Influence of Bile Acids on Digestion and Absorption of Lipids

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THE FOLLOWING discussion is a summary of some late findings concerning the role of bile acids in digestion and absorption of lipids. The results discussed were obtained in the laboratories of Professor Bergström at the Karolinska Institute in Stockholm and of Professor Borgström at the University of Lund. In addition, the recent and interesting findings of Isselbacher and Dawson[19,20] as well as of Olson[21] on the role of bile acids in lipid metabolism in the intestinal wall and at the enzymatic level will be related.

FORMATION AND METABOLISM OF BILE ACIDS

Qualitative and Quantitative Aspects

I would like to begin with a short summary of the qualitative and quantitative aspects of the formation and metabolism of bile acids, with special reference to the conditions in man. As is well known, bile acids represent the major end products of cholesterol catabolism. The comparatively recent interest in cholesterol and cholesterol metabolism has focused attention on the bile acids. The bile acids circulate from the liver to the intestine and back to the liver continuously and in man are partly stored in the gallbladder. In addition, there is a continuous loss of bile acids via the feces. The rate of formation of bile acids in man has been determined by Lindstedt,[1] who found a half-life for cholic acid of approximately three days. This corresponds to a daily production of about 700 mg. of cholic and chenodeoxycholic acids, the pool size being about 3.5 gm.

Rate of Formation

The rate of formation of bile acids is influenced by several factors. Until now, the influence of the state of thyroid activity and diet have been investigated only preliminarily. The half-life of bile acids in the hypothyroid state is considerably prolonged and the daily formation of bile acids is diminished as compared to normal. Conversely, the half-life in the hyperthyroid state is shorter than normal and the daily production of bile acids is greater. Investigations of the influence of diet have just begun. Studies have been made by Dr. Lindstedt in collaboration with Dr. Steinberg at the National Heart Institute. They have found that the half-life of bile acids is prolonged and the daily production decreased when a patient is changed from a free diet to a formula diet containing 60 per cent corn oil as the source of fat. In addition, they found that the composition of the bile acids differs from normal in that deoxycholic acid is absent. Since deoxycholic acid is formed from cholic acid by microbial action, the formula diet must suppress the growth of the bacteria which form deoxycholic acid. If the same patient is changed from the diet containing unsaturated fat to one containing 60 per cent cocoanut oil as the source of fat, the half-life of bile acids is increased and the production of bile is decreased even more.

Concentration

The concentration of bile acids in the intestinal contents sampled by the intubation technic of Blankenhorn and Ahrens[2] has been measured by quantitative paper chromatog-

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raphy by Sjövall.[3] When a test meal is ingested and reaches the duodenum the gallbladder empties and the concentration of bile acids in the duodenum and upper part of the jejunum reaches high levels (18 to 45 mEq. per L., i.e., 10 to 25 mg. per ml.). The concentration, however, is rapidly evened out to values of 5 to 10 mEq. per L. This concentration is in the range observed during fasting. Also, the main absorption of lipids occurs at bile acid concentrations of 5 to 10 mEq. per L.

The ratio of glycine to taurine conjugated acids, as well as that of trihydroxy to dihydroxy acids, remains fairly constant during the passage of intestinal contents from duodenum to the middle of the jejunum. Thereafter, the bile acids are reabsorbed and the concentration further down in the intestine decreases progressively. Using a method of reading optical density at 400 mμ, in which the bile pigments have their main absorption, or following lipid phosphorus originating from the phospholipids in bile,[4] bile acid concentration can be measured in a simpler way than by using quantitative paper chromatography and the results are in good agreement with these determined chromatographically.

Effect on Hydrolysis of Glycerides

The dietary lipids, which constitute about 40 per cent of the total daily caloric intake, consist mainly of triglycerides together with small amounts of phospholipids, cholesterol and phytosterols. By the action of pancreatic lipase the triglycerides are partially hydrolyzed to a mixture of free fatty acids and glycerides. Experiments with doubly labeled glycerides, labeled both in the fatty acid and the glycerol moiety, have shown that about 50 per cent of dietary glycerides have been hydrolyzed completely to glycerol and fatty acids prior to being absorbed. The early experiments of Reiser[5] and of Bernhard[6] et al. were necessarily so designed that the extent of hydrolysis measured also included any hydrolysis taking place in the intestinal mucosa. By feeding glycerides containing small amounts of labeled dimethylstearic or nonadecanoic acid to human subjects and sampling intestinal content after different time intervals,[7] it was found that the extent of complete hydrolysis of glycerides in the lumen amounted to about 50 per cent, i.e., the same figure arrived at by experiments with doubly labeled triglycerides. The rationale of using glycerides with dimethylated long-chain fatty acids is that such ester bonds are not hydrolyzed by pancreatic lipase, hence, the increase in specific activity of the glycerides isolated from intestinal content after different times is a measure of the extent of total hydrolysis.

