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Cholesterol Absorption in Primates as Determined by the Zilversmit Isotope Ratio Method[1] (38365)

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Zilversmit (1) has recently proposed a simple, double isotope method for measuring cholesterol absorption in rats. Basically, the method consists of feeding cholesterol labeled with either ^{14}C or ^{3}H and concomitantly injecting intravenously cholesterol labeled with the isotope not used in the feeding. The ratio of fed to injected isotope (after correcting for degradation or disappearance using labeled sitosterol) is a good measure of absorption and compares well with absorption as determined by direct determination of fecal excretion of orally administered labeled cholesterol (2–4). We have tested this method in baboons and vervet monkeys and our findings are described in this communication.

Materials and Methods. Cholesterol labeled with tritium (1,2-^{3}H) or radioactive carbon (4-^{14}C) and [22,23-^{3}H] -sitosterol were purchased from the New England Nuclear Corp., Boston, MA, and shown to be chromatographically pure before use.

For the oral dose, the labeled cholesterol (50 μCi/baboon; 25 μCi/monkey) was dissolved in propylene glycol and administered by stomach tube. The intravenous dose of cholesterol (10 μCi/baboon; 5 μCi/monkey) was prepared by solubilizing the sterol in saline with a minimum amount of Tween 20. The tritium-labeled sterols were fed and the [4-^{14}C] cholesterol was injected. Sitosterol was fed at the level of 75 μCi per animal.

Four male baboons (*papio ursinus*) of average weight, 21.4 kg, and six male vervet monkeys (*cercopithecus aethiops pygerethrus*), average weight, 3.8 kg were used. The baboons were given the doses of isotope while under Sernylan (1-[1-phenylcyclohexyl]piperdine·HCl anesthesia and the monkeys while under physical restraint. The animals were kept in individual cages. Individual feces were collected daily, weighed, homogenized in a known amount of distilled water and aliquots taken for extraction of neutral steroids (3). The animals were bled daily and the sera extracted and saponified according to the procedure of Abell *et al.* (5). The sterols were extracted in hexane and their radioactivity determined by liquid scintillation spectrometry. In the baboons, the sterol radioactivity was also checked after precipitation of the cholesterol as the digitonide (6) and assaying radioactivity in the precipitate (7).

Results and Discussion. Table I presents the amount of ^{3}H recovered from fecal neutral steroids over a 4 day period. By this method the apparent uncorrected absorption of cholesterol was 90.0% in baboons and 91.8% in monkeys. The amount of [-^{3}H] sitosterol recovered from the feces is shown in Table II. There was an apparent recovery of only 36% of the dose administered the monkeys and 31% of the dose given the baboons. The difference is presumed to be lost by intestinal degradation of this sterol. The levels of sitosterol degraded by the baboons and monkeys (64–69%) are higher than those for man as reported by Grundy *et al.* (8), who found that 37% of a low dose and 23% of a high dose of plant sterols were degraded in man.

Rats degrade only about 5% of an oral dose of β-sitosterol (1). These disparities in sitosterol degradation by different species suggest that

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TABLE I. Tritium Labeled Neutral Steroids Recovered (% of Oral Dose) from Feces of Baboons and Monkeys Fed [1, 2-^{3}H] Cholesterol.

Animal[a]	Days after feeding				Total
	1	2	3	4	
M 203	1.5	9.6	4.7	0.6	16.4
M 204	6.1	3.2	1.3	0.7	11.3
M 205	1.9	1.1	0.4	0.4	3.8
M 206	1.0	1.1	0.3	0.2	2.6
M 207	0.2	5.8	1.1	0.1	7.2
M 208	1.7	3.2	0.6	2.2	7.6
Av	2.1	4.0	1.6	0.7	8.2
B 171	0.02	—[b]	—	3.4	3.4
B 172	0.01	3.2	6.2	5.9	15.3
B 173	1.00	—	6.3	6.0	13.3
B 174	0.50	—	—	7.7	8.2
Av	0.40	0.8	3.1	5.8	10.1

[a] M = Monkey; B = Baboon.
[b] No fecal sample available.

plant sterol absorption and degradation must be determined before applying this method of measuring cholesterol absorption to any particular species.

The ratios of ^{3}H/^{14}C found in the sera of the primates used are presented in Table III. The ratios are fairly constant for each animal over the entire sampling period. For the monkeys, the average ratios over the four day period were: 20.8; 23.9; 27.1 and 26.9; for the baboons, the ratios were: 26.1; 31.8 and 26.3 on days 1, 2 and 4, respectively. The baboons were not bled on day 3. Zilversmit (1) observed similar consistency in these ratios in rat serum.

