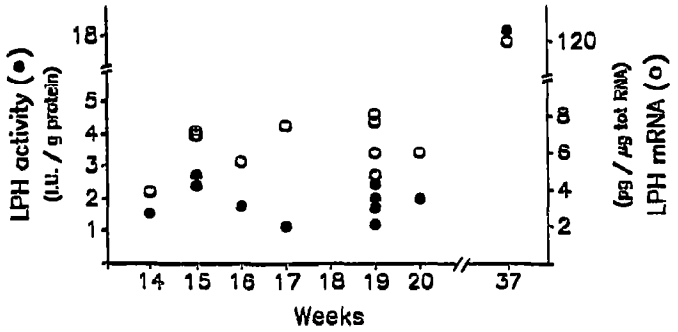

Fig. 1. Levels of lactase activity (●) and of LPH mRNA (○) in developing human jejunum as a function of gestational age in weeks. The correlation coefficient between the two variables is $r = 0.64$.

Table I

Statistical analysis of the effects of organ culture alone or with hydrocortisone on levels of lactase enzymatic activity and LPH mRNA

Condition 1	Condition 2	*P* value*, Condition 2 *vs.* 1 [fold effect]	
		Lactase	LPH mRNA
No organ culture	organ culture	0.455	0.002 [1.9 ×]
No organ culture	organ culture + HC	0.023 [2.1 ×]	0.004 [2.3 ×]
Organ culture	organ culture + HC	0.007 [1.8 ×]	0.198

Fetal jejunum was analyzed without treatment or after 5 days of organ culture with or without 50 ng/ml hydrocortisone (HC). *Conditions 1 and 2 were compared in a paired, two-tailed Student's *t*-test with $n = 10$. In cases where the two conditions differed significantly, the magnitude of the effect is shown in square brackets (condition 2 results divided by condition 1 result).

In control cultures there was no significant variation in lactase activity, whereas a doubling of LPH mRNA level was recorded. The addition of 50 ng/ml hydrocortisone to the culture medium induced a significant increase in lactase activity (2.1-fold) without further increasing the LPH mRNA. Table I summarizes the statistical analysis. In order to assess whether the increased tissue lactase acitivity could have been associated with a modification of the normal release of brush border enzymatic activities during culture [12], lactase activity was determined in the various culture media after 5 days. No significant variation was observed between the control and hydrocortisone-supplemented cultures (data not shown).

Finally, the developmental pattern of the acid-β-galactosidase activity and the effect of hydrocortisone on this enzymatic activity in organ culture was simultaneously studied (Fig. 3). Acid-β-galactosidase activity was present at 14 weeks of gestation and remained low up to 20 weeks. Higher enzymatic activity was noted at 36 weeks (Fig. 3A). During organ culture, the enzymatic activity was maintained or slightly decreased after 5 days and no significant effect of hydrocortisone was observed on acid-β-galactosidase activity (Fig. 3B).

4. DISCUSSION

Our observations have some implications for the regulation of lactase expression during the development of the human intestine.

The relatively high level of lactase activity seen at 37 weeks of gestation compared to 14–20 weeks is accompanied by a correspondingly high level of LPH mRNA (Fig. 1), suggesting that the developmental regulation of lactase during the fetal period is, at least in part, at the level of mRNA accumulation. This conclusion is supported by the fact that even within the period of 14 to 20 weeks, wherein activity varies only a little, the enzymatic activity and the LPH mRNA levels are correlated ($r = 0.64$). However, culturing of explanted human fetal jejunum for 5 days induced by itself a 2-fold increase in LPH mRNA without any significant change in lactase enzymatic activity (Fig. 2). The increase in mRNA may reflect the loss in organ culture of a negative transcriptional regulation operating in vivo. The fact that the enzymatic activity did not increase in parallel implies an additional translational or post-translational regulatory component.

