The Cholesterol Saturation Index of Human Bile

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A line of maximum cholesterol solubility was obtained by plotting the ratios of bile salt to cholesterol *versus* bile salt to phospholipid from model solutions simulating bile containing human biliary lecithin, human bile salts, and cholesterol. The sole factor responsible for the solubility of cholesterol (above the 3% total solid content) was found to be the proportion of bile salts to lecithin. This relationship could be expressed mathematically by a polynomial equation and the bile-salt-to-cholesterol ratio at maximum cholesterol holding capacity for a bile ascertained. From this information the percent cholesterol saturation of human bile could be determined. This method was applied to the bile obtained from 139 patients. The biles from 49 of 53 patients with known cholesterol gallstones had a cholesterol saturation index of 100% or greater, while 53 of 77 patients without evidence of cholesterol gallstones had a cholesterol-un saturated bile. This method quantitates the exact degree of cholesterol saturation in human bile (cholesterol saturation index—CSI), and the availability of this index should be useful for diagnostic and therapeutic purposes.

The methods (1–4) presently available for ascertaining the cholesterol holding capacity of bile are based on the analysis of the bile for the three major biliary constituents (bile salts, lecithin, and cholesterol). Changes in the lipids are then related to known model solutions which simulate human bile. In this manner a number of investigators have devised both pictorial diagrams (2, 3) and numerical indices (1, 4) for estimating the degree of cholesterol saturation of bile. Although these methods have the capability of distinguishing between normal and abnormal bile they are either semiquantitative, depend only on a pictorial representation, or are derived from a measurement made from a diagram. From a clinical point of view, it would be highly desirable to have an accurate, quantitative index of expressing the exact degree of cholesterol saturation of a given bile without resorting to a graphic presentation of the physicochemical state of the bile. Specifically, the availability of a quantitative numerical expression would serve at least two important functions. First, it would provide a simple means of determining whether a patient has or might develop cholesterol gallstones, and second, it would facilitate the evaluation of sequential changes in the cholesterol holding capacity of bile from patients undergoing drug or metabolic manipulation. It is the purpose of this report to introduce a mathematical expression.
(the cholesterol saturation index) which quantitates the exact degree of cholesterol saturation of a given bile.

**METHODS AND MATERIALS**

**In Vitro Incubations**

Model solutions containing bile salts, lecithin, and cholesterol were prepared to determine the exact limits of cholesterol solubility. In order to simulate the conditions of human bile as closely as possible, human biliary lecithin and bile salts were used in these experiments. Human bile lecithin and bile salts were prepared from bile. Lecithin was isolated by silicic acid column chromatography (5) and bile salts were obtained from the methanol–water phase of the Folch extraction (6) of the bile. The latter fraction was analyzed and found to contain 93–95% bile salts with a composition of 38% cholic, 40% chenodeoxycholic, 19% deoxycholic, and 3% lithocholic acids. The experiments on model solutions were designed to ascertain the optimum levels of total solids (or water content) in the ternary system and the influence of the bile-salt-to-lecithin ratio on the solubilization of cholesterol. The incubations were carried out in the following manner: mixtures of bile salts, lecithin, and cholesterol were prepared by adding human bile salt solutions in 0.1 M phosphate buffer pH 7.3 to different amounts of lecithin in the presence of excess crystalline cholesterol. The mixtures were incubated for 4 hr. Incubation periods of up to 3 days did not lead to any additional solubilization of cholesterol. Following the incubation, the lipid mixtures were passed through an 0.22 µm Millipore filter maintained at 37°C, and this gave a clear filtrate. An exact aliquot of this micellar solution was then extracted with 2:1 chloroform–methanol and analyzed for bile salts, lecithin, and cholesterol.

**Bile**

Human bile specimens (duodenal and/or gallbladder bile) were obtained from male and female Caucasians and Indians of the Southwest with and without gallstones. The type of gallstones, pigmented or cholesterol, was verified by cholesterol analysis of the stone. Duodenal bile samples were obtained from patients without evidence of gallstones as determined by cholangiography. Four to five consecutive daily duodenal bile samples were obtained on each of these patients. A previous report (7) has shown that there is no significant difference in the lipid composition between gallbladder and duodenal bile. The bile samples were immediately immersed in ice upon being obtained from the patient. The bile was shaken for 2 min to uniformly disperse any insoluble cholesterol which might be present, and the bile was then extracted with 2:1 methanol. An aliquot of the bile sample was also centrifuged and examined microscopically for the presence of cholesterol crystals.

**Methods**

Bile samples were extracted with 2:1 chloroform–methanol and partitioned with 0.2 volume of water (6). The biliary phospholipid and cholesterol were extracted into the chloroform phase. Cholesterol was measured by the method of Sperry and Webb (8) and phosphorus by the method of Bartlett (9). The bile acid analyses were carried out on the methanol–water phase by thin-layer and gas-liquid chromatography as described earlier (10, 11).

**RESULTS**

**In Vitro Incubations**

Table 1 shows how the total solid content or percentage of water influenced the solubilization of cholesterol. The ratio of bile salts to lecithin was held constant at 2.7 and the total molar amounts of lecithin and bile salts progressively increased. When the total solid content ranged from 3 to 11.4% there was little difference in the maximum amount of cholesterol solubilized (10–10.6 moles %). It was not possible to maintain a stable micellar solution below this level of solid content.

The effect of progressively decreasing the bile-salt-to-lecithin ratio on the solubilization of cholesterol is shown in Table 2. These data em-