JCI The Journal of Clinical Investigation

Usefulness of chromic oxide as an internal standard for balance studies in formula-fed patients and for assessment of colonic function

Jean Davignon, … , W. J. Simmonds, E. H. Ahrens Jr.

J Clin Invest. 1968;47(1):127-138. https://doi.org/10.1172/JCI105703.

Research Article

In 35 patients maintained solely on liquid formula diets, chromic oxide has been evaluated as an internal standard for balance studies that require stool collections. In 28 patients the excretion of chromic oxide was ideal: steady states were attained in which mean daily output was 90% (or more) of mean daily intake. In these patients corrections for fecal flow could validly be applied.

In patients who excreted the marker ideally, the availability of chromic oxide balance data made possible the calculation of pool sizes and turnover rates of unexcreted intestinal content. These indexes bore little relationship to the usual clinical descriptions of bowel habits. In some patient who had daily bowel movements, pool sizes were very large and daily turnover was small, i.e., a large proportion of the colonic contents was not excreted for surprisingly long periods. It is critically important for investigators to recognize this possibility when carrying out balance studies for fecal constituents that may be altered by bacterial action within the gut lumen: for instance, in 6 patients a significant inverse correlation was found between daily fecal turnover and degradative losses of large amounts of dietary β-sitosterol.

7 of 35 patients failed to attain the ideal steady state of chromic oxide excretion. These patients would not have been singled out if an internal standard had not been […]

Usefulness of Chromic Oxide as an Internal Standard for Balance Studies in Formula-Fed Patients and for Assessment of Colonic Function

JEAN DAVIGNON, W. J. SIMMONDS, and E. H. AHRENS, JR.

From The Rockefeller University, New York

ABSTRACT In 35 patients maintained solely on liquid formula diets, chromic oxide has been evaluated as an internal standard for balance studies that require stool collections. In 28 patients the excretion of chromic oxide was ideal: steady states were attained in which mean daily output was 90% (or more) of mean daily intake. In these patients corrections for fecal flow could validly be applied.

In patients who excreted the marker ideally, the availability of chromic oxide balance data made possible the calculation of pool sizes and turnover rates of unexcreted intestinal content. These indexes bore little relationship to the usual clinical descriptions of bowel habits. In some patient who had daily bowel movements, pool sizes were very large and daily turnover was small, i.e., a large proportion of the colonic contents was not excreted for surprisingly long periods. It is critically important for investigators to recognize this possibility when carrying out balance studies for fecal constituents that may be altered by bacterial action within the gut lumen: for instance, in 6 patients a significant inverse correlation was found between daily fecal turnover and degradative losses of large amounts of dietary β-sitosterol.

7 of 35 patients failed to attain the ideal steady state of chromic oxide excretion. These patients would not have been singled out if an internal standard had not been used. In such patients balance studies that require analysis of fecal constituents must be interpreted with great caution, since the constituents in question may be handled in the same nonideal fashion as the internal standard.

Dr. Davignon's address is The Clinical Research Institute, 110 Pine Avenue West, Montreal 18, P.Q., Canada. Dr. Simmonds' present address is the Department of Physiology, University of Western Australia, Nedlands, Western Australia. Address requests for reprints to Dr. E. H. Ahrens, Jr., The Rockefeller University, New York 10021.

Received for publication 5 May 1967 and in revised form 21 August 1967.

INTRODUCTION

In metabolic balance studies stools collected during a given period of time do not necessarily reflect the biological events that take place in the intestine during the collection period; even 4-day collections rarely represent exactly 96 hr in the transit of any particular portion of the intestinal contents. Hence, there is a need for an inert marker which can be incorporated into the food intake, the excretion of which can indicate the completeness of stool collections and permit corrections for variations in fecal flow. Chromic oxide (Cr_2O_3) has been widely used in man since 1947 (1) for these purposes, since it is nontoxic, readily measurable in the feces, and appears to be completely unabsorbable.

In view of the widening use of formula feeding in metabolic balance studies, we have evaluated Cr_2O_3 as a marker in patients fed a residue-free diet. In the course of that exploration, we noted that the pool size of unexcreted colonic contents, as well as the daily percentage turnover of that pool, could be estimated from Cr_2O_3 excretion data. It then became clear that these estimates bore little relationship to such common clinical indexes as the size and frequency of bowel movements

which seem, therefore, to give a poor indication of the residence time of intestinal contents within the colon. Nevertheless, this residence time determines to some extent the degree of bacterial growth in the colon; this, in turn, could affect the degree of degradation of various metabolites by bacteria before evacuation.