We also examined the effect of hydrocortisone on

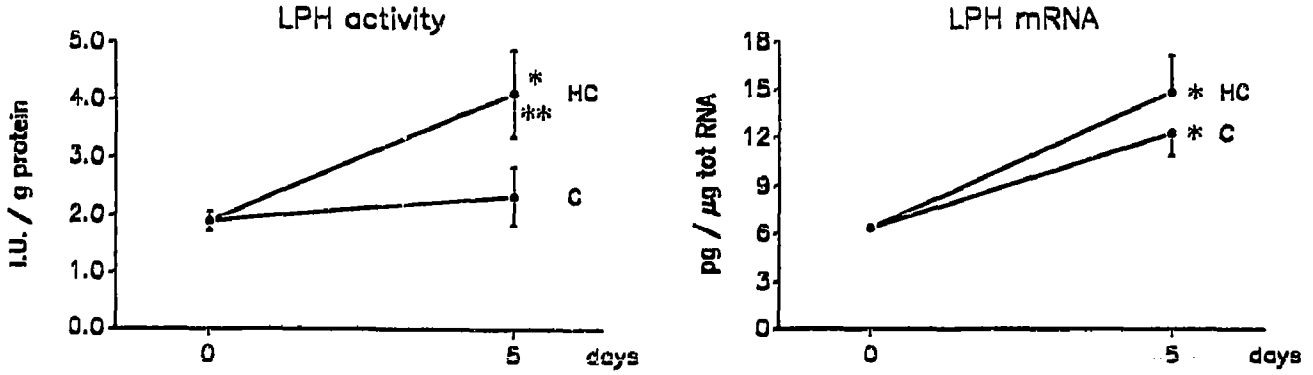

Fig. 2. Effect of hydrocortisone on the level of lactase activity and LPH mRNA during organ culture. *Significant ($P<0.05$) variation between the explants cultured for 5 days compared to control values without culture. **Significant variation between the hydrocortisone-supplemented cultures (line HC) and the 5-day control cultures (line C).

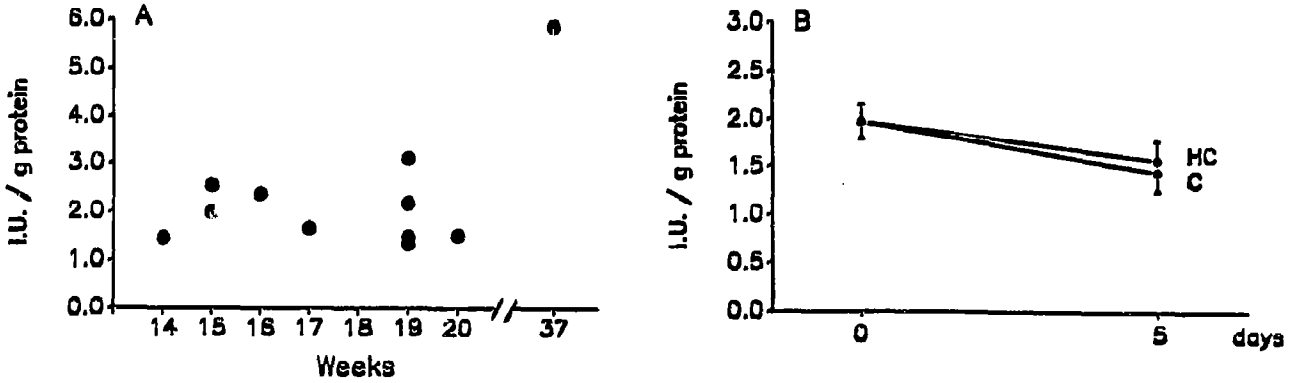

Fig. 3. (A) Acid-β-galactosidase activity in developing human jejunum as a function of gestational age in weeks. (B) Effect of organ culture alone (line C) or in the presence of hydrocortisone (line HC) on the level of acid-β-galactosidase. In all cases the measured activities have been corrected to pH 6 to allow direct comparison with the lactase activities (see Materials and Methods).

lactase expression. In the presence of 50 ng/ml hydrocortisone during 5 days of organ culture, lactase activity doubled, in agreement with previous results [14]. However, this increase was not accompanied by an increase of LPH mRNA beyond that seen during culture without hormone (Fig. 2). These results suggest that hydrocortisone modulates lactase expression at a step beyond mRNA accumulation, possibly post-translationally. In this connection, it is noteworthy that no consensus binding site for glucocorticoid hormone receptor was found upstream of the human LPH gene, at least within the 1 kb segment sequenced [3]. However, the existence of a binding site elsewhere in the gene, e.g. in an intron, remains a possibility. Recently, it has been reported that the thyroxine-induced precocious decline in lactase activity in suckling rats was not correlated with any modification of the mRNA levels, and the authors suggested a post-transcriptional regulation of lactase expression by thyroxine in this animal model [27]. This modulation of lactase expression contrasts with that of another well-known brush border enzyme, namely sucrase-isomaltase. Indeed, dexamethasone (a synthetic glucocorticoid) elicited parallel increases in the levels of sucrase activity and of sucrase–isomaltase mRNA in suckling rabbits which would be, therefore, consistent with a purely transcriptional control [28].

In parallel, we have studied the developmental profile and the effect of hydrocortisone on the intestinal acid-β-galactosidase, a lysosomal enzyme also able to hydrolyze lactose. The recorded profile illustrated in Fig. 3 is in agreement with that reported for many lysosomal enzymes, including acid-β-galactosidase, in developing human fetal jejunum [29]. Few data are available regarding the modulators involved in the development of acid-β-galactosidase activity in rats [30] and none for human. Acid-β-galactosidase activity was found to have decreased after cortisone treatment in suckling rats [30]. In the present investigation, hydrocortisone did not affect the activity of acid-β-galactosidase, while lactase (neutral-β-galactosidase) activity was stimulated. The result clearly illustrates that the two intestinal β-galactosidases are regulated diffrently in developing human jejunum.

