Cholesterol synthesis in freshly isolated human leukocytes

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Summary. Cholesterol synthesis has been studied in human leukocytes shortly after the isolation from healthy subjects. Not delipidated human serum reduced the cholesterol synthesis when added to the incubation medium. A similar effect was obtained when the leukocytes were incubated in the presence of physiological concentrations of low density lipoproteins.

In the last few years, there has been a growing interest in the study of the regulation of cholesterol biosynthesis in human cells growing in culture[1-5]. Recent studies carried out in cultured human fibroblasts have demonstrated that cholesterol biosynthesis in these cells is under the control of serum low density lipoproteins (LDL). LDL bind to specific cells receptors, are internalized by endocytosis and then catabolized. Free cholesterol derived from LDL regulates the synthesis of cholesterol by suppressing the activity of the enzyme 3-methyl-3-glutaryl Coenzyme A reductase (HMG-CoA reductase, E.I. I.I.I.34) which is the rate limiting enzyme of cholesterol biosynthesis[6,7]. In cultured fibroblasts isolated from patients with homozygous familial hypercholesterolemia, the regulation of cholesterol biosynthesis is lacking because of a defect in the specific high affinity receptors for LDL[8-10].

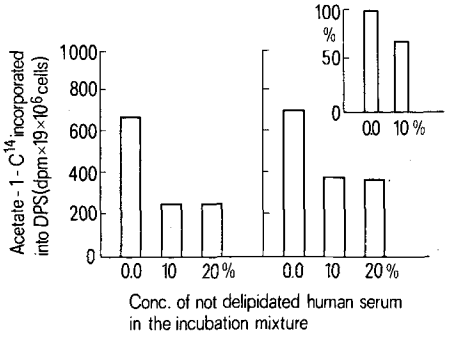

Fig. 1. Leukocytes isolated from 2 healthy donors were incubated in Eagle's medium supplemented with 10% delipidated and defibrinated human AB serum which had been inactivated at 56 °C for 1 h. Full (not delipidated) AB serum was added to the incubation mixture at the start of the incubation at a final concentration of 10 and 20% respectively. Incubation time: 4 h. The inset shows the percent reduction of the incorporation of ^3H_2O into cholesterol by leukocytes incubated with 10% full AB serum. The concentration of total cholesterol in the full serum was 131 mg ml^{-1}.

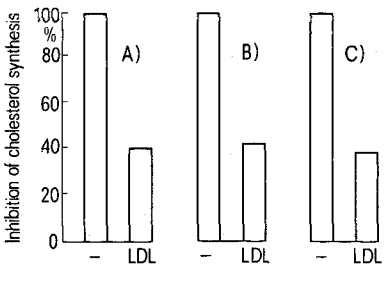

Fig. 2. Freshly isolated leukocytes isolated from 3 healthy donors were incubated in Eagle's medium supplemented with 10% delipidated AB serum with or without the addition of LDL at a final concentration of 560 µg of LDL protein per ml of incubation mixture.

Since the metabolic state of the cultured cells does not necessarily reflect the in vivo situation, we have investigated the synthesis of cholesterol and its regulation in freshly isolated leukocytes on the assumption that their metabolism better represented the situation in vivo.

Materials and methods. 40–60 ml of heparinized human blood were collected in silanized glass cylinders from fasting healthy subjects of both sexes. Leukocytes were separated by the dextran-mixing technique[11] at 4 °C and harvested by low speed centrifugation. After the removal of the contaminating erythrocytes[12] and 2 washings in cold isotonic phosphate buffer (pH 7.5), the cells were suspended in sterile Eagle's medium up to a final concentration ranging from 4 to 15×10^6 cells per ml. Leukocytes were then incubated in Eagle's medium containing 10% lipoprotein free-inactivated human serum at 37 °C in an atmosphere of 95% O_2 and 5% CO_2. As cholesterol precursors, we used either acetate-1-^{14}C (specific activity 266 µCi/0.1 mmole) or mevalonate-2-^{14}C (specific activity 10/µCi/0.1 mmole) or ^3H_2O (2 mCi/mmole). The incorporation of labelled acetate into CO_2, fatty acids and digitonin precipitable sterols was carried out as described previously[13]. Serum low density lipoproteins were isolated by preparative ultracentrifugation[14]. Lipoprotein-free serum was obtained by ultracentrifugation after the isolation of the various lipoprotein classes.

