Regulation of mevalonate 5-pyrophosphate decarboxylase in isolated cells from chick intestinal epithelium

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The present studies were undertaken to determine whether mevalonate 5-pyrophosphate decarboxylase (EC 4.1.1.33) is subject to physiological regulation in the intestinal mucosa. Activity was determined in epithelial cells isolated in a villus-to-crypt gradient from chicks fed on different diets in order to vary the sterol flux across the intestinal epithelium. When animals were fed on cholesterol, decarboxylase activity was decreased in all the cell fractions studied, although percentages of inhibition were maximum in crypts of jejunum and ileum. In contrast, decreased sterol flux as a consequence of cholestyramine feeding stimulated decarboxylase activity, especially in villi of the duodenum, where values increased 3-fold with respect to controls. On the other hand, the total cellular sterol content was significantly increased by the cholesterol diet. In duodenum and jejunum, 20–30 % of the total cholesterol was in the esterified form under these conditions. However, dietary cholestyramine did not significantly affect amounts of total cellular cholesterol in any of the cell fractions. These results demonstrate that mevalonate 5-pyrophosphate decarboxylase activity changes considerably under different dietary situations and that the existence of secondary sites in the physiological regulation of sterol synthesis in the intestinal mucosa should be considered.

INTRODUCTION

Although 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase is considered the rate-limiting enzyme for cholesterol synthesis in mammalian tissues, it has been repeatedly demonstrated that steps that follow mevalonate production are also subject to physiological regulation [1,2]. Consistent with this observation are the findings by Jabalquinto & Cardemil [3] that mevalonate kinase (EC 2.7.1.36), mevalonate 5-phosphate kinase (EC 2.7.4.2) and mevalonate 5-pyrophosphate (MVAPP) decarboxylase (EC 4.1.1.33) are down-regulated by cholesterol feeding, and those by Faust *et al.* [4] that squalene synthetase activity is decreased by low-density lipoprotein. Mitchell & Avignan [5] have reported that mevalonate kinase and MVAPP decarboxylase in human skin fibroblasts are increased when whole fetal-calf serum in the incubation medium was replaced with lipid-deficient serum.

On the other hand, it has been established that the intestine contributes a significant proportion of the total amount of cholesterol synthesized [6]; therefore interest in the regulation of sterol synthesis in this tissue seems justified. However, little is known about the involvement of steps after that catalysed by HMG-CoA reductase, and the nature of the mechanisms that regulate sterol synthesis in this tissue is somewhat controversial. Studies previously performed in our laboratory have demonstrated that both fasting and a cholesterol-enriched diet suppress MVAPP decarboxylase activity in chick duodenum [7], but few other investigations have been performed with regard to the existence of secondary sites of regulation beyond mevalonic acid formation.

An important point to be taken into account when studying the regulation of intestinal sterol synthesis is the

existence of different anatomical regions, both along the length of the intestine and vertically along the axis of the villus. These regions are considerably distinct with regard to their contribution towards total cholesterol synthesis in the small bowel, and, in addition, respond differently to certain dietary situations [8].

The distribution of the activity of MVAPP decarboxylase between the different anatomical regions of the intestinal mucosa has been previously reported [9]. The purpose of the present study was to investigate in detail the possible physiological regulation of MVAPP decarboxylase in chick duodenum, jejunum and ileum. Epithelium cells were isolated sequentially from the villi to the crypts, and the effects of different dietary situations were evaluated.

MATERIALS AND METHODS

Materials

D-[2-¹⁴C]Mevalonate 5-pyrophosphate (ammonium salt) and [6-³H]thymidine were supplied by Amersham International (Amersham, Bucks., U.K.). Cholestyramine, ATP, EDTA and alkaline phosphatase were from Sigma. All other reagents used were of analytical grade.

Animals and diets

White Leghorn male chicks (*Gallus domesticus*), 35 days old, were used. Newborn animals were obtained from a commercial hatchery and fed *ad libitum* on a commercial diet (Sanders A-00) which contained (w/w) 45.2 % carbohydrate (mainly starch), 3.5 % fat and 20.5 % protein. Chicks were maintained in a chamber with light cycle from 09.00 to 21.00 h and with controlled temperature (28 °C). When required, cholesterol (5 %,

Abbreviations used: MVAPP, mevalonate 5-pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.

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w/w) or cholestyramine (3%, w/w) was added to the commercial diet and given to the animals for 5 days before the experiments were started. Food consumption was similar in the three groups of chicks.