Table IV presents a comparison of the fecal (F) and serum isotope ratio (S) methods for measuring cholesterol absorption. To compute levels of unabsorbed cholesterol, the percent of intravenous dose (^{14}C) found in the feces was subtracted from the percent of oral dose (^{3}H) recovered in the feces and the apparent physiologic loss (based upon recoveries of fecal sitosterol) was added. The average spillover into the feces of intravenously administered cholesterol (^{14}C) was 1.3 ± 0.13% for monkeys and 1.6 ± 0.48% for the baboons. It is apparent the two methods are comparable. Cholesterol absorption in vervet monkeys was found to be 29.1% by the fecal method and 26.9% by the isotope ratio method. In baboons absorption was 22.2% and 26.3% as measured by methods F and S, respectively.

TABLE II. Tritium Labeled Neutral Steroids Recovered (% of Oral Dose) from Feces of Primates Fed [22,23-^{3}H] Sitosterol.

Animal[a]	Days after feeding				Recovery	Degraded
	1	2	3	4	%	%
M 210	29.8	4.5	6.8	7.5	48.7	55.3
M 211	2.1	18.7	5.9	4.7	31.4	68.6
M 212	4.0	23.6	11.5	5.7	44.8	55.2
M 213	10.9	9.3	6.2	0.9	27.3	72.7
M 214	3.6	17.6	2.1	9.1	32.4	67.6
M 215	8.1	10.8	8.5	3.5	30.9	69.1
Av ± SD					35.9	64.1 ± 2.8
B 175	51.1	6.4	—	7.6	65.1	34.9
B 176	0.3	15.7	8.2	0.9	25.1	74.9
B 177	0.1	7.1	7.1	2.7	17.1	82.9
B 178	0.1	11.0	3.2	1.2	15.5	84.5
Av ± SD					30.7	69.3 ± 10.1

[a] M = Monkey; B = Baboon.

TABLE III. Ratio of ^{3}H/^{14}C in Sera of Primates Fed [1, 2-^{3}H] Cholesterol and Intravenously Injected with [4-^{14}C] Cholesterol.[a]

Animal[b]	Days after dosage			
	1	2	3	4
M 203	23.1	25.2	25.1	26.1
M 204	21.4	25.1	24.1	26.7
M 205	22.9	29.4	30.1	30.6
M 206	7.0	6.0	10.8	21.4
M 207	22.8	23.4	29.9	24.5
M 208	27.5	34.3	43.9	32.2
B 171	25.9	39.9	—	39.0
B 172	31.2	32.2	—	20.5
B 173	41.2	47.1	—	34.7
B 174	6.3	12.0	—	10.9

[a] Oral dose 50 μCi/baboon; 25 μCi/monkey. Injected dose 10 μCi/baboon; 5 μCi/monkey. Serum cholesterol was precipitated as the digitonide and the ratio of dpm of ^{3}H to dpm of ^{14}C determined.
[b] M = Monkey; B = Baboon.

TABLE IV. Comparison of Cholesterol Absorption Measured by Fecal Excretion (F) or Serum Isotope Ratio (S).

Animal[a]	Isotope		Unabsorbed cholesterol (%)		Absorbed cholesterol (%)		Recovery (%)
	Oral	IV	Uncorrected[b]	Corrected	F	S	
M 203	^{3}H	^{14}C	16.4	78.8	21.2	26.1	104.9
M 204	^{3}H	^{14}C	11.3	73.8	26.2	26.7	100.5
M 205	^{3}H	^{14}C	3.8	67.1	33.0	30.6	97.6
M 206	^{3}H	^{14}C	2.6	65.5	34.5	21.4	86.9
M 207	^{3}H	^{14}C	7.2	70.4	29.7	24.5	94.9
M 208	^{3}H	^{14}C	7.6	70.2	29.8	32.2	102.4
	Av ± SD		8.2 ± 1.9	70.9 ± 1.8	29.1 ± 1.8	26.9 ± 1.5	97.9 ± 2.4
B 171	^{3}H	^{14}C	3.4	72.5	27.5	39.0	111.5
B 172	^{3}H	^{14}C	15.3	82.6	17.4	20.5	103.1
B 173	^{3}H	^{14}C	13.3	79.7	20.3	34.7	114.4
B 174	^{3}H	^{14}C	8.2	76.4	23.6	10.9	87.3
	Av ± SD		10.0 ± 2.3	77.8 ± 1.9	22.2 ± 1.9	26.3 ± 5.6	104.1 ± 5.3

[a] M = Monkey; B = Baboon.

[b] Corrected for bacterial degradation and intestinal reexcretion.

Summary. The Zilversmit isotope ratio method for measuring cholesterol absorption has been applied to baboons (*Papio ursinus*) and vervet monkeys (*cercopithecus aethiops pygerethrus*) and found to compare well with the more complicated method involving fecal excretion. Cholesterol absorption in the baboon was found to be 26.3% by the Zilversmit method and 22.2% by the fecal excretion method. In vervet monkeys the Zilversmit method showed 26.9% absorption as compared with 29.1% by the other. Sitosterol degradation (which is used to assay degradation) was found to be 64.1 and 69.3% in monkeys and baboons, respectively. Sitosterol degradation is about 5% in rats (1) and 23–27% in man (8). The correction for sterol degradation must, therefore, be determined for each species (and possibly in each experiment).

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