Results and discussion. Freshly isolated human leukocytes were capable of incorporating labelled acetate into CO_2, fatty acids and cholesterol (digitonin precipitable sterols, DPS) for several hours after the isolation from the donors. The rate of incorporation of acetate, mevalonate and tritiated water into cholesterol was found to be linear over a 4-h-period. It should be pointed out that the

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amount of cholesterol and fatty acid synthetized from acetate was very low (from 100 to 160 pmoles $\times 10^7$ cells per h and from 345 to 743 pmoles $\times 10^7$ cells per h respectively) as compared to the amount of acetate incorporated into CO_2 (0.166 to 0.301 μmoles $\times 10^7$ cells per h). Under our experimental conditions, cholesterol synthesis was directly related to the concentration of leukocytes in the incubation mixture.

When leukocytes were incubated in a medium containing full human serum (figure 1), the rate of cholesterol synthesis from acetate was greatly reduced without any significant reduction in the production of CO_2. The incorporation of tritiated water into cholesterol was also reduced but to a lesser extent (figure 1, inset). The addition of fatty-acid-free albumin (40 mg ml^{-1}) to the incubation medium failed to reduce the synthesis of cholesterol.

The addition of serum low density lipoproteins (LDL) at the concentration usually found in normal serum caused a 50% reduction of the rate of incorporation of acetate into cholesterol and a slight (10–20%) reduction of the production of CO_2. When leukocytes were incubated with lower concentrations of LDL (from 25 to 170 μgml^{-1} of LDL protein), the rate of cholesterol synthesis changed very little. The degree of suppression varied from 0 to 15% in leukocytes isolated from different donors. Higher concentrations of LDL did not cause a further reduction of cholesterol synthesis. The addition of ethanol : acetone solutions of free cholesterol up to 100 μgml^{-1} was ineffective in suppressing sterol synthesis in leukocytes under our experimental conditions.

Our findings confirm the observations of Williams et al.[1] and Fogelman et al.[15,16], who originally documented the inhibitory effect of full serum on cholesterol synthesis from acetate in freshly isolated human leukocytes. In addition we found that, as it has been observed in cultured human cells, cholesterol synthesis in freshly isolated leukocytes is reduced by serum low density lipoproteins. Surprisingly, however, the amount of LDL which is needed to cause a 50% reduction of cholesterol synthesis (figure 2) is 10 times higher than that found to be capable of suppressing HMG-CoA reductase and cholesterol synthesis in cultured cells[5,8] and in uncultured leukocytes which had been maintained in a lipoprotein-free medium for several hours prior to the incubation with LDL[17]. One possible explanation for this discrepancy emerges from recent studies carried out in cultured fibroblasts[18] which indicate that cells which had been cultured in a medium deprived of lipoprotein before being exposed to serum LDL possess a high number of high affinity receptors for LDL, whereas those cells which have been pre-incubated in a medium containing LDL or high concentrations of free cholesterol lose their ability to bind LDL at the high affinity sites. It is not surprising, therefore, that freshly isolated leukocytes which had been exposed in vivo to the high concentrations of LDL present in the human serum, showed a low rate of cholesterol synthesis in vitro and required (figure 2) a high concentration of LDL to reduce their synthesis of cholesterol. This interpretation seems in accord with the observation that the number of high affinity binding sites is very low in freshly isolated lymphocytes[19] but it increases if these cells are incubated for 72 h in a medium deprived of lipoproteins[20].

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Effects of hypo- and hyperthyroidism on the activity of cystathionase in mammalian parenchymatous organs during early development

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Summary. In rats after neonatal thyroid destruction, cystathionase in liver and pancreas increased, but the enzyme activity in kidneys decreased. Substitution with thyroxine corrected these changes. Excess of thyroxine, too, had an effect on tissue cystathionase.

In mammalian organism L-cysteine is synthesized from L-methionine through the transsulphuration pathway. The last step in this pathway, cleavage of L-cystathionine into L-cysteine and L-homoserine, is catalyzed by vitamin B_6-dependent enzyme cystathionase (L-cystathionine cysteinelyase [deaminating] EC 4.4.1.1.).

Previous studies have demonstrated the influence of thyroid hormones on the activity of various enzymes in developing mammalian brain[1]. After the destruction of thyroid gland by radioiodine at birth, diminished activity of cystathionine synthase and decreased amounts of L-cystathionine were found in rat brain tissue during early postnatal life, whereas the activity of brain cystathionase remained unaltered[2]. Thus, thyroid hormone supply is necessary for the normal development of transsulphuration in mammalian central nervous system.

The present study reports the effects of radioiodine-induced neonatal hypothyroidism on the activity of cystathionine-cleaving enzyme (cystathionase) in liver, pancreas and kidney of developing rats during 3 first postnatal weeks of life. In addition, the effects of substitution treatment with L-thyroxine were evaluated. The activity of cystathionase in parenchymatous organs was also investigated during experimentally induced hyperthyroidism of newborn rats.

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