These experiments also indicated that the dietary glycerides were absorbed by the intestinal mucosa as free fatty acids and monoglycerides and to some extent as diglycerides. In the early 1940's Frazer and his collaborators[8] found that the bile acids, together with products of lipolysis, i.e., fatty acids and monoglycerides, provided an effective emulsifying system for triglycerides and compounds of similar polarity. This emulsification was found to promote the rate of lipolysis effected by pancreatic lipase. In studies of the action of pancreatic lipase *in vitro* bile salts usually have been added to the substrate in order to stimulate the hydrolysis. The mechanism of the activation of pancreatic lipase is not known entirely. An important factor seems to be the emulsifying properties of bile salts, as pancreatic lipase acts only on emulsified substrates and not on soluble ones.[9] An additional effect of bile salts is the shift in pH-optimum of pancreatic lipase in presence of bile salts. Using rat pancreatic juice as the source of enzyme it was found [10] that in the absence of bile salts the pH-optimum for hydrolysis of triolein was about 8.5, while addition of taurocholate shifted the pH-optimum to 6.5, the pH ordinarily found in the duodenum and upper part of the jejunum.

When it was realized that the dietary lipids were only partially hydrolyzed prior to absorption, the function of the emulsifying system described by Frazer was later suggested[11] to be one of emulsification of dietary lipids to a particle size of less than 0.5 μ. Such particles would then be able to penetrate the mucosal epithelium. According to current theories this would be accomplished by the process of pinocytosis.

Micelle Formation

Another concept of the physical state of the lipids when absorbed by the intestinal mucosa is that they are present as micelles and solubilized by micelles. Molecules possessing water-solubility and both polar and nonpolar groups like soaps, detergents, bile salts and so on, aggregrate in aqueous solution above a certain critical concentration. Such molecular aggregrates, discovered and named micelles by McBain in 1913, are in reversible equilibrium with nonaggregrated molecules. The concentration at which aggregation begins is termed the critical micellar concentration (CMC).

The site of digestion and absorption of lipids has been found to be the upper 100 cm. or so of the jejunum. This has been shown by Turner[12] in studies of absorption of iodinated fat in dogs and also by Borgström[4] in man. In these intestinal segments the concentration of bile salts is 5 to 10 mEq. per L., as mentioned earlier. This concentration is well within the limits for micelle formation of bile salts as shown by Ekwall and his associates[13,14] and by Norman[15] using pure systems. The beginning association, i.e., the critical micellar concentration, was found to be 5 mEq. per L. for both free and conjugated deoxycholic acid and 12 mEq. per L. for free and conjugated cholate. In the upper part of the intestine the bile acids are present as conjugates with glycine and taurine, and the conjugates are completely ionized. Recently, studies have been initiated on micelle formation and solubilization of glycerides and fatty acids by bile salts under conditions more similar to the ones prevailing during digestion and absorption of lipids. Micelle formation is studied in solutions of pure bile salts with sodium ion concentration of 0.15 M, a pH of 6.3 and at a temperature of 37°c. Under these conditions the critical micellar concentration for conjugated bile salts as measured by solubilization of palmitate is lower than in aqueous solutions of bile salts: for deoxycholate 2 mEq. per L; and for taurocholate 7 mEq. per L; as compared to 5 and 12 mEq. per L; respectively. Palmitate itself does not form micellar solutions under these conditions as the solubility of long-chain fatty acids is too low. Solubilization of α-monoolein by bile salts begins at a lower concentration of bile salt, 0.8 mEq. per L. for deoxycholate and 5 mEq. per L. for cholate, and rises much more steeply with increasing bile salt concentration than does the solubilization of palmitate. Interestingly enough, however, α-monopalmitin is solubilized by bile salts to the same degree as palmitate. Monoglycerides are examples of compounds showing amphiphilic properties, i.e., compounds which are polar but nonionized in one region of the molecule and nonpolar in the other. Amphiphilics are thought to be solubilized between the oriented detergent molecules in the periphery of the micelles. Nonpolar compounds, on the other hand, are thought to be solubilized in the interior of the micelles.

The capacity of mixed micelles of bile salts and α-monoolein to solubilize palmitate is greater than that of micelles of bile salts. The properties of other types of monoglycerides as well as of lysolecithins in micellar systems are being studied currently but no definite data are available as yet.

The possibility that micellar structures are involved in the absorption of glycerides and fatty acids seems strengthened by the fact that dietary triglycerides are absorbed after partial hydrolysis to a mixture of monoglycerides and fatty acids. In addition, centrifugation at high speed of intestinal contents collected during absorption of triglycerides has shown that triglycerides and hydrolysis products thereof are distributed between the oily top layer and the clear subnatant, and that the level monoglycerides and fatty acids in the clear subnatant is comparatively higher than that of the triglycerides.

