**Bile acids and vitamin A absorption in man: the effects of two bile acid-binding agents, cholestyramine and lignin**

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SUMMARY In 14 healthy volunteers, the addition of 12 g cholestyramine to a vitamin A-containing test meal reduced the expected rise in serum vitamin A by 59.5% (\(p<0.001\)). By contrast, lignin had no significant effect in 12 subjects. This study confirms the importance of bile acids in vitamin A absorption and the ineffectiveness of lignin as a sequestrator of conjugated bile acids.

It is generally accepted that the absorption of fat-soluble vitamins is dependent on the presence of a micellar phase and hence of conjugated bile acids in the intestinal lumen (Hofmann, 1968; Heaton, 1972). It is also well documented that cholestyramine binds conjugated bile acids both in vitro (Johns and Bates, 1970) and in vivo (Kenney and Garbutt, 1970; Heaton, Heaton, and Barry, 1971). Lignin, a natural phenylpropane polymer present in plant fibre, has undoubted, if rather slow, bile acid-binding activity in vitro, this activity being greater with free than with conjugated bile acids (Eastwood and Hamilton, 1968). Clinical experience in a small number of cases has suggested that lignin may be effective in two disorders in which cholestyramine is very useful, namely, type II hyperlipoproteinaemia and post-ileectomy diarrhoea (Thiffault, Bélanger, and Pouliot, 1970; Eastwood and Girdwood, 1968). However, it has not yet been shown that lignin sequestrates conjugated or free bile acids in vivo. In a previous study in normal subjects, we were unable to detect any effect of lignin on the recirculation of radioactively labelled taurocholate and its metabolites (Heaton et al., 1971).

The present study was designed to re-assess whether lignin binds conjugated bile acids in vivo by observing its effect on vitamin A absorption, as measured by the vitamin A tolerance test. For comparison, cholestyramine was studied in the same way. It was expected that cholestyramine would have an easily detectable effect, although scant data have been published on how this agent affects vitamin A absorption (Longenecker and Basu, 1965).

**Methods**

**DESIGN OF THE STUDY**

Healthy volunteers of both sexes aged 19-28 were studied, each acting as his or her own control. Most subjects received three test meals, given in random order at intervals of not less than a week. Fourteen subjects received a control test meal and one containing cholestyramine. Twelve subjects also received a test meal containing lignin.

After an overnight fast the test meal was consumed within 10 minutes. Venous blood samples, taken immediately before the meal and again at four, five, and six hours, were kept in the dark until analysis on the same day.

**PREPARATION OF TEST MEALS**

A stock solution of vitamin A was prepared by adding 1 g vitamin A palmitate (Oily Preparation, Roche, containing \(10^4\) iu/g) to 99 g arachis oil and mixing thoroughly. This solution was found to keep satisfactorily for several weeks at 4°C in the dark, but the concentration of vitamin A was checked immediately before preparing the test meal. Each test meal contained 5000 iu vitamin A per kilogram body weight, 0.5 g/kg Casilan, 0.5 g/kg glucose, and 5 g/kg water. No additions were made to the control test meal, but to the other test meals either 12 g of...
lignin (Therapharm Ltd) or 12 g of cholestyramine (in the form of Questran 27 g) were added. All test meals were thoroughly homogenized immediately before being taken.

**ESTIMATION OF INCREASE IN SERUM VITAMIN A**

A simple method was used which depends on there being no change in the serum level of other heptane-extractable compounds that absorb at 327 nm during the period of the test. Studies on 120 patients using this method gave results identical with those obtained by measuring the absolute levels of vitamin A (Chawla and Wiggins, 1969). Absolute alcohol, 4·0 ml, was added to 2·0 ml of serum in a glass-stoppered tube and the contents mixed. Heptane, 4·0 ml, was then added and the tube shaken on an automatic shaker for 10 minutes. The tube was centrifuged and the absorbance of the upper heptane phase containing the vitamin A was read at 327 nm. The difference between the absorbance at four, five, and six hours and that of the fasting sample was divided by 0·00031 to give a curve for the rise in serum vitamin A in IU per 100 ml. All estimates were made in duplicate and showed good agreement.

The possibility that lignin breakdown products are absorbed and give rise to significant heptane-extractable serum absorbance at 327 nm was investigated in two subjects. This absorbance was measured before, and four, five, and six hours after, ingestion of a test meal containing 12 g lignin but no vitamin A. No change in absorbance was noted in either subject.

The possibility that cholestyramine and lignin bound vitamin A in the test meal was investigated by studies *in vitro*. Standard vitamin A test meals were prepared with and without cholestyramine. After standing for four hours, the mixtures were rehomogenized and aliquots were extracted with heptane and their absorbance was measured at 327 nm. Identical readings were obtained whether or not cholestyramine was present in the test meal. Similarly, the presence of cholestyramine made no difference to the readings when phosphate buffers of pH5 and 7·6, homogenized with vitamin A in arachis oil, were used in place of the test meal. Parallel studies with lignin gave the same results.