Isolation of epithelial cells

Chicks were killed by decapitation at the same time every day (14:00 h). When the light/dark cycle mentioned above was used, no significant differences were found in intestinal MVAPP decarboxylase activity, whereas for other enzymes with a diurnal rhythm of activity, such as HMG-CoA reductase, maximal activity was found at about 14:00 h [10,11]. Because of this, animals were always killed at this time for simultaneous determinations of other enzyme activities than the decarboxylase.

Intestines were removed, chilled and then divided into three segments, designated duodenum (between gizzard and bile-duct junction), jejunum (a 12 cm region from 15 cm after the bile-duct junction) and ileum (a 12 cm region proximal to the ileo-caecal junction). Enriched isolated epithelial-cell fractions from different locations along the villus-crypt axis were prepared as described by Weiser [12], with the modification suggested by Raul *et al.* [13], consisting of the eversion of the intestine fragment before incubation in the chelation buffers. To define the enterocyte fraction isolated after various time intervals, alkaline phosphatase was determined as marker enzyme for differentiated villus cells, thymidine kinase was regarded as an indicator for crypt cells, and acid phosphatase was characteristic for both cell types [14].

Incubations carried out as described by Weiser [12] for 20 min intervals up to a total of 160 min yielded successive cell fractions with varying activities of the marker enzymes. Thus specific activity of alkaline phosphatase decreased gradually in cells progressively harvested, whereas that of thymidine kinase increased in the final cell fraction relative to the initial fraction. Acid phosphatase showed a similar specific activity in all fractions recovered, so that the observed differences in alkaline phosphatase and thymidine kinase were not due to differences in cell stability [15].

According to these results, the various cell fractions were combined in four separate pools, designated as upper (0–40 min), mid (40–80 min) and lower (80–120 min) villus cells and as crypt cells (120–160 min) from duodenum and jejunum. In the ileum, similar pools were designated according to the following time intervals: 0–30 min, 30–60 min, 60–90 min and 90–120 min respectively. These terms represented only a functional classification based on the enzyme content of these groups of enterocytes, and probably should not be taken as a literal indication of the anatomical sources of the various cells [16].

MVAPP decarboxylase assay

This was assayed with [2-¹⁴C]MVAPP as substrate. The incubation mixture consisted of 125 µg of protein, 5 nmol of [2-¹⁴C]MVAPP (0.265 µCi), 3.5 µmol of ATP and 5.5 µmol of MgCl₂ in a final volume of 1 ml in 0.1 M-potassium phosphate buffer (pH 5.7). This pH value was used because the optimum pH of chick MVAPP decarboxylase is between 4.0 and 6.5 [17,18]. At this pH value, formation of alcohol derivatives other than those of isopentenol was minimal, because the optimum pH of isopentenyl-pyrophosphate isomerase (EC 5.3.3.2) was clearly higher (7–8) [19]. Likewise, eukaryotic prenyl-

transferase (EC 2.5.1.1) activities are normally assayed at pH 7.0–7.2 [1,20]. After incubation at 37 °C for 15 min, 9.8 units of bovine alkaline phosphatase (type I) in 1 M-Tris/HCl buffer (pH 8.4) were added to each tube and incubated for another 2.5 h at 37 °C. The final incubation mixture was extracted with 3 × 2 ml of diethyl ether. Samples of the combined extracts were assayed for radioactivity in scintillation fluid containing 0.125 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene and 4 g of 2,5-diphenyloxazole in 1 litre of toluene/ethanol (10:1, v/v). In 'blank' experiments carried out in our laboratory by adding boiled homogenate to the incubation mixture, no radioactivity from [2-¹⁴C]MVAPP was detected in the diethyl ether fraction, whereas all radioactivity was recovered as mevalonate in the aqueous phase. Decarboxylase activity was expressed as nmol of alcohol liberated/min per mg of protein.

Protein contents were determined by the method of Lowry *et al.* [21], with bovine albumin as standard.

Determination of free and esterified cholesterol

Lipids were extracted with chloroform/methanol (2:1, v/v) as described by Folch *et al.* [22]. Total and free cholesterol contents were determined by enzymic colorimetric methods by using 'Test-Combination Cholesterol' or 'Test-Combination Free Cholesterol' respectively, from Boehringer Mannheim G.m.b.H. (Mannheim, Germany).

