HYPOVITAMINOSIS C AND CHOLELITHIASIS IN GUINEA PIGS

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SUMMARY

Hypovitaminotic C guinea pigs fed a high cholesterol diet for 5 weeks developed gallstones (81% cholesterol by weight), whereas no concretements were observed in vitamin C replete animals fed the same diet. Gallstone formation was associated with qualitative changes in the gallbladder bile, namely, a higher cholesterol concentration, a lowered bile acid content, and diminished phospholipid: cholesterol and bile acid: cholesterol ratios. No differences in 7α-ketolithocholic acid levels between groups was observed, but the chronic hypovitaminotic C animals had significantly lower levels of chenodeoxycholic acid. It is concluded that in the chronically hypotivaminotic C guinea pig there is a reduction in gallbladder bile acid: cholesterol and phospholipid: cholesterol ratios arising from an impaired conversion of cholesterol to chenodeoxycholic acid, and that consequently cholesterol precipitation and gallstone formation are favoured.

INTRODUCTION

The formation of cholesterol gallstones (cholelithiasis) has been induced experimentally in several species of animal by modifications to their diet (1,2,3,4,5). Although studies on the formation of gallstones have been in progress for many years, a complete understanding of the underlying lesion or lesions has not been achieved. It seems clear, however, that cholesterol is held in micellar solution in bile in combination with phospholipids and bile acids, and the postulate that the bile acid: cholesterol and phospholipid to cholesterol ratios determines its solubility rather than the absolute value (6) has been verified in a number of experimental situations. Thus the bile of animals in which cholelithiasis has been induced, has both reduced bile acid: cholesterol and phospholipid: cholesterol ratios (2,5,7). Although experimentally induced vitamin A and, to a lesser extent vitamin D deficiency, have been shown to promote gallstone formation (8), no evidence for a relationship between vitamin C status and gallstone formation has appeared. This is perhaps sur-

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prising in view of the considerable amount of experimental evidence linking vitamin C with cholesterol metabolism (9,10,11,12,13).

During the course of a wide-ranging study of the effects of atherogenic diets in guinea pigs, a high incidence of gallstone formation was observed in animals with latent vitamin C-deficiency. We report here on observations which we believe will advance the understanding of the aetiology of cholelithiasis and which suggest possible preventive and curative measures.

MATERIALS AND METHODS

Twenty-six male guinea pigs (Dunkin Hartley) aged eighteen days and weighing approximately 200 g. were given access to water and a standard pelleted laboratory ad libitum for two weeks, and then transferred to a pelleted high cholesterol (0.5%) vitamin C-deficient diet (Cooper Nutrition). Because it is difficult to distinguish between the metabolic consequences of the inanition induced by vitamin C-deficiency and vitamin C-deficiency per se, a state of chronic hypovitaminosis C was induced in thirteen of the animals by the daily peroral administration of 0.5 mg. l-ascorbic acid (Sigma Ltd.) in 0.2 ml. of 20% sucrose solution. The remaining animals were similarly dosed with 5.0 mg. of the vitamin in the same volume of vehicle. This dietary regimen lasted for five weeks after which time the animals were weighed, anaesthetised with ether, and the bilary tree inspected for concretements. Following ligation of the cystic ducts the gallbladders were removed and the contents centrifuged at 5,000 r.p.m. for 20 minutes to separate the bile from gallstones and associated debris. Bile phospholipids were measured by the method of Zilversmit and Davis (14) and cholesterol by the method of Watson (15) after digitonin precipitation and reconstitution in distilled water. Bile acids were determined by gas liquid chromatography (16) following enzymatic hydrolysis with Clostridium welchii powder (Sigma). The gallstones and associated debris were pooled, washed in distilled water, dried in a vacuum oven, weighed and extracted with ethanol. An aliquot of the extract was partitioned between 70% alcohol and

petroleum ether, and the upper phase analysed for cholesterol in the same manner as described above for bile.

RESULTS AND DISCUSSION

During the experimental period the increase in weight of the vitamin C-replete animals (28 g.) was considerably greater than that of animals subjected to the low vitamin C regimen (7 g.), but inanition was not observed in any of the animals.

Cholesterol gallstones (81% cholesterol by weight) were present in the gallbladders and occasionally in the lumen of both the cystic and common bile duct of ten of the twelve survivors in the hypovitaminotic-C group, whereas no concretements occurred in the guinea pigs receiving 5.0 mg. of the vitamin daily. In the chronic hypovitaminotic C group, bile cholesterol concentration was significantly higher ($P < 0.01$) and bile acid concentration significantly lower ($P < 0.01$) than in the vitamin C-replete group, whereas the phospholipid content did not differ significantly (table 1). Consequently both the bile acid: cholesterol and phospholipid: cholesterol ratios were significantly lower ($P < 0.01$) in the chronic vitamin C deficient group, conditions predisposing to cholelithiasis.