**Results**

In most subjects given a control test meal the peak serum vitamin A level was observed at five hours, but in a few cases at four or six hours after ingestion of the meal. The timing of the peak was unchanged when lignin or cholestyramine was added to the test meal.

In 14 subjects the five hour increase in serum vitamin A was measured with and without cholestyramine (fig 1). The increase with cholestyramine was

![Fig 1](http://gut.bmj.com/)

**Fig 1** The effect of cholestyramine on the five-hour rise in serum vitamin A in 14 subjects. The result for a control test is plotted against the result when cholestyramine 12 g was added to the test meal. The scale indicates international units of vitamin A.

In 12 subjects the five hour rise in serum vitamin A was measured with and without lignin (fig 2). The increase with lignin was

![Fig 2](http://gut.bmj.com/)

**Fig 2** The effect of lignin on the five-hour rise in serum vitamin A in 12 subjects. The result for a control test is plotted against the result when lignin 12 g was added to the test meal. The scale indicates international units of vitamin A.
smaller in all subjects, markedly so in most cases. The mean increase with cholestyramine was 40.5 ±
17.0% of the increase for the control test (t = 13.07, p < 0.001).

In 12 subjects the five hour increase in serum vitamin A was measured with and without lignin
(fig 2). The increase with lignin was smaller in eight and larger in four subjects. Overall, no significant
difference was found. The mean increase with lignin was 93.2 ± 29.5% of the control test increase (t =
0.798).

If the data are calculated using the peak rise (that is, the highest of the four, five, and six hour values)
instead of the five hour rise in serum vitamin A, lignin again produced no significant change, whereas
cholestyramine caused a highly significant reduction (mean value with cholestyramine 40.4 ± 15.4% of
the control value; t = 14.48, p < 0.001).

Discussion

In this study we have shown that cholestyramine, in
an amount equivalent to a modest daily dose, markedly reduces vitamin A absorption as assessed
by the vitamin A tolerance test. It acts presumably by
removing bile acids from solution and so preventing
the formation of a micellar phase in the small bowel
lumen. This finding was predictable and confirms
earlier preliminary studies (Longenecker and Basu,
1965). Although cholestyramine is not very palatable,
it is unlikely that delayed gastric emptying played a
significant role since adding this agent to the test
meal did not delay the peak rise in serum vitamin A.

It is probable that cholestyramine also impairs the
absorption of the other fat-soluble vitamins D, E,
and K. Direct studies of this question are lacking,
but cases are on record in which cholestyramine
appeared to cause osteomalacia (Heaton, Lever,
and Barnard, 1972) and hypoprothrombinemia (Gross
and Brotman, 1970), which were reversed by treat-
ment with vitamins D and K respectively.

Although the vitamin A tolerance test is crude and
unphysiological, the results with cholestyramine
show that it can be used to detect pharmacological
binding of conjugated bile acids. The failure of
lignin, even when given in an amount two or three
times greater than the suggested daily dose (Thiffault
et al, 1970; Eastwood and Girdwood, 1968), to have
any significant effect on the rise in serum vitamin A
suggests that in vivo lignin does not bind conjugated
bile acids to an important extent. A similar conclusion
was reached in a previous study in which lignin
failed to accelerate the removal from the entero-
hepatic circulation of labelled taurocholate and its
metabolites (Heaton et al, 1971).

Small-scale studies have led to the claim that lignin
is effective in lowering elevated serum cholesterol
levels (Thiffault et al, 1970) and in relieving the bile
acid catharsis of ileal resection (Eastwood and Gird-
wood, 1968). If these effects are substantiated, the
mode of action of lignin must be different from that
of cholestyramine. There are marked physico-
chemical differences between the two materials.
Cholestyramine is a polybasic anion exchange resin
which is believed to act mainly by exchanging chloride
ions for bile acid ions, though a lesser non-electro-
static component has been postulated (Johns and
Bates, 1970). In contrast, lignin is a relatively non-
polar, aromatic polymer, which is thought to act by
hydrophobic bonding (Eastwood and Hamilton,
1968). In vitro, lignin binds most effectively the least
polar bile acids and it may be speculated that any
therapeutic properties of lignin are due to binding of
mono-hydroxy or other bacterially degraded bile
acids.

We are grateful to the medical students and tech-
nicians who volunteered for this study and to the
dieticians Miss Gleghorn and Miss Martin who
prepared the test meals. We are indebted to Miss
E. H. L. Duncan and Mrs A. F. Morris for statistical
help. Lignin, prepared from pine wood, was kindly
donated by Therapharm Ltd, Downham Market,
Norfolk.

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_Gut_ 1973 14: 316-318
doi: 10.1136/gut.14.4.316

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