This paper reports the usefulness of Cr_2O_3 as a marker in formula-fed patients, and describes certain anomalies in the physiology of colonic function which can cause errors in balance experiments but which can be recognized if an internal standard is being used.

METHODS

Clinical aspects

The study was carried out on 35 patients (Table I), 15 males and 20 males, 11–70 yr of age, admitted to the metabolic ward of The Rockefeller University Hospital for long-term investigation of cholesterol metabolism. 4 patients were normocholesterolemic; all others were affected with some form of hypercholesterolemia and (or) hyperglyceridemia, and 19 had ischemic heart disease. In none was there clinical evidence of gastrointestinal disease. All were ambulatory and were urged to exercise within the limitations set by the severity of their coronary artery disease.

Patients were maintained solely on orally administered liquid formula feedings, supplemented with vitamins and minerals as previously described (3), throughout their hospital stay. 26 different dietary regimens were imposed; in all but 3 regimens the protein intake was 15% of total calories, and in these 3, fed to children, protein intake was increased to 20%. 16 formulas supplied 40% of calories as fat, and in the others the fat content varied from 0 to 34%; 8 different dietary fats were used. These variations had no effect on the aims or results obtained in the present study. In all cases, caloric intakes were adjusted in order to maintain the patient's body weight constant.

Five feedings were given at 3-hr intervals each day, and with each feeding one or two tablets (see below) each containing 60 mg of Cr_2O_3 were dispensed: a 300 mg per day dosage was chosen in most cases because it permitted an accurate estimation of the chromium content in a 0.5–1.5 g aliquot of fecal homogenate. Complete fecal collections were made, and in most cases were pooled in 4-day collections throughout the study period, except for the 1st and last 8 days, when 24-hr collections were made. Stools were homogenized as previously described (4); 6-oz aliquots were immediately transferred to screw-cap bottles for storage at 4°C. The duration of study varied from 33 to 302 days.

6 patients received approximately 10 μc of $^{51}Cr_2O_3$, either as a single tablet or in divided doses (2–5 tablets). In 5 of the 6 patients the radioactive tablets were substituted for nonlabeled tablets during chronic administration of the marker; the 6th patient received radioactive Cr_2O_3 without prior exposure to the marker.

Preparation of chromic oxide for tableting

Tablets of reagent grade Cr_2O_3 (chromium sesquioxide, Fisher Scientific Company, Pittsburgh, Pa.) were prepared in large batches according to standard pharmaceutical techniques by Lederle Laboratories Div. (American Cyanamid Co., Pearl River, N. Y.; Mr. Adolph E. Tiesler). Each tablet contained 60 mg of Cr_2O_3, 120 mg of microcrystalline cellulose, and 5 mg of talc. The coefficient of variation for a total tablet weight of 185 mg was less than 2% (number of tablets = 50), and random sampling throughout the study confirmed the manufacturer's estimate of Cr_2O_3 content, within the error of our analytical method. If held in the mouth, the tablet would rapidly disintegrate into powder.

Analyses were made for trace metals by flame emission spectroscopy through the generous cooperation of Dr. Bert L. Vallee, Harvard Medical School. Elements other than chromium were not found in the milled or unmilled powder, or in acid washings, in concentrations that could cause concern from a toxicological viewpoint.

Preparation of radioactive Cr_2O_3 tablets

The tablets of Cr_2O_3 described above were bombarded in an atomic pile in order to generate the ^{51}Cr isotope with an activity level of approximately 10–20 μc per tablet. (This isotope has a half-life of 27.8 days and emits γ-radiation with an energy of 0.3 Mev.) Radiopurity was assessed by neutron activation and by γ-ray scintillation spectrometery: no long-lived γ-emitting species other than ^{51}Cr were detected, and the radiochemical purity was better than 99.9%. Irradiations and analyses were carried out by Dr. Werner H. Wahl, Union Carbide Corporation, Suffern, N. J.

