Intestinal cholesterol synthesis and mobilisation to blood in sucrose-feeding and alcohol-intake

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Small intestines synthesize cholesterol to a greater extent than liver. Between starch-fed and sucrose-fed rats, using \( ^{14} \text{C} \) glucose, it was found that the synthesis of cholesterol by the jejunum of small intestines was greater in the sucrose-fed group than starch-fed group. By a novel experimental technique and using \( ^{14} \text{C} \) glucose, it was found that the contribution towards buffer representing lymph was greater in the sucrose-fed group (13.3 \%) than the controls(11\%). Hypercholesterolemia on sucrose feeding may be at least partly due to contribution by the small intestines. Regarding alcohol, using \( ^{14} \text{C} \) glucose it was found that total synthesis of cholesterol by the small intestines was decreased in alcohol-fed rats. There was no difference in the cholesterol retained by the intestinal tissue between the controls and alcohol-fed animals while, the secretion towards buffer (lymph) was 9 \% as against 11. This indicates that there is contribution of cholesterol to blood from small intestines in alcohol-intake also but due to overall decrease in the intestinal synthesis of cholesterol, contribution of intestines to hypercholesterolemia may not be substantial as in the case of sucrose feeding. This is because in sucrose-feeding there is increased cholesterol synthesis.

Many tissues may contribute to blood cholesterol but the contribution by liver and small intestines is quite high (\( >91\% \))\(^1\). Recently, it has been shown that small intestines synthesize cholesterol even to greater extent than that of liver. Venugopala Rao and Ramakrishnan\(^2\) have shown that small intestines synthesize cholesterol nearly 6 times that of liver in humans and 1.5 times that of liver in rats.

Sucrose in diet in excessive amounts causes hypercholesterolemia\(^3\). Increase of cholesterol in blood has been reported in alcohol-intake also\(^4-6\).

Venugopala Rao and Ramakrishnan\(^7\) have shown that small intestines synthesized more cholesterol than liver in sucrose feeding. They have also reported that in alcohol treatment intestinal cholesterol content was not altered but the \textit{in vivo} synthesis decreased\(^7\).

However there is no information as to how much of intestinal cholesterol is diverted to lumen and lymph in these conditions. One is concerned with the amount diverted to lymph, as it will directly add to blood cholesterol.

Materials and Methods

Male albino rats of 80-100 g weight were divided into 3 groups of 6 rats, one each for control and for studies on sucrose feeding and alcohol-intake.

The control rats were given diet of following composition(gm).

Starch, 670; Casein, 180; Salt\(^8\), 40; Ground Nut oil, 80; Shark liver oil, 20 and Vitamin mixture\(^9, 10\).

Each rat was given 8g of diet in 2 spells of 4 g each.

For the sucrose-fed group, the quantity of starch was reduced to 268 from 670 and the balance of 402 was made good with sucrose.

For alcohol-feeding, each rat was given 4 ml of 9\% alcohol by gastric intubation while the control rats were given 4 ml of water by the same route.

The duration of experiment was 45 days.

After the stipulated period, the sucrose-fed rats and the corresponding controls were sacrificed by decapitation after 12 hr of fasting. In respect of alcohol-fed and the corresponding control group, the rats were ether anaesthetised. From all the sacrificed rats, 7 to 8 cm of jejunum was removed by opening the abdomen. Part of the jejunum was used for the assay of HMG CoA reductase.

For the study of contribution of cholesterol synthesized by the intestines to blood, the experimental design of Venugopala Rao and Ramakrishnan\(^2, 10\) was used (Fig. 1).

In this design, the isolated jejunum of the intestines was allowed to survive by suspending in oxygenated Krebs-Ringer phosphate buffer. For this, the lumen of the jejunum was washed with ice-cold oxygenated
buffer pH 7.4. The entire length was bent in the shape of U. 1.5 ml of Krebs-Ringer phosphate buffer containing 1 mM glucose (5 μCi of \((U^{14}C)\) glucose) was injected into the lumen and then both ends of the segment were tied using a thread. It was then suspended in a beaker containing 50 ml of oxygenated Krebs-Ringer phosphate buffer at 37°C and shaken at 100 oscillations per minute. At the end of the incubation period, the segment was removed and washed into the flask with the buffer. The portions of segment which were above the buffer surface were discarded.

Cholesterol content could change either due to synthesis or imbibition of circulating cholesterol as low density lipoproteins. Hence HMG CoA Reductase (NADPH) in intestinal tissue was assayed by the method of Venugopala Rao and Ramakrishnan\(^1\)\(^1\) to follow the synthesis of cholesterol. HMG-CoA was determined by reaction with hydroxylamine at pH 5.5 and subsequent colorimetric measurement of the resulting hydroxamic acid by formation of complexes with ferric salts. Because, mevalonate interferes in this estimation at acid or neutral pH, alkaline hydroxylamine was used to estimate specifically HMG-CoA only. Possible interference by coenzyme A is also minimal when readings are taken at 540 nm.

