same[25]. Meanwhile, it is known that the distribution and the number of receptors vary not only with the degree of differentiation of myotubes[26], but also with its state of innervation[27], the same holds true for cholinesterase[28]. Recently is has been shown[10] that the number of acetylcholine receptors of neuromuscular junction-free muscle strips of rat soleus (red) was almost 2 times greater than that of the extensor digitorum longus (white). The difference between the AChE activity in the 2 cultures suggest that this is related to the different structural properties of excitable membranes of pectoralis and adductor muscle differentiated in vitro. These differences in the AChE activity, as in ICDH, show that myoblasts obtained from 2 different homogeneous muscles differentiate, in vitro, under the same conditions of development, into muscle fibres with different functional and metabolic properties. This result between the 2 cultures may be related to the existence of myogenic cells with different potentialities.

In a histochemical study of in vitro differentiated muscle fibres from breast and leg muscles myoblasts of 10-day-old chick embryos, Askanas et al.[11] found no difference in phosphorylase (glycolytic metabolism) staining between the 2 cultures, this result being in agreement with our observation for aldolase, another enzyme of the glycolytic pathway. As we observed significant differences in ICDH activities (citric acid cycle), these authors also observed a difference in succinate dehydrogenase (oxidative metabolism): staining for this enzyme was more pronounced in cultured leg muscles than cultured breast muscle, and this difference could be related to a greater amount of mitochondria as observed in electron micrographs of cultured leg muscles. However, these leg muscle cultures were established from ill defined populations of myogenic cells coming from heterogeneous muscles samples, the majority of them being of mixed fibre populations: In these circumstances it is difficult to relate the observations to the type of myogenic cell. We feel that with our results, particularly in the case of adductor muscle cultures, we can with certainty relate the differences to the type of myoblast from which they originated.

This is evidence, therefore, that myoblasts from potentially different skeletal muscle differentiate in different ways in tissue culture, even in the absence of the nerve supply. This certainly seems to be true as far as enzyme activities are concerned. From a general point of view, this seems to be the expression of those characteristics which are myogenically determined, other characteristics may well be determined by exogenous factors including the nerve supply.

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Reduction of gallstone formation by ascorbic acid in hamsters

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Summary. The addition of 0.5% of ascorbic acid to the lithogenic diet of golden hamsters whose body pool was labelled with 26-^{14}C-cholesterol, lowered the formation of gallstones, the cholesterol concentration and half-life in blood plasma and in the liver, and accelerated cholesterol transformation to bile acids.

Bile acids and lecithin are the major solubilizing agents for biliary cholesterol, the predominant component of at least 90% of gallstones. An initial phase in gallstone formation is a disorder of liver cell metabolism, the production of bile that is supersaturated with cholesterol[1]. Persons with cholesterol gallstones have a decrease in bile acid pool size[2]. The increase in biliary cholesterol secretion and the reduction of bile acid pool size could lead to a decrease in the proportion of bile salts to cholesterol which could result in the precipitation of cholesterol and aggregation of cholesterol crystals into gallstones. A similar metabolic situation occurs in guinea-pigs during a chronic latent vitamin C deficiency: the rate of cholesterol transformation to bile acids in the liver is decreased[3,4] and the size of the bile acid pool is also reduced[5]. Marginal vitamin C deficiency interferes with the biosynthesis of bile acids at the stage of microsomal 7 α-hydroxylation of the cholesterol nucleus[6]. Björkhem and Kallner[7] have suggested that ascorbate affects the synthesis or breakdown of the cholesterol 7 α-hydroxylating system, in particular the cytochrome P-450 component. In scorbutic guinea-pigs, frequent occurence of gallstones was observed[8–10]. These facts have prompted us to consider the potential role of a chronic latent vitamin C deficiency in the pathogenesis of cholelithiasis and the possibility of preventing the formation of gallstones by a permanent supply of vitamin C[11]. The aim of the experiment reported here has been to verify this hypothesis.

Materials and methods. 200 male golden hamsters aged approximately 6 weeks (body weight about 70 g) were put on a semi-purified fat-free, high-glucose diet[12] known to produce cholesterol gallstones. 100 animals were fed this diet without any vitamin C addition, the others had the same diet plus 5 g of ascorbic acid per kg of diet. The

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weight curves and mortality in both the groups were similar (7% within 8 weeks). After 8 weeks, 50 prospering animals with a minimum body weight range (90–110 g), were picked out from each group. Body weight was the only selection criterium. The animals were all given an i.p. injection of 26-^{14}C-cholesterol (Radiochemical Center, Amersham; specific activity 55.8 mCi/mM) emulsified with tween 20 in saline, in a dose of 3.36 μCi per animal. The nutritional regimen in both the groups remained unaltered. At weekly intervals from week 1 to 8 following administration of labeled cholesterol, the 24-h output of $^{14}CO_2$, vitamin C concentration in the liver, the concentration and specific activity of cholesterol in blood plasma and liver were determined by procedures similar to those used in our previous study[4]. 6 animals were autopsied in each week (8 in week 8) of both the groups. The presence of gallstones was registered: the occurence of small stones, approximately 0.2 mm in diameter, was classified as incipient cholelithiasis, while that of a larger number of minute stones or of one large aggregate above 1 mm in diameter was classified as advanced cholelithiasis. The decline of the log specific activity of plasma and liver cholesterol in time from the second week after labelled cholesterol administration proved to be linear (correlation coefficient of log specific activity-time was 0.985 in both the groups). These data served for a calculation of the half-life of plasma and liver cholesterol and further parameters of cholesterol turnover in terms of one-pool model[13]. The rate of cholesterol transformation to bile acids was determined as the ratio of disintegrations in the $^{14}CO_2$ to the cholesterol specific activity similarly as in our preceding study[4]. The results were statistically evaluated by means of standardized programmes (Student's t-test, linear regression, χ^2-test) on a computer.