Measurement of chromic oxide

Mass measurements of Cr_2O_3 were carried out according to the method of Bolin, King, and Klosterman (5), with some modifications. Thick-wall Vycor digestion tubes calibrated at 25 ml were used instead of Kjeldahl flasks, and the tubes were heated in heating block at 215°C, instead of with microburners. Aliquots (0.5–1.5 g) of fecal homogenate containing 1–3 mg of Cr_2O_3 were digested, and the resultant yellow solution (dichromate) was read against a reagent blank in a Coleman Jr. Spectrophotometer (Coleman Instrument Corp., Maywood, Ill.) at 440 mμ. A standard calibration curve was prepared with each set of analyses, using a standard suspension of Cr_2O_3 in water. 14 such curves obtained over a 2 yr period with freshly prepared standard suspensions of Cr_2O_3 showed a coefficient of variation of 2%. All analyses were performed in duplicate; when disagreements between duplicates exceeded 8%, the determination was repeated. In some cases insoluble inorganic salts formed a fine precipitate during digestion; in these in-

TABLE I

Clinical Data

Patient No.	Patient Initials	Age	Sex	Weight	Height	Diagnosis	Length of study period: On ward	Length of study period: On Cr_2O_3
				kg	*cm*		*days*	
1	J.St.	35	M	70	175	IHD,* hypercholesteremia (Type II‡)	138	120
2	J.Sh.	68	F	63	165	IHD, normocholesteremia	182	172
3	A.M.	60	F	66	164	IHD, PVD,* hyperglyceridemia (Type IV), chronic lymphocytic leukemia	139	109
4	E.Y.	49	F	53	164	Hypercholesteremia (Type II)	180	65
5	R.T.	48	M	65	164	IHD, hyperglyceridemia (Type IV)	191	179
6	H.Sp.	50	F	78	164	IHD, hypercholesteremia (Type II), essential hypertension	214	180
7	H.Sa.	54	M	78	169	Hyperglyceridemia (Type V)	84	80
8	H.R.§	41	F	65	165	Hyperglyceridemia (Type IV)	145	137
9	L.B.§	14	F	32	149	IHD, PVD, xanthomatosis, hypercholesteremia (Type II)	197	184
10	R.F.§	55	F	64	154	IHD, hypercholesteremia (Type II)	126	43
11	A.C.§	15	F	33	146	IHD, PVD, xanthomatosis, hypercholesteremia (Type II)	190	172
12	R.G.	58	F	61	147	IHD, xanthomatosis, hypercholesteremia (Type II), essential hypertension	193	168
13	N.A.	30	M	67	170	IHD, hypercholesteremia (Type II)	244	229
14	N.R.	36	F	67	168	PVD, hypercholesteremia (Type II)	147	118
15	J.R.	36	F	53	164	IHD, PVD, xanthomatosis, hypercholesteremia (Type II)	169	159
16	D.R.	56	F	53	157	Hyperglyceridemia (Type V)	156	148
17	D.A.§	55	M	64	168	IHD, hyperglyceridemia (Type V)	325	302
18	L.Bo.	48	M	95	172	IHD, hypercholesteremia (Type II)	70	44
19	R.B.	37	F	51	169	Primary biliary cirrhosis	34	33
20	J.H.	38	M	72	172	Hypercholesteremia (Type II)	210	203
21	J.J.§	39	F	71	162	Hyperglyceridemia (Type V)	229	220
22	M.K.§	45	F	54	159	Primary biliary cirrhosis	90	90
23	E.K.	68	M	73	165	IHD, hypercholesteremia (Type III)	121	100
24	L.M.	58	F	50	157	Xanthomatosis, hypercholesteremia (Type II)	119	111
25	C.P.	51	F	59	155	Primary biliary cirrhosis	186	171
26	N.S.	21	F	52	166	IHD, xanthomatosis, hypercholesteremia (Type II)	169	130
27	H.T.	57	M	45	166	Retinopathy of unknown origin, hypercholesteremia (Type II)	180	90
28	J.Ca.	39	M	64	176	IHD, hypercholesteremia (Type II)	221	200
29	B.K.	53	F	38	154	Xanthomatosis, hypercholesteremia (Type II)	209	207
30	E.S.	20	M	70	178	Xanthomatosis, hypercholesteremia (Type III)	85	69
31	I.G.	46	F	55	155	Normal	118	91
32	R.I.	11	M	23	131	Hyperglyceridemia (Type I)	97	95
33	S.I.	11	M	29	139	Hyperglyceridemia (Type I)	97	95
34	J.T.	70	M	72	166	Cerebral arteriosclerosis	180	150
35	A.G.	48	M	95	186	IHD, hyperglyceridemia (Type IV)	150	128

* IHD, ischemic heart disease; PVD, peripheral vascular disease.

‡ Typing of hyperlipidemias according to Fredrickson, Levy, and Lees (2).

§ Patients in whom a steady state of Cr_2O_3 excretion was not achieved.

stances the final reaction mixture was centrifuged, and the clear supernatant solution was used for colorimetry.