RESULTS

Studies were undertaken to determine the effect of both cholesterol and cholestyramine feeding on MVAPP decarboxylase in isolated enterocytes obtained from different levels of the mucosa of duodenum, jejunum and ileum. The different diets were used with the idea of altering the flux of sterol across the intestinal epithelial cells. Cholesterol feeding decreases decarboxylase activity considerably in all regions of the intestinal mucosa. Thus, inhibition was 50% in duodenum (Table 1), although this lowering effect was particularly pronounced in the crypt region of jejunum (Table 2) and ileum (Table 3), where activity in cholesterol-fed animals was approx.

Table 1. Effect of cholesterol and cholestyramine feeding on MVAPP decarboxylase activity in villus and crypt cell fractions from chick duodenum

Duodenums of three different animals were used. Enterocytes were isolated in a villus-to-crypt gradient as described in the Materials and methods section. Cells from the same localization were pooled. Homogenates were obtained by sonication and used as enzyme source. Results are expressed as means ± S.E.M. for three determinations. Statistical significance is indicated by ***P < 0.001 for the effect of the different dietary treatments.

Cell fraction	MVAPP decarboxylase specific activity (pmol/min per mg of protein)		
	Control	Cholesterol	Cholestyramine
Upper villus	52.6 ± 0.8	51.1 ± 3.2	169.0 ± 5.7***
Mid villus	84.8 ± 1.3	53.2 ± 1.0***	170.7 ± 6.4***
Lower villus	118.8 ± 2.2	61.6 ± 4.4***	203.9 ± 10.0***
Crypt	136.0 ± 0.8	72.4 ± 3.0***	224.6 ± 15.0***

Table 2. Effect of cholesterol and cholestyramine feeding on MVAPP decarboxylase activity in villus and crypt cell fractions from chick jejunum

Portions (12 cm) from jejunums of three different animals were used. Enterocytes were isolated in a villus-to-crypt gradient as described in the Materials and methods section. Cells from the same localization were pooled. Homogenates were obtained by sonication and used as enzyme source. Results are expressed as means $\pm$ S.E.M. for three determinations. Statistical significance is indicated by ** $P < 0.01$ and *** $P < 0.001$ for the effect of the different dietary treatments.

Cell fraction	MVAPP decarboxylase specific activity (pmol/min per mg of protein)		
	Control	Cholesterol	Cholestyramine
Upper villus	44.3 $\pm$ 1.0	26.8 $\pm$ 1.0***	61.3 $\pm$ 2.2**
Mid villus	54.0 $\pm$ 3.5	26.3 $\pm$ 0.3**	63.4 $\pm$ 1.0
Lower villus	68.9 $\pm$ 2.0	30.6 $\pm$ 1.0***	65.1 $\pm$ 1.3
Crypt	83.1 $\pm$ 5.8	33.3 $\pm$ 0.8***	82.6 $\pm$ 3.0

Table 3. Effect of cholesterol and cholestyramine feeding on MVAPP decarboxylase activity in villus and crypt cell fractions from chick ileum

Portions (12 cm) from ileums of three different animals were used. Enterocytes were isolated in a villus-to-crypt gradient as described in the Materials and methods section. Cells from the same localization were pooled. Homogenates were obtained by sonication and used as enzyme source. Results are expressed as means $\pm$ S.E.M. for three determinations. Statistical significance is indicated by * $P < 0.05$ and *** $P < 0.001$ for the effect of the different dietary treatments.

Cell fraction	MVAPP decarboxylase specific activity (pmol/min per mg of protein)		
	Control	Cholesterol	Cholestyramine
Upper villus	48.6 $\pm$ 2.4	41.9 $\pm$ 0.7	54.0 $\pm$ 0.6
Mid villus	50.4 $\pm$ 5.9	31.1 $\pm$ 3.6*	72.2 $\pm$ 2.7*
Lower villus	68.6 $\pm$ 6.3	39.3 $\pm$ 1.6*	80.0 $\pm$ 1.9
Crypt	84.7 $\pm$ 0.2	34.5 $\pm$ 2.2***	95.6 $\pm$ 5.1

30% that of controls. Hence the situation that arises in conditions of cholesterol feeding is that the crypts, mainly in jejunum and ileum, are no longer the site of maximum specific decarboxylase activity, but show similar values to those observed in the upper villus.

