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].

15 A. M. Fogelman, J. Edmond, A. Polito and G. Popjack, J. biol. Chem. *248*, 6928 (1973).
16 A. M. Fogelman, J. Edmond, J. Seager and G. Popjack, J. biol. Chem. *250*, 2045 (1975).
17 M. J. P. Higgins, D. S. Lecamwasam and D. J. Galton, Lancet *2*, 737 (1975).
18 M. S. Brown and J. L. Goldstein, Cell *6*, 307 (1975).
19 D. Reichl, A. Postiglione and N. B. Myant, Nature *260*, 634 (1976).
20 Y. K. Ho, M. S. Brown, D. W. Bilheimer and J. L. Goldstein, J. clin. Invest. *58*, 1465 (1976).

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

Kirsti Heinonen

Kuopio University Central Hospital, Kuopio (Finland), 25 March 1977

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.

1 J. Gourdon, J. Clos, C. Coste, J. Dainat and J. Legrand, J. Neurochem. *21*, 861 (1973).
2 K. Heinonen, Biochim. biophys. Acta *399*, 113 (1975).

Materials and methods. L-cystathionine and dithiotreitol were from Calbiochem (Los Angeles, Calif., USA). Pyridoxal-5′-phosphate was from Sigma Chemical Co. (St. Louis, Mo, USA). Carrier-free ^{131}I was from Medica OY (Helsinki, Finland).

Rats of Wistar strain were used. The experiments were performed on split litters, with half of littermates as an experimental group and remaining animals as controls. Immediately after birth, 17 litters were paired into 8 pups each. Half of the animals in each litter were made hypothyroid by a single i.p. injection of radioactive iodine (100 μCi carrier-free ^{131}I)[3]. The litters were kept with their mothers until the experiments were terminated at the 5th, 10th, 15th and 23rd postnatal day.

In order to evaluate the effects of substitution therapy, one group of hypothyroid animals received daily s.c. injections of L-thyroxine, beginning from the 5th postnatal day[4]. Hypothyroid and normothyroid controls received injections of the vehicle only. The experiment was terminated when the animals were 10, 15 and 23 days old.

The effects of L-thyroxine treatment were also examined on split litters. Half the normal rats were given s.c. injections of L-thyroxine as described above. Normothyroid control littermates received injections of the vehicle only. The experiments were terminated when the animals were 10, 15 or 23 days old.

Activity of cystathionase (nmoles L-cysteine/mg protein/h) in liver, pancreas and kidney from rats after neonatal thyroid destruction (HT), from rats receiving substitution therapy with L-thyroxine after neonatal thyroid destruction (HT + ST), from rats treated with daily injections of L-thyroxine (TT) and from normothyroid control rats (NT)

Activity of cystathionase in liver tissue

Age (days)	HT	HT + ST	NT	TT
5	1301 ± 247 (N = 4)		1080 ± 37 (N = 4)	
10	**1367 ± 24 (N = 4)	911 ± 65 (N = 4)	985 ± 62 (N = 4)	***588 ± 21 (N = 4)
15	**1592 ± 90 (N = 4)	964 ± 63 (N = 4)	1009 ± 72 (N = 4)	***607 ± 36 (N = 4)
23	***1652 ± 71 (N = 4)	1001 ± 114 (N = 4)	977 ± 42 (N = 4)	***611 ± 38 (N = 4)

Activity of cystathionase in pancreatic tissue

Age (days)	HT	HT + ST	NT	TT
15	1019 ± 75 (N = 4)	869 ± 59 (N = 4)	850 ± 43 (N = 4)	866 ± 30 (N = 4)
23	***1434 ± 29 (N = 4)	841 ± 68 (N = 4)	886 ± 62 (N = 4)	765 ± 53 (N = 4)

Activity of cystathionase in kidney tissue

Age (days)	HT	HT + ST	NT	TT
10	*168 ± 30 (N = 4)		356 ± 57 (N = 4)	
15	*268 ± 36 (N = 4)	471 ± 17 (N = 4)	412 ± 37 (N = 4)	**804 ± 58 (N = 4)
23	*246 ± 51 (N = 4)	388 ± 56 (N = 4)	420 ± 36 (N = 4)	***762 ± 31 (N = 4)

Significance of difference between experimental and control groups was calculated by Student's t-test ($p \geqslant 0.05$ not significant). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Liver, pancreas and kidneys were dissected at −4 °C. Tissues were prepared for enzyme assay as described previously[5]. The activity of cystathionase was determined by the method of Gaull et al.[6]. The protein concentrations were measured by the method of Lowry et al.[7], with bovine serum albumin as a standard. The activity of cystathionase was expressed as nmoles L-cysteine/mg protein/h.

Results and discussion. In newborn rats, the highest activities of cystathionase are found in liver and pancreas. The activity of cystathionase in kidney is approximately half the values found in liver and pancreatic tissues. Throughout the first 3 weeks of life, the activities of parenchymatous cystathionase remain on similar levels as at birth. This pattern of cystathionase development in the rat differs from that observed in other species[8]. Particularly in human liver from foetuses and newborn infants[9], the activity of cystathionase is very low at birth and increases some time thereafter.

Effects of neonatal thyroid destruction on the activity of cystathionase on parenchymatous tissues from 5-, 10-, 15- and 23-day-old rats are given in the table. At these ages, liver cystathionase is markedly more active in hypothyroid rats than in normothyroid littermates. Similarly, the activity of cystathionase in pancreatic tissues is higher in hypothyroid rats than in normothyroid controls. On the contrary, the activity of cystathionase in kidney tissue is significantly less in hypothyroid animals than in normothyroid littermates. The activities of cystathionase in parenchymatous organs from hypothyroid animals which receive substitution treatment with L-thyroxine are similar to the values observed in tissues from normothyroid controls.

When normothyroid rats were treated with daily injections of L-thyroxine from the 5th postnatal day, some significant changes in the activities of parenchymatous cystathionase were observed at the age of 10, 15 and 23 days. These data are given in the table. The activities of cystathionase in liver tissue are significantly less in thyroxine-treated animals than in normothyroid controls. The activities of pancreatic cystathionase in thyroxine-treated rats are not significantly different from the values observed in control animals. Kidney cystathionase, on the other hand, is markedly more active in thyroxine-treated animals than in control littermates.

Previous studies have shown that the presence of thyroid hormones is necessary for the normal development of transsulphuration enzymes in mammalian brain[2]. The present data indicate that even the activity of cystathionase in mammalian parenchymatous organs is influenced by thyroid destruction or excess of thyroid hormones. These findings further emphasize the importance of adequate thyroid function during critical early development of mammalian organism.

3 R. C. Goldberg and I. L. Chaikoff, Endocrinology *45*, 64 (1949).
4 S. E. Geel and P. S. Timiras, Brain Res. *4*, 135 (1967).
5 S. H. Mudd, J. D. Finkelstein, F. Irreverre and L. Laster, J. biol. Chem. *240*, 4382 (1965).
6 G. E. Gaull, D. K. Rassin and J. A. Sturman, Neuropädiatrie *1*, 199 (1969).
7 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
8 J. A. Sturman, D. K. Rassin and G. E. Gaull, Int. J. Biochem. *1*, 251 (1970).
9 G. E. Gaull, J. A. Sturman and N. C. R. Räihä, Pediat. Res. *6*, 538 (1972).