papers and notes on methodology

Rapid computation with the personal computer of the percent cholesterol saturation of bile samples

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Abstract A microcomputer program to calculate the cholesterol saturation of bile is described. The program is designed to accept most of the conventional concentration units for bile salts, phospholipid, and cholesterol. The calculated cholesterol saturation can be corrected for the presence of ursodeoxycholic acid conjugates in bile. The program is designed to make appropriate statements when the input data produce results that are out of range of the solubility data available in the published literature. The program makes possible not only a very rapid calculation of the cholesterol saturation of bile, but eliminates arithmetical errors that are occasionally encountered during conventional calculations. —**Kuroki, S., B. I. Cohen, M. C. Carey, and E. H. Mosbach.** Rapid computation with the personal computer of the percent cholesterol saturation of bile samples. *J. Lipid Res.* 1986. **27:** 442–446.

Supplementary key words bile acid • cholelithiasis • cholesterol • computer program • lecithin • lithogenic index • phospholipid • ursodeoxycholic acid

It is now clearly established that, under carefully defined conditions, chenodeoxycholic and ursodeoxycholic acids are effective gallstone-dissolving agents in man and in animal models (1–5). The etiology of cholesterol cholelithiasis is not known with certainty, but the development of the disease requires the presence of gallbladder bile that is supersaturated with respect to cholesterol (6–8). The presence of nucleating factors (or the absence of antinucleating factors) also appears to play an important role (9–11).

Recently, several laboratories have focused on the task of developing new cholelitholytic bile acid analogs in the hope of producing drugs that are more effective than the known compounds, chenodeoxycholic acid and ursodeoxycholic acid (12–14). To test these new drugs in gallstone prevention and gallstone dissolution, recent studies have employed specific animal models of cholesterol cholelithiasis (12–15). Such studies, like clinical trials in man, require the determination of the cholesterol saturation of bile in order to evaluate the mechanisms involved.

The critical tables of Carey have been widely used to calculate the cholesterol saturation of bile (the lithogenic index or saturation index) (16). The popularity and usefulness of these tables is based upon the observation of Carey and Small (8) that the solubility of cholesterol in model systems and in bile is not merely a function of the bile salt/lecithin ratio, but depends also on several additional factors, such as bile salt composition, ionic strength, temperature, and total lipid concentration. With the exception of bile containing large proportions of ursodeoxycholic acid conjugates or other hydrophilic bile salts, the type of common bile salt plays a relatively minor role in determining cholesterol solubility, whereas the total lipid concentration and bile salt/lecithin ratio appear to be the most important factors (17).

The critical tables of Carey (16) provide a means of calculating maximum cholesterol solubility of model solutions and of bile as a function of total lipid content at 37°C in 0.15 M NaCl (pH ~ 7.0). In the absence of ursodeoxycholic acid conjugates, determination of the cholesterol saturation of bile requires merely a knowledge of the concentration of total bile salts, phospholipids (lecithin), and cholesterol in the bile sample (8, 16). It is possible to introduce a single correction factor for ursodeoxycholic acid conjugates provided the total lipid content is within the range of 5–15 g/dl and the glycine/taurine ratio ranges from about 3:1 to 5:1. At higher glycine/taurine ratios, separate correction factors for the individual contents of

Abbreviation: UDA, ursodeoxycholic acid.

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glycine and taurine conjugates of ursodeoxycholic acid should be introduced.

Obviously, if the cholesterol saturation has to be calculated for very many bile samples, as in extensive clinical trials or in elaborate studies of animal models, it would be very convenient to employ a personal computer for these calculations. This report describes a computer program, written in BASIC, for the rapid calculation of the percent cholesterol saturation of bile. The program accepts several units of measurement of the biliary lipids and cues for an ursodeoxycholic acid correction, if desired.[3]

MATERIALS AND METHODS

An IBM PC-XT computer, equipped with an IBM color display (monitor), Epson RX-80 parallel printer, and 256KB internal memory was used. IBM PC BASIC (Version 1.10, Microsoft) was employed to create the program (Appendix). If a different personal computer is used to create the program, it will be necessary to refer to the pertinent instruction book to start BASIC. The time required to enter the program is about 2–3 hr.

RESULTS AND DISCUSSION

The program is written in IBM Personal Computer BASIC (Microsoft). The commands, statements, variables, and functions used in the program are essentially compatible with BASIC as utilized in other personal computers.

A summary of the program is as follows:

1000–1530: Remarks and initiation of program.
2000–2080: Input of biliary lipid values.
3000–3040: Calculation #1, generation of mole percent of each biliary lipid and total lipid concentration.
4000–4080: Computer decides which line of data should be used for calculating maximal equilibrium cholesterol solubility (from lines 9010–9290).
5000–5050: Calculation #2, generation of maximal cholesterol solubility and percent cholesterol saturation.
7000–7120: Printout of the results.
7500–7640: Correction factor for ursodeoxycholic acid conjugates (16, 17).
8000–8020: End calculation or continue.
9000–9480: Data from Carey and Small (8) and string variables.

