A Simple Method for Calculating Absorption of Dietary Cholesterol in Man (35560)

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The current methods available for calculating the absorption of dietary cholesterol in man are so difficult and time-consuming that only a few satisfactory studies under rather restricted experimental conditions have been conducted (1). This is due to the following difficulties: (a) To ensure quantitative collection of the unabsorbed cholesterol from the diet, the fecal samples must be collected for a number of days and this results in the contamination of the unabsorbed fraction with the cholesterol secreted in the bile. (b) During its passage in the gastrointestinal (GI) tract, the unabsorbed dietary cholesterol is converted by bacterial flora to a number of different compounds (2). (c) Intestinal bacteria can also degrade the sterol ring so that some of the unabsorbed dietary cholesterol is not recovered by the methods employed to extract neutral sterols (3). (d) Exfoliation of the intestinal cells adds to both dietary and endogenous cholesterol.

The proposed method of calculating cholesterol absorption circumvents these difficulties and is based on the determination of two isotopes in a single unmeasured "test" sample of feces. Cholesterol and β-sitosterol quantitatively and qualitatively undergo identical conversions and degradations in the GI tract (3, 4). Less than 5% of the β-sitosterol is absorbed; whereas, the absorption of cholesterol may be 10 times greater than that of β-sitosterol. After an oral dose of cholesterol-1-2-^{3}H and β-sitosterol-4-^{14}C, the difference in the ^{3}H/^{14}C ratios between the fed and fecal sterols is a measure of the difference in the absorption of the two sterols.

Materials and Methods. Cholesterol-1-2-^{3}H (500 mCi/mmole) and β-sitosterol-4-^{14}C (61 mCi/mmole) (>99% pure) were obtained from Amersham/Searle Corporation. The subjects were fed a mixture of cholesterol-1-2-^{3}H (5 μCi), β-sitosterol-4-^{14}C (1 μCi) and 300 mg or more of carmine red. As long as the ratios of the two isotopes are known it is not necessary to determine their exact doses. The first specimen of feces showing the red color of the carmine red which is not necessarily the first sample of feces collected after giving the radioisotopes and carmine red, was examined by determining the ^{3}H/^{14}C ratios in this "test" sample. The fecal samples were collected individually and the radioactivity in the neutral and acidic sterols was determined by the methods of Miettinen *et al.* (5) and Grundy *et al.* (6). The standard error in 12 aliquots from the same pool by these methods was 1.5%. The plasma lipids were extracted in chloroform:methanol (7); and aliquots of the extract were taken for determination of the concentrations (8) and radioactivity in total cholesterol (9). The fraction of dietary cholesterol absorbed was then calculated from the equation, $(x - y)/x \times 100$, where x and y are the ratios of ^{3}H/^{14}C in the administered dose and the "test" sample of feces, respectively.

Experimental procedures. Four studies were conducted. Two subjects received 5 μCi of cholesterol-1-2-^{3}H and 1 μCi of β-sitosterol-4-^{14}C each; and the others received undetermined amounts of the same mixture. The sterol content of their diets was not examined. The ^{3}H specific activity of plasma cholesterol increased slowly to reach its peak (229 to 292 dpm/mg of cholesterol) on about the third day and the ^{14}C activity was not enough to be detected in the samples of plasma cholesterol. The total activity in the fecal neutral sterols and the bile acids in different subjects is shown in Table I. There

TABLE I. Total Radioactivity in Fecal Neutral Sterols and Bile Acids in Subjects Given Cholesterol-1-2-^{3}H and β-sitosterol-4-^{14}C.

Subject	Time of fecal collection (hr)[a]	Total ^{3}H activity (dpm × 10^{3})		Total ^{14}C activity (dpm × 10^{3})		^{3}H/^{14}C ratio
		Neutral sterols	Bile acids	Neutral sterols	Bile acids	
1	21	2078.6	7.1	836.5	2.0	2.48
	44	473.4	2.7	124.4	1.7	3.81
	46	829.6	4.9	291.0	1.3	2.85
	60–168[b]	3884.7	24.3	1205.9	6.8	3.22
	Total 7 days	7266.3	39.0	2457.8	11.9	
2	30	0.0	0.0	0.0	0.7	
	59	823.5	0.0	299.7	1.1	2.75
	59–91[b]	5426.4	53.5	1819.4	4.5	2.98
	91–222[b]	973.5	14.8	223.8	7.8	4.35
	Total 9 days	7223.4	68.3	2345.6	14.8	
3	35	1523.6	34.8	483.0	5.2	3.15
	47	626.5	6.5	206.4	0.7	3.04
	84	2460.6	39.8	740.0	3.3	3.33
	98	817.4	14.2	200.4	1.0	4.08
	144	636.7	118.2	67.0	0.0	9.50
	203	27.5	5.1	0.6	0.0	
4	24	3990.7	ND[c]	1458.2	ND	2.74
	48	1950.1	ND	563.1	ND	3.46
	72	611.7	ND	109.2	ND	5.60
	96	524.2	ND	31.6	ND	16.59

[a] Time after feeding radioactive sample and carmine red.