Because of the high density of the marker (5.21), the possibility that sedimentation might occur during homogenization, storage, and sampling was investigated. When feces were diluted 1:1 or 1:2 (w/w) with water, no sedimentataion of Cr_2O_3 could be detected within 15 min after homogenization; this allowed sufficient time for transfer of a representative sample of feces to a storage jar. In stored samples vigorously shaken by hand, aliquots taken at the surface and at the bottom of the storage jar showed no significant differences in chromium content.

Storage at 4°C for long periods did not influence Cr_2O_3 measurements in fecal samples: replicate determinations over several months showed a coefficient of variation of less than 5%. 10 replicate determinations over a 7 month period in feces diluted 1:2 showed a coefficient of variation of 2.8% with a mean difference between duplicates of 2.2% (± 1.5%).

Measurement of γ-emission in feces containing $^{51}Cr_2O_3$ was carried out in a well-type scintillation counter at a constant volume of 3.5 ml. Fecal homogenates were counted directly, since Roche, Perez-Gimenez, Layrisse, and Di Prisco (6) had shown that in fecal homogenates diluted 1:1 there was no self-absorption of radioactivity. Counting efficiency was 3.1%; samples and background were counted for sufficiently long times that the error of counting was lower than 5%.

Counts in fecal homogenates of 3.5 ml volume were always related, for decay correction, to counts obtained with a $^{51}Cr_2O_3$ tablet set aside as a standard. It was necessary to increase the count in feces by 12.5% because of the difference in geometry between a tablet and 3.5 ml of suspension. This correction factor was obtained by counting soluble Na radiochromate in a volume equal to that of a tablet, then adding water to bring the volume to 3.5 ml and recounting.

Gamma-ray counting of urine was carried out on the residue obtained by lyophilization; blood samples were hemolyzed with small amounts of sodium dodecyl sulfate, then counted as described above for feces.

Terms and calculations

Steady state of Cr_2O_3 excretion refers only to the part of the study period when the mean daily excretion equals the daily intake.

Idcality of Cr_2O_3 excretion is, by definition, achieved only if the steady state is attained. Any patient not achieving the steady state is considered nonideal with regard to excretion of Cr_2O_3; this finding should serve to warn the investigator that the excretion of other fecal constituents may also be nonideal.

Cr_2O_3 pool size refers to the amount of marker present in the intestinal tract at any given time after the steady state is attained. It is measured directly as the amount collected in the feces after the intake has been discontinued. Also, it can be calculated at any time during the study as the difference between the total administered and total recovered; however, this estimate is subject to greater inaccuracy than the former direct measurement because of the accumulation of errors made in analyses of many stool collections.

Fecal pool size represents the weight (g) of unexcreted intestinal contents (presumably that mainly in the colon). During the steady state it is equal to the Cr_2O_3 pool size (mg) divided by the mean daily concentration of Cr_2O_3 (mg/g of feces). Clearly, the fecal pool calculation is an oversimplification: since there must be a gradient of Cr_2O_3 concentration from the cecal to the sigmoid end of the large bowel, because of water absorption during colonic transit, pool sizes calculated in this manner are minimum estimates.

Daily Cr_2O_3 turnover (%) represents an average figure for the percentage of the Cr_2O_3 pool which is excreted daily. In the steady state it is the ratio of the mean daily Cr_2O_3 output to the Cr_2O_3 pool size, multiplied by 100.

Daily fecal turnover represents the percentage of the fecal pool that is excreted daily, on the average. In the steady state it is the ratio of the average daily fecal weight (g) to the fecal pool size, multiplied by 100.

Fractional turnover rate of $^{51}Cr_2O_3$ was calculated on the assumption that the elimination of $^{51}Cr_2O_3$ follows first-order kinetics:

$$k_1 = -\frac{1}{t}\left[\log\left(1 - \frac{u_t}{u_{max}}\right)\right],$$

where u_t is the total amount of $^{51}Cr_2O_3$ excreted at time t, and u_{max} the total amount of radioactivity recovered from the feces. Taking into account the transit time c of the marker in the intestine (which is considered to be a constant for a given patient) and subtracting this value from t, so that the curve of slope k_1 will pass through the origin,

$$k_1 = -\frac{1}{(t-c)}\left[\log\left(1 - \frac{u_t}{u_{max}}\right)\right].$$

The value $\left[-\log\left(1 - \frac{u_t}{u_{max}}\right)\right]$ was plotted against $(t-c)$, and the fractional turnover rate was derived by measurement of the slope k_1 on a graph depicting the line of best fit by the least squares method. Lindstedt and Norman (7) made the same general assumptions in their calculation of bile acid turnover in man.