The data values used in lines 9010–9290 are the fifth-degree polynomial coefficients generated by Carey and Small (Table 2 in ref. 8). Fifth-degree polynomial equations for maximum equilibrium cholesterol solubility are defined as a function of variations in total lipid concentration and bile salt–lecithin ratio (37°C, 0.15 M NaCl, pH 7.0) (8). The equations have the form

$$y = a + bx + cx^2 + dx^3 + ex^4 + fx^5$$

where y = moles percent cholesterol, i.e., [cholesterol]/([bile salt] + [lecithin] + [cholesterol]) × 100 and x-molar [lecithin]/([bile salt] + [lecithin]) ratio and a to f are coefficients mentioned above. Twenty-nine regression curves at different total lipid concentrations (ranging from 0.30 to 30.00 g/dl) are now available (Fig. 14 in ref. 8). Solution of the equations for y, employing the physiological range of x (0.085–0.425) in 0.005 increments, gives the Critical Tables (Table 1 in ref. 16). Instead of using the tables for maximum cholesterol solubility, the program generates two y values at the exact x value corresponding to total lipid concentrations immediately above and below that of the bile sample for which published regressions are available (lines 5000–5020). Interpolation between the two values (line 5030) gives the maximum cholesterol solubility of the bile (16).

The biliary lipids have been calculated in the paper of Carey and Small (8) using weight concentrations of the sodium salts of conjugated bile acids, phospholipid, and anhydrous cholesterol. The program, however, is written to accept different concentration units, e.g., mg/ml (or μg/μl) or mM (millimoles per liter) of either conjugated or unconjugated bile salts, mg/ml or mM of either phospholipid or inorganic phosphorus, and mg/ml or mM of anhydrous cholesterol. After executing the basic command RUN, the computer will read the data (lines 9000–9290) and then ask for the type and the dimensions of the biliary lipid data (lines 1100–1350). This feature makes it possible to determine the percent cholesterol saturation directly from the original laboratory data. The computer will then prompt which total lipid concentration should be used, that is, either the "classical" concentration of 10 g/dl on which most of the earlier calculations were based (6, 7) or the actual total lipid concentration (8) (lines 1400–1450). This option is very useful, especially when duodenal aspirates are analyzed in which native bile is diluted with gastric juice, liquid formulas, and/or pancreatic juice. In this case it is commonly assumed that original bile had a total lipid concentration of approximately 10 g/dl.

Use of the printer is optional (lines 1500–1530), and printout of the results is possible at any time (lines 6150–

[3]A print-out of the program will be sent free of charge to those requesting reprints. The program is also available on 5.25" diskettes for users of IBM PC or compatible computers. To obtain the diskette, send a check for $10.00 payable to the Department of Surgery (Beth Israel Medical Center) to cover cost of the diskette, postage, and handling.

TABLE 1. Absolute and total biliary lipid composition data and calculated percent cholesterol saturation values of gallbladder biles from gallstone patients

Sample	Composition			Total Lipids	Percent Cholesterol Saturation			
	Bile Salt	Lecithin	Cholesterol		Triangular Graphs[a]	Polynomial Equations[a]	Critical Tables[b]	Computer Program[c]
		mg/ml		*g/dl*		*%*		
1	54.5	18.6	5.10	7.82	159	158	153	157
2	76.8	42.5	9.05	12.84	130	128	127	127
3	115.9	43.1	11.15	17.02	130	131	131	131
4	43.7	22.8	6.80	7.33	184	184	189	185
5	40.5	20.4	3.30	6.42	111	105	108	108
6	89.6	38.3	7.90	13.58	114	116	104	114
7	60.1	26.0	4.75	9.09	112	108	110	110
8	103.1	49.8	9.25	16.22	105	105	103	104
9	71.4	25.1	5.27	10.18	118	118	118	117
10	33.1	19.0	3.68	5.58	136	138	136	135
11	120.3	47.3	12.67	18.03	137	137	136	136
12	59.9	42.6	9.97	11.25	151	151	153	153
13	27.5	20.3	2.48	5.03	101	100	100	99
14	50.2	24.7	5.02	7.99	129	128	126	128
15	102.3	48.7	14.65	16.57	159	155	158	159
16	68.4	28.6	6.80	10.38	139	136	135	136
Mean	69.8	32.7	7.37	10.96	132	131	131	131

[a]Data published in reference 8.
[b]Data published in reference 16.
[c]Percent cholesterol saturation values calculated with the program.

6170). Concentration units of the biliary lipid data can be changed during successive computations (line 8000). Any errors can be corrected before the final calculations are made by the computer. The results are displayed on the screen, and a printed output is obtained for each set of values if the printing option has been selected (lines 1500–1530). Examples of typical printouts are shown in the Appendix. A comparison between the published calculations (8, 16) and results of the present computer calculations is shown in **Table 1.** Since the computation is based on the same fifth-degree polynomial equations (8), differences between the two can be attributed to truncation of the significant figures employed.