In order to decrease cholesterol absorption across the epithelial cells, 3% cholestyramine was included in the diet. Feeding this polymer increased decarboxylase activity pronouncedly in most of the cell fractions studied, although this effect was greater in cells from the duodenum (Table 1), but there were only small differences between values for control and cholestyramine-fed in cells from the jejunum (Table 2) and ileum (Table 3).

With regard to the cholesterol content of cells from the different regions, cholesterol feeding resulted in enhanced contents of total cholesterol in duodenum (Table 4) and jejunum (Table 5), whereas in ileum (Table 6) this increase was smaller. These changes were due to an increase in free and especially esterified cholesterol, a fraction that reached approx. 30% of total cholesterol in cells from the duodenum of cholesterol-fed animals. In contrast, decreasing the sterol flux by cholestyramine feeding had practically no effect on the cellular contents of free and esterified cholesterol.

DISCUSSION

The results presented here give detailed information about the regulation of MVAPP decarboxylase in different regions of the chick intestine. Cholesterol feeding resulted in an increase in the cellular sterol content and an inhibition of decarboxylase activity in almost all the cell fractions studied. A previous report indicated a 20–30% decrease in decarboxylase activity in duodenum segments as a consequence of cholesterol feeding [7]. In the present study, inhibition was nearly 70% in crypt cells of jejunum and ileum, and 50% in duodenum, observations that demonstrate the convenience of performing studies taking into account the different anatomical distributions of cholesterologenesis activities in the intestine. On the other hand, it is clear that, in the chick, dietary cholesterol inhibits the activity of enzymes involved in sterol synthesis in the intestinal mucosa, in contrast with the rat, where cholesterol feeding did not affect the rate of synthesis [23] or HMG-CoA reductase

Table 4. Effect of cholesterol and cholestyramine feeding on cholesterol content in villus and crypt cell fractions from chick duodenum

Duodenums of three different animals were used. Enterocytes were isolated in a villus-to-crypt gradient as described in the Materials and methods section. Cells from the same localization were pooled. Homogenates were obtained by sonication and used for determinations of free and total cholesterol. Results are expressed as means $\pm$ S.E.M. of three determinations. Statistical significance is indicated by ** $P < 0.01$ and *** $P < 0.001$ for the effect of the different dietary treatments.

Treatment ...	Cholesterol content (μ g/mg of protein)								
	Control			Cholesterol			Cholestyramine		
	Total	Free	% esterified	Total	Free	% esterified	Total	Free	% esterified
Upper villus	19.4 $\pm$ 0.2	16.7 $\pm$ 0.2	14	41.2 $\pm$ 0.2***	29.1 $\pm$ 1.6**	29	19.7 $\pm$ 0.7	16.6 $\pm$ 0.4	16
Mid villus	23.2 $\pm$ 0.7	21.5 $\pm$ 0.5	7	55.4 $\pm$ 2.0***	39.1 $\pm$ 2.3**	29	20.6 $\pm$ 0.9	17.5 $\pm$ 0.8	15
Lower villus	24.8 $\pm$ 0.8	22.1 $\pm$ 1.1	11	51.6 $\pm$ 2.3***	36.6 $\pm$ 0.1***	29	22.0 $\pm$ 0.6	19.5 $\pm$ 0.3	11
Crypt	21.5 $\pm$ 0.1	19.9 $\pm$ 0.5	8	52.2 $\pm$ 2.1***	36.7 $\pm$ 0.5***	30	21.1 $\pm$ 0.9	20.0 $\pm$ 1.7	7

Table 5. Effect of cholesterol and cholestyramine feeding on cholesterol content in villus and crypt cell fractions from chick jejunum

Portions (12 cm) from jejunums of three different animals were used. Enterocytes were isolated in a villus-to-crypt gradient as described in the Materials and methods section. Cells from the same localization were pooled. Homogenates were obtained by sonication and used for determinations of free and total cholesterol. Results are expressed as means $\pm$ S.E.M. for three determinations. Statistical significance is indicated by $^{**}P < 0.01$ and $^{***}P < 0.001$ for the effect of the different dietary treatments.