The effect of vitamin C on the incidence of gallstones and on cholesterol metabolism in golden hamsters fed fat-free high-glucose diet

Parameter	Ascorbic acid in the diet	
	Zero	0.5%
Body weight (g)	92 ± 1	93 ± 1
Animals free of gallstones (%)	32 (16)	56 (28)*
Animals with incipient cholelithiasis (%)	26 (13)	20 (10)
Animals with advanced cholelithiasis (%)	42 (21)	24 (12)*
Ascorbic acid in the liver (mg/100 g wet tissue)	14.7 ± 0.5	26.8 ± 0.6**
Total cholesterol in plasma (mg/100 ml)	210 ± 8	181 ± 4 (49)**
Total cholesterol in the liver (mg/100 g wet tissue)	751 ± 49	582 ± 26**
Cholesterol transformation to bile acids (mg/24 h/100 g b.wt)	2.16 ± 0.12 (32)	2.53 ± 0.11 (30)*
Body pool of miscible cholesterol (mg per animal)	162	128
Half-life of plasma cholesterol (days)	21.5	15.7**
Half-life of liver cholesterol (days)	21.5	16.3**
Cholesterol turnover rate (mg per 24 h per animal)	5.23	5.67
Fractional turnover rate (% of pool renewed in 24 h)	3.2	4.4

Figures represent mean values ± SEM. The number of animals observed for each determination was 50, unless indicated otherwise in parenthesis. *$p < 0.05$–0.02; **$p < 0.01$–0.001.

Results and discussion. The body weight from the time of the administration of labelled cholesterol until death remained unchanged and was almost identical in the 2 groups. Not a single one of the 100 animals perished. $^2/_3$ of the hamsters kept on vitamin C-free diet had gallstones. The addition of ascorbic acid to the diet significantly depressed both the occurence and the degree of experimental cholelithiasis (table). Even though hamsters possess the capacity to synthetize ascorbate in the liver, a long-term administration of an unbalanced diet deprived of vitamin C produced in them a relative vitamin C deficiency: their ascorbate level in the liver was substantially lower than in hamsters fed on cereals and vegetables[14]. The addition of 0.5% ascorbic acid to the diet doubled the vitamin C concentration in the liver. Under the effect of ascorbic acid, the concentration and half-life of cholesterol decreased in the plasma and liver, and simultaneously the rate of cholesterol transformation to bile acids slightly increased. The effect of vitamin C on cholesterol metabolism in hamsters is thus very similar to that seen in guinea-pigs[4,6]. Data from a kinetic analysis of cholesterol turnover in terms of one-pool model overestimate the true size of miscible pools[15]. However, the decrease in the size of miscible pool in animals receiving vitamin C is in good correlation with the decline in the levels of plasma and liver cholesterol.

The metabolic situation developing in hamsters put on a fat-free, ascorbate-free, high-glucose diet, is probably as follows: the intake of enormous quantities of glucose enhances the endogenous synthesis of cholesterol[16]. As a consequence of a deficiency of essential fatty acids and vitamin C, these animals are incapable of compensating for the enhanced cholesterol biosynthesis by increasing its catabolism to bile acids[4,17]. Hypercholesterolemia sets in these animals, with an accumulation of cholesterol in the liver, a prolongation of the half-life of plasma and liver cholesterol, an increase in the ratio cholesterol/bile salts in the bile[12], and ultimately leads to the formation of gallstones. Similarly in guinea-pigs latent ascorbate deficiency increases the propensity for gallstone formation (unpublished results). On the other hand, addition of vitamin C to the diet enhances the concentration of ascorbate in hepatic cells so that the liver becomes at least partially capable of compensating for the enhanced synthesis of endogenous cholesterol by its increased transformation to bile acids. As a result of this, the cholesterol accumulation in the plasma and liver is smaller and the increase in the ratio cholesterol/bile salts in the gallbladder bile is less striking. The overall outcome is a lowered gallstone formation under the effect of vitamin C. The results obtained indicate that saturation of the liver with vitamin C significantly depresses the occurrence of gallstones in the hamster. The key question that remains open is whether these results may be extrapolated to the human organism. Large doses of vitamin C did not significantly alter the chemical composition of the bile in healthy subjects[18], but in these experiments ascorbic acid was unfortunately administered only over a very short period (1–2 weeks).

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