Mevalonate was estimated by reaction with the same reagent, but at pH 2.1. At this pH, the lactone form of mevalonate readily reacts with hydroxylamine to form the hydroxamate.

Analytical procedure: Equal volumes of the fresh 10% tissue homogenate and diluted perchloric acid were mixed, allowed to stand for 5 min. and centrifuged (2000 rpm, 10 min). One ml of filtrate was treated with 0.5 ml of freshly prepared hydroxylamine reagent (alkaline hydroxylamine reagent in the case of HMG-CoA), mixed and after 5 min 1.5 ml of ferric chloride reagent added to the same tube and shaken well. Readings were taken after 10 min. at 540 nm vs. a similarly treated saline/arsenate blank. The activity of HMG CoA is indirectly given by the ratio of HMG CoA and mevalonate. The less the ratio the greater the activity and \textit{vice versa}.

\((^{14}C)\)-Cholesterol synthesis was assessed in the washings of the lumen, the buffer in the flask and the segment separately. The \((^{14}C)\) labelled cholesterol in the buffer of the container was taken as secretion across the muscle wall (lymph). The total synthesis by the segment was computed by summing up the three values.

Estimation of \((^{14}C)\)-cholesterol synthesized: The buffer in the flask and the washings of the lumen were dried separately. The tissue was ground well. The lipids in each were extracted by Folch's procedure\(^12\). After extracting the lipids, the proteins of the segment were estimated by the method of Lowry \textit{et al.}\(^13\). An aliquot of the extracted lipids was washed, the solvents evaporated and the lipids redissolved in 0.5 ml of chloroform. This solution was applied along with standards on a thin layer of silica gel\(^10\),\(^14\) previously activated. The bands were marked on the TLC plate and scraped into a vial containing scintillant and counted in a Beta counter (LKB, Wallace 1211 Beta Liquid Scintillation Counter). The disintegrations per minute (dpm) were expressed per g of protein. Student's \(t\) test was used for statistical analysis.

Results

(i) \textit{Sucrose-feeding}: In terms of percentage, \((^{14}C)\) Cholesterol synthesis was increased in the intestinal tissue. There was increased secretion towards buffer (lymph), (13.3%) but less was excreted into the lumen (8.5%) (Table 2). In terms of \((^{14}C)\) cholesterol synthesis as dpm/g of protein shows increased synthesis by the intestines (Table 3). This was confirmed by increased activity of HMG CoA Reductase (Table 1) in animals fed with sucrose for 45 days.

(ii) \textit{Alcohol – intake}: In respect of alcohol feeding, \((^{14}C)\) cholesterol synthesis in different compartments are expressed in terms of disintegrations/min/g of protein (Table 3). The contribution of \((^{14}C)\) cholesterol to each compartment is decreased significantly. If the three values are summed up, the value shows the total synthesis which is also
explained due to non-availability of adequate energy

feeding (Tables explain greater contribution of intestinal cholesterol to decreased. This was confirmed by decreased activity of HMG CoA Reductase (Table 1) in animals given alcohol for 45 days.

In terms of percentage, it is found that in the intestinal tissue (14C) cholesterol synthesis is unaltered, secretion into the lumen is increased (28%) and muscle wall secretion is decreased (9%) in alcohol-fed rats (Table 2).

**Discussion**

Increased synthesis of cholesterol in sucrrose feeding (Tables 1 and 2) confirms the work of Venugopala Rao and Ramakrishnan7. Increased movement into buffer representing lymph (13.3%) and retention in the tissue (78.2%) can be taken to explain greater contribution of intestinal cholesterol to blood cholesterol via lymph.

The augmented synthesis, retention in the tissue and secretion into lymph might be responsible to a significant extent for the hypercholesterolemia in sucrrose feeding.

Regarding alcohol-treatment the decreased secretion by muscle wall in alcohol intake may be explained due to non-availability of adequate energy in the form of ATP as alcohol treatment has been shown to impair glucose oxidation15.

As in the case of total synthesis, the contribution to lymph is also decreased (9%). So when synthesis is decreased, secretion into lymph may not contribute to hypercholesterolemia which is reported in alcohol-intake in experimental animals5 and humans. Increased secretion into lumen may be due to accelerated sloughing of intestinal7 cells in the presence of alcohol. There is no change in the cholesterol content of intestinal tissue. Our work shows that intestines does contribute some amount of cholesterol directly via lymph to the blood in alcohol-intake(9%) though it is less than the control(11%).

**References:**

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