Effect on Cholesterol Absorption

The absorption of cholesterol requires the presence of bile salts as shown by Chaikoff and his collaborators in 1952.[16] No definite data as yet are available concerning the mechanism of action of bile salts in sterol absorption. In studies of cholesterol absorption in man[17] it was found that the dietary cholesterol

was completely mixed in the intestine with endogenous cholesterol excreted with bile. The cholesterol in the intestinal content was present both in the top fatty layer and in the clear subnatant, indicating the possibility that micellar solubilization is involved in cholesterol absorption.

In a preliminary communication Suzuki[18] has reported on the absorption of cholesterol in rats in the presence of different bile salts. Appreciable absorption was found only with free and conjugated cholate. However, the other cholanic acids tested were all added as free acids, and it remains to be established if the same results will be obtained with the conjugates of these acids.

Role in Lipid Metabolism

Recently, Dawson and Isselbacher[19,20] have described the preparation of slices and homogenates of intestinal mucosa that carry out active synthesis of triglycerides. In addition to their surface-active properties, conjugated bile salts also stimulate the formation of glycerides from free fatty acids by affecting the metabolism of the mucosal cell.

Olson[21] recently investigated the role of glycocholate in the absorption of β-carotene and the formation of vitamin A ester in rat intestine. Although an effect of glycocholate on the conversion of β-carotene to vitamin A in the mucosal cell cannot be excluded, the main site of action of bile salts appears to be in the transport of β-carotene into the cell.

SUMMARY

The qualitative and quantitative aspects of bile acid formation in man under different conditions are discussed. It is suggested that bile acids play an important role in lipid absorption by virtue of their ability to form micelles which solubilize the products of lipolysis, *viz.*, monoglycerides and free fatty acids and likely also cholesterol.

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DISCUSSION

Dr. Albert J. Mendeloff (*Baltimore, Maryland*): Two people have asked to discuss this paper. Dr. Isselbacher?

Dr. Kurt J. Isselbacher (*Boston, Massachusetts*): It is a pleasure to comment on Dr. Danielsson's fine paper and presentation on the excellent work that is emanating from the laboratories at Lund and Stockholm.

We now appreciate the fact that in considering the action of bile salts it is necessary to think of a number of actions within the lumen and in the mucosa proper: (1) bile salts act as detergents at low concentrations or have a surface active property; and (2) bile salts stimulate lipase and, as demonstrated by Dr. Borgström and his colleagues, also shift the pH-optimum of the lipase, such that the pH which obtains in the duodenum is the most favorable environment.

Dr. Danielsson has emphasized that there is another probably crucial function of bile salts which needs to be considered, namely, micellar formation. He indicates that the concentration of bile salts that obtain in the intestinal mucosa is consistent with such a theory, based on *in vitro* studies of the critical concentration of bile salts favoring the formation of micelles.

Recently, Dr. Alexander Rich at the Massachusetts Institute of Technology emphasized that bile salts, at a given concentration, when they aggregate in micelles may form a helical structure, much as we see in the structure of DNA, and suggests that there may be some definite structural component to micelles.

I have a number of thoughts and questions that I would like to ask briefly.

One, ionized fatty acids would make better micelles than unionized fatty acids, yet at the pH that obtains in the gut, most of the fatty acids would be in the unionized form. I wonder what Dr. Danielsson thinks about this particular aspect in relation to micelle formation.

We have been impressed by the fact that conjugated bile salts, as indicated, appear to be the physiologic excretory product of the liver; I wish to emphasize that in carrying out studies with bile salts one has to be extremely careful not to use commercial mixtures without testing them first, because free or unconjugated bile salts may have a deleterious effect, on the mucosa proper in terms of structure, and on numerous metabolic functions of the mucosa.

Dr. Danielsson has indicated that we believe there is an additional action of bile salts beyond what has been mentioned, namely, on the mucosa proper. At least it would appear that detergents, such as Tween, Trion and bile salts, have an effect which can be measured by the stimulation of triglyceride formation or palmitate esterification, and a pronounced effect on increasing glucose utilization. This effect would then facilitate the triglyceride synthesis during absorption which was emphasized by Dr. Turner.

Finally, Dr. Danielsson, would you care to speculate about differences in function between dihydroxy and trihydroxy cholanic acids? Do you believe that their presence in man and their specific structure has any specific function in terms of what we know at present of their physiologic function.