Corrections of maximum cholesterol solubility and percent cholesterol saturation in the presence of ursodeoxycholic acid conjugates in bile can be made during each operation (lines 7500–7640). This option is available only when total lipid concentration lies between 5 and 15 g/dl (8, 17), otherwise an error statement will be displayed and the calculation will be terminated. Because correction factors for bile salts not commonly found in human bile, such as salts of hyodeoxycholic acid and muricholic acids, are not available so far, it should be noted that the percent cholesterol saturation calculated may be inaccurate when these bile salts account for a considerable proportion of the total.

Since the Critical Tables (8) were generated essentially for human gallbladder bile, the "n" numbers included in the tables are between 0.085 and 0.425. Therefore, maximum cholesterol solubility of bile with much smaller or greater phospholipid concentration cannot be calculated. However, the present program generates the cholesterol solubility directly from the original fifth-degree polynomial equations (8) that are valid for a much wider range of "n" numbers. This makes it possible to calculate the cholesterol saturation of bile of certain animal models [hamsters (15) and prairie dogs (12)] which may exhibit low phospholipid concentrations. If the "n" number calculated is out of range of the Critical Tables, an error statement (line 9430) will be displayed. When total lipid concentrations of samples are too low (< 0.3 g/dl) or too high (> 30 g/dl), the computer gives an absolute error statement (line 9440) and no result will be obtained.

The program is written to calculate the percent cholesterol saturation as fast, precisely, and easily as possible and to reduce the effort required to scan the Critical Tables for the maximum saturating cholesterol concentrations, thus making it easier to avoid calculation errors. Although the program is rather long and takes an appreciable amount of time to load into the computer, we have found it most facile and useful in practical applications.

In summary, we have developed a computer program that allows us to calculate the percent cholesterol saturation (lithogenic index) for human bile. The program also has applicability for biles of various experimental animals and for ursodeoxycholic acid treatment.

```
Data  1        12-05-1985
                                        Bile Salt       Lecithin        Cholesterol
Original data                            54.500          18.600           5.100
Conc. by weight      (mg/ml)             54.500          18.600           5.100
Millimolar conc.       (mM)             110.998          24.000          13.178
Mole percent            (%)              74.909          16.197           8.894
Total lipid conc.    (g/dl)   7.820
N number       ((PL/(BA+PL))  0.178
Maximum cholesterol solubility (mol%)   5.666
Percent cholesterol saturation     (%)   157
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Data  2        12-05-1985
                                        Bile Salt       Lecithin        Cholesterol
Original data                            76.800          42.500           9.050
Conc. by weight      (mg/ml)             76.800          42.500           9.050
Millimolar conc.       (mM)             156.415          54.839          23.385
Mole percent            (%)              66.662          23.372           9.966
Total lipid conc.    (g/dl)  12.835
N number       ((PL/(BA+PL))   0.260
Maximum cholesterol solubility (mol%)   7.861
Percent cholesterol saturation     (%)   127
Percent of UDA in bile acid composition 47
Maximum cholesterol solubility (mol%) (Corrected for UDA)   6.122
Percent cholesterol saturation     (%) (Corrected for UDA)   163
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Data  3        12-05-1985
                                        Bile Salt       Lecithin        Cholesterol
Original data                             9.950           1.450           0.085
Conc. by weight      (mg/ml)              9.950           1.450           0.085
Millimolar conc.       (mM)              20.265           1.871           0.220
Mole percent            (%)              90.648           8.369           0.982
Total lipid conc.    (g/dl)   1.149
N number       ((PL/(BA+PL))   0.085
Maximum cholesterol solubility (mol%)   2.239
Percent cholesterol saturation     (%)    44
Your data are out of range of Carey's Critical Tables [JLR. 19: 945-955, (1978)]
and calculated by fifth-degree polynomial equations [JCI. 61: 998-1026, (1978)]
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Data  4        12-05-1985
                                        Bile Salt       Lecithin        Cholesterol
Original data                             1.560           0.980           0.084
Conc. by weight      (mg/ml)              1.560           0.980           0.084
Millimolar conc.       (mM)               3.177           1.265           0.217
Mole percent            (%)              68.198          27.143           4.659
Total lipid conc.    (g/dl)   0.262
N number       ((PL/(BA+PL))   0.285
Maximum cholesterol solubility (mol%)   0.000
Percent cholesterol saturation     (%)     0
Your data cannot be calculated correctly  because total lipid concentration is
out of range of fifth-degree polynomial equations [JCI. 61: 998-1026, (1978)].
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Data 1: Gallbladder bile of cholesterol gallstone patient.

Data 2: Gallbladder bile of cholesterol gallstone patient. Ursodeoxycholic acid (UDA) conjugates accounted for 47% of total bile salts.

Data 3: Hepatic bile of a bile fistula hamster. Lecithin concentration was too low for application of the Critical Tables (ref. 16), but percent cholesterol saturation could be calculated by the fifth-degree polynomial equations (ref. 8).

Data 4: Hepatic bile of a bile fistula hamster. After 3 hr of biliary drainage, biliary lipid concentration became very low and percent cholesterol saturation could not be calculated.

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