[b] Pooled samples.

[c] Not determined.

was practically no radioactivity in the bile acids present in the "test" samples. In the two subjects where exact quantities of isotopes ingested were known (first two subjects of Table I), the recovery of β-sitosterol in the two subjects was about 90% and that of cholesterol was about 50%. The sterols not recovered in the feces were either absorbed or degraded or were still in the GI tract. The ^{3}H/^{14}C ratios in the "test" samples from four subjects were 2.48, 2.75, 3.15, and 2.74 so that the absorption calculated by the ratio method was 54.9, 50.0, 42.7 and 47.6 of the total cholesterol present in the meal with which the radioactive tracer was given. The increase in ^{3}H/^{14}C ratios in successive fecal samples (Table I) could be explained by the progressive increase in the contributions of biliary cholesterol-1-2-^{3}H to the feces. In the third study where all fecal samples were collected individually, the day 8 sample had very little β-sitosterol-4-^{14}C but significant amounts of cholesterol-1-2-^{3}H suggesting that most of the latter had come from the bile.

Discussion. The only difference between the structure of β-sitosterol and cholesterol is the presence of an ethyl group at the 24th position in the former, and the only difference in the disposition of these sterols in the GI tract is that the absorption of β-sitosterol is less than 5% and that of cholesterol is much greater (4). Except for this difference, the two sterols exist as if in a single pool in the lumen of the GI tract. Grundy and associates (3) were so impressed by the qualitative and quantitative similarity in metabolic conversions and degradations of the two sterols in the GI tract that they suggested that β-sitosterol can be used as a standard for estimating the amounts of degradation of dietary cholesterol in the GI tract. The principle of the method is a logical consequence

of the above observations provided the ^{3}H activity in the "test" sample came only from the unabsorbed dietary cholesterol and from nowhere else. The biliary cholesterol can only mix with the contents of the duodenum; and, most of the absorption of the dietary cholesterol occurs after the food has left the duodenum. Moreover, the absorbed cholesterol-1-2-^{3}H is diluted in a pool of about 16 g (1) so that when it begins to appear in significant amounts in the duodenum, the first part of the meal mixed with the tracer would have moved well along into the lower part of the small intestine. These physiological facts would appear to rule out the possibility of significant contamination of the "test" sample of feces with the absorbed cholesterol-^{3}H secreted in the bile. From these and previous studies (9) it can be deduced that the specific activity of plasma (or bile) cholesterol at the time when the first part of the meal enters the duodenum will be insignificant. The absence of radioactivity in the fecal bile acids of the "test" sample supports this assumption. The increasing ratios of $^{3}H/^{14}C$ in the fecal samples associated with reductions in the total activity indicate that progressively greater amounts of the absorbed cholesterol-^{3}H are contributed through the enterohepatic circulation to the fecal samples. This explains the necessity of obtaining the very first sample of feces containing the unabsorbed radioactivity. Carmine red is used as a marker just for this purpose.

Some evidence validating the principle of this method is provided by the results of the two studies where exact doses of the administered isotopes were known. The fecal recoveries of β-sitosterol were 91.5 and 88.0%. The remainder may have been either absorbed or degraded or still have been in the GI tract. The recoveries of 49.2 and 49.0% of cholesterol include very small amounts of radioactive cholesterol secreted in the bile (1), but the values are similar to 55 and 50% calculated from the $^{3}H/^{14}C$ ratios in the "test" sample.

Summary. The currently available methods for estimating the absorption of dietary cholesterol are so difficult that only a few studies under rather restricted experimental conditions have been conducted. A very simple approach circumventing these difficulties is based on the determination of two isotopes (^{3}H and ^{14}C) in a single unmeasured "test" sample of feces after feeding a mixture of cholesterol-1-2-^{3}H (5 μCi), β-sitosterol-4-^{14}C (1 μCi) and carmine red (300 mg). The two sterols undergo similar conversions and degradations in the gastrointestinal tract, except for the differences in their absorption. Thus, the fraction of dietary cholesterol which is absorbed in excess of the absorption of β-sitosterol can be calculated merely from the difference in the $^{3}H/^{14}C$ ratios between the mixture given by mouth and that obtained from the feces. It is important to examine the first sample of feces containing the red color of the carmine red since this "test" sample contains the unabsorbed sterols uncontaminated by radioactivity from the biliary cholesterol and bile acids.

This work was supported by grants (MA3431 and MA-2721) from the Medical Research Council of Canada. We greatly appreciate the kind help of Dr. L. B. Jaques in the preparation of this manuscript.

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Received Sept. 17, 1970. P.S.E.B.M., 1971, Vol. 137.