The bile acid profile of the gallbladder bile of both groups of guinea pigs revealed chenodeoxycholic and 7α-ketolithocholic acids as the major components. Other bile acids were present in such minute quantities that they were not readily detectable. No differences in 7α-ketolithocholic acid levels between groups was observed, but the chronic hypovitaminotic C animals had significantly lower levels of chenodeoxycholic acid. It would appear, therefore, that in the vitamin C-deficient guinea pig, cholesterol conversion to chenodeoxycholic acid (the major primary bile acid in this species) is impaired. It has been suggested that an early event in this conversion is the introduction into the cholesterol molecule of an hydroxyl group at C7, and this step, catalysed by cholesterol 7α-hydroxylase, may be rate limiting (17). The 7α-hydroxylase system is located in the microsomal fraction of the liver and

Table 1. Bile acid and cholesterol content of the gallbladder bile of guinea pigs given either 0.5 or 5.0 mg. vitamin C daily. (Values are m.mol/l ± S.E.M.)

Group	Daily dose of vitamin C (mg.)	Phospholipids (P)	Chenodeoxycholic acid (CDC)	7α-ketolithocholic acid (7-KL)	Total bile acid (BA)	Cholesterol (C)	Ratios BA:C	CDC:C	7-KL:C	P:C
A	0.5	4.6 ± 0.6 NS	4.99 ± 0.31 *	4.24 ± 0.26 NS	9.20 ± 0.44*	1.73 ± 0.14*	5.3	2.9	2.5	2.7
B	5	5.2 ± 0.3	8.02 ± 0.54	4.09 ± 0.42	12.10 ± 0.75	0.99 ± 0.15	12.2	8.1	4.1	5.3

* Significantly different from group B values at $P < 0.01$ ("t" test).

NS Not significantly different from group B values at $P > 0.05$ ("t" test).

is cytochrome P450 dependant (18). A reduced ability of the livers of scorbutic guinea pigs to hydroxylate acetanilide *in vitro* has been attributed to either a reduction in the concentration (19) or activity (20) of cytochrome P450. Thus the impaired conversion of cholesterol to chenodeoxycholic acid observed in this study may be due to a vitamin C-related effect on the cytochrome P450 dependant 7α-hydroxylase system.

We conclude, therefore, that in chronic hypovitaminosis C in the guinea pig there is a reduction in gallbladder bile acid: cholesterol and phospholipid to cholesterol ratios arising from an impaired conversion of cholesterol to chenodeoxycholic acid, and that consequently cholesterol precipitation and gallstone formation are favoured. Further, vitamin C appears to be able to prevent cholelithiasis even when dietary cholesterol is high, an observation which may be of considerable significance in view of the high incidence of cholesterol gallstones in Western communities. It remains to be determined whether or not the administration of high doses of the vitamin can effect solubilisation of gallstones.

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REFERENCES

1. Borgman, R.F. Am. J. Vet. Res. 26, 1167-1171 (1965).
2. Caldwell, F.J. Jr., Levitsky, K. and Rosenberg, B. Am. J. Physiol. 209, 473-478 (1965).
3. Christensen, F, and Dam, H. Acta. Physiol. 211, 1069-1074 (1966).
4. Schoenfield, L.G. and Sjövall, K.J. Am. J. Physiol. 211, 1069-1074 (1966).
5. Osuga, T. and Portman, O. Proc. Soc. exptl. Biol. Med. 136, 722-726 (1971).
6. Isaksson, B. Acta. Soc. Med. Upsaliensis. 59, 296-306 (1954).
7. Dam, H. Proc. Intern. Cong. Nutr., Edinburgh. 6-23 (1964).
8. Fortner, J. G. Surgery 36, 932-940 (1954).
9. Chatterjee, G.C. In the Vitamins. Vol. 1. New York and London. (Academic Press) 399-457 (1967).
10. Ginter, E. Science. 179, 702-704 (1973).
11. Ginter, E., Babala, J. and Cerven, J. J. Atheroscler. Res. 10, 341-352 (1967).
12. Ginter, E., Bobek, P. and Ovecka, M. Internat. Z. Vit. Forschung. 38, 104-113 (1967).

13. Hughes, R.E. J. Hum. Nutr. 30, 315-319 (1976).
14. Zilversmit, D.B. and Davis, A.K. J. Lab. Clin. Med. 35, 155-160 (1950).
15. Watson, D. Clin. Chim. Acta. 5, 637-643 (1960).
16. Klassen, C.D. Clin. Chim. Acta. 35, 225-229 (1971).
17. Lindstedt, S. Acta. Chem. Scan. 11, 417 (1957).
18. Boyd, G.S. and Percy-Robb, I.W. Am. J. Med. 51, 580-587 (1971).
19. Degkwitz, E. and Staudinger, H.J. In Vitamin C. (Ed. G.G. Birch and K. Parker). Applied Science Publishers Ltd. London (1974).
20. Fielding, A.M. and Hughes, R.E. Experientia 31, 1394-1395 (1975).