Dr. Danielsson: First of all, I agree on the points raised by Dr. Isselbacher. He asked whether the free fatty acids would take place in micelle solubilization and micelle formation. I think that under these conditions, that is, a pH of 6.3 and a sodium concentration of 0.15 M, the solubility of a free acid is low. However, the little that is soluble is completely ionized, the pK being about 4.0. However, the amount that is ionized and is in solution is not large enough to reach the micellar concentration. Current opinion is that under these conditions, free fatty acids, particularly long-chain fatty acids, do not take part in micellar formation, but are solubilized.

As to the biologic function of different bile salts, I think it can only be said that we find a difference in the critical micellar concentration for dihydroxy and trihydroxy acids; that is, with dihydroxy acids the critical micellar concentration is considerably lower than with trihydroxy acids. However, I would not speculate on any difference in biologic function at this moment.

Dr. Mendeloff: Dr. Hashim has asked to make some comments.

Dr. Sami A. Hashim (*New York, New York*): Our group is located at St. Luke's Hospital in the

Institute of Nutritional Science at Columbia University. We believe we can offer some form of an explanation of the mechanism of the role of bile acids—an indirect one—in man in the digestion and absorption of dietary fat.

We owe a great deal—and I would like to second Dr. Isselbacher's comment—of our current knowledge of the bile acids to the Swedish group.

If we assume that there is an enterohepatic cycle of bile acids, and this enterohepatic cycle has a regulatory influence on the conversion of cholesterol to bile acids, it is possible to influence such a conversion by various means. We have tried to sequester bile acids in the gut and thus influence this conversion.

In a search for various other materials that can sequester bile acids, Dr. Tennant of our group has been able to identify such an agent, called MK 135, with which we have been working.

When MK 135 is fed to dogs, there is a tenfold increase of bile acids in the stools. Thus, we took this material and fed it to a series of persons with various hypercholesterolemias. It definitely does reduce the cholesterol level. We then went ahead and doubled the amount of the material fed, thinking that if it was possible to interfere seriously with the reabsorption of bile acids, that is, if it was possible to block this reabsorption totally, or to block a good measure of it, it might be possible to interfere with the digestion and perhaps absorption of triglycerides.

One subject was given a formula diet in which the fat was butter. The fat was homogenized under 5,000 pounds pressure, and was given as a constant amount per day to this person, who was otherwise normal. After an initial period, the sequestrate was given in doses of 30 gm. per day, a large dose. In one day, 20 gm. of fat was excreted in the stools. Upon the institution of a placebo in another feeding period, fat excretion returned to more or less normal. In addition, there was a depression in the serum cholesterol level.

In another study, the fat fed was exclusively corn oil. Again the same pattern of steatorrhea was observed. This then is an experimental type of steatorrhea. At this point I will say that the fat in the stools is almost exclusively triglyceride. In this subject, the total serum cholesterol level decreased to 50 mg. per cent while he was on both the corn oil formula and resin diet. This is one of the lowest levels we have ever encountered in a North American Yankee.

From these studies we believe that it is possible, through bile acid sequestration in man, to produce steatorrhea experimentally without any immediate untoward effect on the individual.

DR. DANIELSSON: In 1958 we showed that the formation of bile acids was regulated by the amount of bile acids reaching the portal circulation. For example, when a bile fistula is induced in any kind of an animal, the bile acid synthesis will increase 10 to 20 times the original amount. This means an increased cholesterol synthesis and a decreased half-life of cholesterol. (This latter finding has not been published.) Then, when bile salts are administered into the intestine, so that they eventually are absorbed by the portal route, the bile salt formation in bile fistula rats is brought down to normal levels.

In such cases, when bile acids are continuously removed from the animal you have the danger of creating a largely increased cholesterol synthesis.

DR. JAMES A. OLSON (*Gainesville, Florida*): I will limit myself to a few comments. The first relates to the possibility that bile salts are also stimulating intramucosal metabolism. We have found in the uptake of β-carotene and its conversion into vitamin A in the gut, that bile salts are essential for this over-all reaction to take place. If we suspend our β-carotene in one of the Tweens, no uptake of β-carotene conversion into vitamin A occurs. If glycocholic acid or bile is present, decided conversion takes place.

We have measured the micelle size of Tweens, something that lipid chemists have been working with for a long time, but few physical chemical experiments are made on these compounds. We found values of about 100,000 to 130,000 for the micelle or particle size of Tween® 80.

Under the conditions in which β-carotene uptake and conversion to vitamin A are maximal, we have found no stimulation of several metabolic processes in the gut. That is, the conversion of radioactive acetate into cholesterol or fatty acid, of radioactive leucine into protein and of radioactive glycerol into triglyceride glycerol were not stimulated under our conditions. So I would like to summarize our findings in two ways: (1) We believe that the effect of bile salts is on the absorption of β-carotene rather than on the general stimulation of intramucosal metabolism. (2) By studying a number of bile salts and detergents, we believe that there is some specificity in the nature of the bile salt action. Just how specific this is requires further investigation.