Treatment...	Cholesterol content ($\mu\text{g}/\text{mg}$ of protein)								
	Control			Cholesterol			Cholestyramine		
	Total	Free	% esterified	Total	Free	% esterified	Total	Free	% esterified
Upper villus	25.5 $\pm$ 0.9	24.9 $\pm$ 0.3	2	50.0 $\pm$ 0.4 ***	40.4 $\pm$ 0.9 ***	19	28.7 $\pm$ 1.2	27.7 $\pm$ 1.2	3
Mid villus	26.2 $\pm$ 0.2	25.4 $\pm$ 1.1	3	41.1 $\pm$ 0.9 ***	34.4 $\pm$ 1.0 **	16	28.0 $\pm$ 0.8	26.8 $\pm$ 1.9	4
Lower villus	23.8 $\pm$ 0.7	23.4 $\pm$ 0.5	2	45.9 $\pm$ 1.8 ***	37.0 $\pm$ 0.4 ***	19	27.6 $\pm$ 1.4	26.6 $\pm$ 1.2	4
Crypt	28.4 $\pm$ 0.9	26.6 $\pm$ 1.6	6	42.6 $\pm$ 1.2 ***	35.2 $\pm$ 0.2 **	17	25.3 $\pm$ 0.7	23.8 $\pm$ 0.4	6

Table 6. Effect of cholesterol and cholestyramine feeding on cholesterol content in villus and crypt cell fractions from chick ileum

Portions (12 cm) from ileums of three different animals were used. Enterocytes were isolated in a villus-to-crypt gradient as described in the Materials and methods section. Cells from the same localization were pooled. Homogenates were obtained by sonication and used for determinations of free and total cholesterol. Results are expressed as means $\pm$ S.E.M. for three determinations. Statistical significance is indicated by $^{*}P < 0.05$ and $^{**}P < 0.01$ and $^{***}P < 0.001$ for the effect of the different dietary treatments.

Treatment...	Cholesterol content ($\mu\text{g}/\text{mg}$ of protein)								
	Control			Cholesterol			Cholestyramine		
	Total	Free	% esterified	Total	Free	% esterified	Total	Free	% esterified
Upper villus	19.5 $\pm$ 0.1	19.1 $\pm$ 1.4	2	18.4 $\pm$ 0.7	17.8 $\pm$ 0.5	3	18.7 $\pm$ 0.5	17.2 $\pm$ 2.2	8
Mid villus	21.6 $\pm$ 0.4	20.7 $\pm$ 0.4	4	26.5 $\pm$ 1.1 *	22.5 $\pm$ 0.5 *	15	21.2 $\pm$ 0.5	19.6 $\pm$ 0.1	8
Lower villus	23.5 $\pm$ 0.3	22.8 $\pm$ 0.6	3	31.0 $\pm$ 1.1 **	28.1 $\pm$ 0.8 **	9	20.2 $\pm$ 0.8 *	18.8 $\pm$ 1.0 *	7
Crypt	18.1 $\pm$ 0.4	16.7 $\pm$ 0.1	8	29.3 $\pm$ 0.9 ***	25.7 $\pm$ 0.2 ***	12	19.3 $\pm$ 0.5	17.0 $\pm$ 0.3	12

activity [24]. In this sense, the chick is reported to be highly sensitive to dietary cholesterol [25]. Cholesterol supplementation of the diet induces in chickens a constant and significantly elevated hypercholesterolaemia [26]. It is noteworthy that HMG-CoA reductase from chick duodenum is also strongly inhibited by cholesterol feeding [27].

When chicks were given cholestyramine, there were significant increases in the decarboxylase activity in all cell fractions of the duodenum, whereas in jejunum and ileum no increase was observed in the lower villus and crypt fractions. Maximal activation of decarboxylase activity in conditions of decreased luminal cholesterol uptake was observed in villus cells of the duodenum, where values were increased 3-fold with respect to controls. As the standard diet used in this study is essentially cholesterol-free, luminal sterol under these conditions is derived from biliary secretion and from the mucosa itself via desquamation of epithelial cells or direct secretion [28]. On the other hand, the absorption of nutrients is well known to take place in the villi, and in the chick the proximal region of the intestine is the preferential site of cholesterol absorption [29].

It is possible that the marked increase in decarboxylase

activity, especially in villi of the duodenum, is to compensate for decreased cholesterol uptake, in order to maintain intracellular cholesterol needs in conditions of decreased cholesterol flux across the intestinal mucosa. This idea would be confirmed by the fact that cholesterol contents remain practically unaltered in cells from the cholestyramine-fed animals. The nature of the physiological regulator involved is not clear. Either intracellular cholesterol and/or decreased exposure of these absorptive villus cells to bile acids may be responsible for the effects of cholestyramine feeding.