The present observations, especially the dissociation of LPH mRNA levels and LPH enzymatic activity in organ culture, could therefore lead to the following suggestion for the interpretation of human LPH development: between 14–20 weeks of gestation there is a negative transcriptional regulation, possibly mediated by a factor which is either not present or is inactivated during culture. This factor declines during the course of gestation, leading to the accumulation of LPH messengers as observed at 37 weeks of gestation. Between 35 and 40 weeks of gestation the doubling of the fetal serum hydrocortisone levels modulates LPH activity expression at a post-transcriptional level. A post-transcriptional control of lactase expression is likely to be a regulatory mechanism for adult life [9].

Acknowledgements: We thank Mr. Werner Boll and Dr. Hans Wacker for the helpful advice. The financial support of the Swiss National Science Foundation, Berne, and the Medical Research Council of Canada (D.M.) is gratefully acknowledged.

REFERENCES

[1] Mantei, N., Villa, M., Enzler, T., Wacker, H., Boll, W., James, P., Hunziker, W. and Semenza, G. (1988) EMBO J. 7, 2705–2713.
[2] Duluc, I., Boukamel, R., Mantei, N., Semenza, G., Raul, F. and Freund, J.N. (1991) Gene 103, 275–276.
[3] Boll, W., Wagner, P. and Mantei, N. (1991) Am. J. Human Genet. 48, 889–902.
[4] Kruse, T.A., Bolund, L., Grzschik, H.H. Ropers, H.H., Sjöström, H., Norén, O., Mantei, N. and Semenza, G. (1988) FEBS Lett. 240, 123–126.
[5] Semenza, G. (1986) Annu. Rev. Cell Biol. 2, 255–313.
[6] Semenza, G. (1987) in: Mammalian Ectoenzymes (Kenny, A.J. and Turner, A.J. eds.) pp. 265–287, Elsevier, Amsterdam.
[7] Doell, R.J. and Kretchmer, N. (1962) Biochim. Biophys. Acta 62, 353–362.
[8] Toofanian, F. (1984) Animal Lab. Sci. 34, 268–271.

[9] Sebastio, G., Villa, M., Sartorio, R., Guzzetta, V., Poggi, V., Auricchio, S., Boll, W., Mantei, N. and Semenza, G. (1989) Am. J. Human Genet. 45, 489–497.
[10] Grand, R.J., Watkins, J.B. and Torti, F.M. (1976) Gastroenterology 70, 790–810.
[11] Buller, H.A., Montgomery, R.K., Sasak, W.V. and Grand, R.J. (1987) H. Biol. Chem. 262, 17206–17211.
[12] Naim, H.Y., Sterchi, E.D. and Lentze, M.J. (1987) Biochem. J. 241, 427–434.
[13] Ménard, D. (1989) in: Human Gastrointestinal Development (Lebenthal, E. ed.) pp. 123–150, Raven Press, NY.
[14] Arsenault, P. and Ménard, D. (1985) J. Pediatr. Gastroenterol. Nutr. 4, 893–901.
[15] Semenza, G. and Auricchio, S. (1989) in: The Metabolic Basis of Inherited Disease (Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D. eds.) pp. 2975–2997, Mc Growth-Hill, NY.
[16] Sterchi, E.E., Mills, P.R., Fransen, J.A.M., Hauri, H.P., Lentze, M.J., Naim, H.Y., Ginsel, L. and Bond, J. (1990) J. Clin. Invest. 86, 1329–1337.
[17] Witte, J., Lloyd, M., Lorenson, V., Korsmo, H. and Olsen, W. (1990) J. Clin. Invest. 86, 1338–1342.
[18] Lloyd, M., Mevissen, G., Fisher, M., Olsen, W., Pitot, H., Genini, M., Boll, W., Semenza, G. and Mantei, N. (1992) J. Clin. Invest. 89, 524–592.
[19] Ménard, D. and Arsenault, P. (1985) Gastroenterology 94, 656–663.
[20] Asp, N.G. and Dahlqvist, A. (1972) Anal. Biochem. 47, 527–538.
[21] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[22] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[23] Peterson, G.L. (1977) Anal. Biochem. 83, 346–356.
[24] Dahlqvist, A. and Asp, N.G. (1971) Anal. Biochem. 44, 654–657.
[25] Asp, N.G. and Dahlqvist, A. (1971) Anal. Biochem. 42, 275–280.
[26] Auffray, C. and Rougeon, F. (1980) Eur. J. Biochem. 107, 303–314.
[27] Freund, J.N., Duluc, I. and Raul, F. (1989) FEBS Lett. 248, 39–42.
[28] Sebastio, G., Hunziker, W., O'Neil, B., Malo, C., Ménard, D., Auricchio, S. and Semenza, G. (1987) Biochem. Biophys. Res. Commun. 149, 830–839.
[29] Antonowicz, I., Chang, S.K. and Grand, R.J. (1974) Gastroenterology 67, 51–58.
[30] Koldovsky, O. and Sunshine, P. (1970) Biochem. J. 117, 467–471.