In summary, these data show that MVAPP decarboxylase is subject to regulation by cholesterol and cholestyramine feeding in chick enterocytes. Changes observed in intestinal decarboxylase activity are similar to those observed in HMG-CoA reductase. Thus supplementation of the diet with 2% cholesterol suppressed the increase observed in the intestinal reductase from normally fed chicks during the first days after hatching, whereas cholestyramine feeding clearly increased intestinal reductase [27]. Therefore the existence of secondary sites of regulation should be taken into account when studying the physiological regulation of sterol synthesis in the intestinal mucosa.

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REFERENCES

- Slakey, L. L., Craig, M. G., Beytia, E., Briedis, A., Feldbruege, D. H., Dugan, R. E., Qureshi, A. A., Subbarayan, C. & Porter, J. W. (1972) *J. Biol. Chem.* **247**, 3014-3022
- Ramachandran, C. K. & Shah, S. N. (1976) *Biochem. Biophys. Res. Commun.* **69**, 42-47
- Jabalquinto, A. M. & Cardemil, E. (1981) *Arch. Biochem. Biophys.* **210**, 132-139
- Faust, J. R., Goldstein, J. L. & Brown, M. S. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5018-5022
- Mitchell, E. D., Jr. & Avignan, J. (1981) *J. Biol. Chem.* **256**, 6170-6173
- Turley, S. D., Andersen, J. M. & Dietschy, J. M. (1981) *J. Lipid Res.* **22**, 551-569
- Gonzalez-Pacanowska, D., Marco, C., Garcia-Martinez, J. & Garcia-Peregrin, E. (1985) *Biochim. Biophys. Acta* **833**, 449-455
- Dietschy, J. M. & Siperstein, M. D. (1965) *J. Clin. Invest.* **44**, 1311-1327
- Iglesias, J., Gonzalez-Pacanowska, D., Caamaño, G. & Garcia-Peregrin, E. (1988) *Lipids* **23**, 291-294
- Ramírez, H., Alejandre, M. J. & García-Peregrín, E. (1982) *Lipids* **17**, 434-436
- Ramírez, H., Alejandre, M. J., Zafra, M. F., Segovia, J. L. & García-Peregrín, E. (1984) *Nutr. Rep. Int.* **30**, 725-734
- Weiser, M. M. (1973) *J. Biol. Chem.* **248**, 2536-2541
- Raul, F., Simon, P., Kedlinger, M. & Haffen, K. (1977) *Cell Tissue Res.* **176**, 167-178
- Fortin-Magana, R., Hurtwitz, R., Herbst, J. J. & Kretchner, N. (1970) *Science* **167**, 1627-1628
- Merchant, J. L. & Heller, R. A. (1977) *J. Lipid Res.* **18**, 722-733
- Stange, E. F. & Dietschy, J. M. (1983) *J. Lipid Res.* **24**, 72-82
- González-Pacanowska, D., García-Martínez, J. & García-Peregrín, E. (1981) *Enzyme* **26**, 136-143
- Alvear, M., Jabalquinto, A. M., Eyzaguirre, J. & Cardemil, E. (1982) *Biochemistry* **21**, 4646-4650
- Satterwhite, D. M. (1985) *Methods Enzymol.* **110**, 92-98
- Rilling, H. C. (1985) *Methods Enzymol.* **110**, 145-152
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497-509
- Stange, E. F., Suckling, E. E. & Dietschy, J. M. (1983) *J. Biol. Chem.* **258**, 12868-12875
- Shefer, S., Hauser, S., Lapar, V. & Mosbach, E. H. (1973) *J. Lipid Res.* **14**, 400-405
- Kritchevsky, D. (1975) *Handb. Exp. Pharmacol.* **41**, 215-220
- Chandler, R. F., Hooper, S. N. & Ismail, H. A. (1979) *Can. J. Pharm. Sci.* **14**, 15-17
- Alejandre, M. J., Ramírez, H., Segovia, J. L. & García-Peregrín, E. (1985) *Ann. Nutr. Metab.* **29**, 111-118
- Thompson, A. B. R. & Dietschy, J. M. (1981) in *Physiology of the Gastrointestinal Tract* (Johnson, L. R., ed.), pp. 1147-1220, Raven Press, New York
- Sklan, D., Budowsky, P., Ascarelli, I. & Hurtwitz, S. (1973) *J. Nutr.* **103**, 1299-1305

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