Indexes of bowel habits. The following terms were used to compare the bowel habits of different patients. The *average daily fecal weight* (*AFW*) represents the mean daily wet stool weight before dilution and homogenization. The *average bowel movement weight* (*BMW*) is the total weight of stools passed during a given period divided by the number of movements over the same period. The *regularity index* is the percentage of the number of days with at least one bowel movement. The *frequency index* is the percentage of the number of days with more than one bowel movement, i.e., a frequency index of 40% means that during 100 days of observation a patient had more than one bowel movement per day on 40 days.

Measurements of fecal steroids

Fecal neutral and acidic steroids were measured by techniques developed in this laboratory and published in detail elsewhere (4, 8). The raw data were corrected for degradation of the sterol ring structure as previously described (9–11).

RESULTS

Cr_2O_3 balance data in 28 patients with ideal excretion of the marker transit time

In 9 patients who had daily bowel movements and who later were shown to excrete Cr_2O_3 ideally, the transit time of the marker (measured chemically) was 2 days. However, there was no relationship between appearance time, average daily fecal weight, and percentage of daily dose excreted when the marker was first detected. When $^{51}Cr_2O_3$ was given later to 4 of these patients, radioactivity was first detectable in the stools on the 2nd day in each case.

Attainment of steady state. Under ideal balance conditions with daily administration of the marker, the concentration of Cr_2O_3 in the feces should increase rapidly to reach a plateau. Similarly, daily output of Cr_2O_3 should rise until it first exceeds daily intake and then fluctuates around 100% of intake, at which time a steady state of balance is attained and the amount of Cr_2O_3 retained in the intestinal tract (the pool size) has become constant.

Although attainment of the steady state is reflected by a tendency for a cumulative recovery curve to be hyperbolic and to approach the 100% limit with time, that limit is necessarily reached at a later time. By contrast, if there is a continual retention of marker, the concentration of Cr_2O_3 in feces may still reach a plateau and so may a cumulative recovery curve. However, the plateau for cumulative recovery will be less than 100% (for example, 66% if ⅓ of the intake is retained each day); even when the daily retention of marker is a constant fraction of the daily intake, the balance is nonideal and a true steady state is not reached.

Fig. 1 illustrates these points with data obtained in patient 2, in whom the Cr_2O_3 balance data met the criteria of ideality. The concentration of marker in the feces reached a maximum in 4 days, and remained relatively constant thereafter until the end of this 172 day study. Balance data exceeded

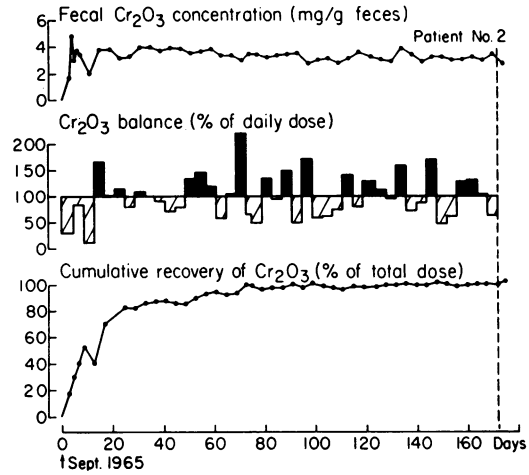

FIGURE 1 Excretion pattern of Cr_2O_3 administered daily in an "ideal" patient. Patient 2, given 300 mg of Cr_2O_3 per day, had a transit time of 2 days and rapidly attained a plateau of fecal concentration of Cr_2O_3 (4 days); the balance data showed that the steady state was reached by 16 days.

100% of the daily dose for the first time in the fourth 4-day stool collection; clearly, the steady state could not have been reached until that time. Thereafter, the balance data fluctuated between 50 and 150% of the daily dose in 35 or 40 collection periods, and it appeared that the steady state was reached after 16 days. The cumulative recovery curve reached 100% only after 72 days, even though the steady state was reached in 16 days; the plateau at 100% indicated ideality of Cr_2O_3 excretion.

Table II describes studies of 65–180 days' duration in 6 patients in whom the steady state was examined in detail. This analysis required total stool collections during the entire period of Cr_2O_3 administration as well as for several days thereafter, in order to determine the total amount of marker retained in the intestine after oral intake ceased. In these 6 patients at least 93% of the daily intake of Cr_2O_3 was recovered in the feces during the steady state, and in the 4 who were given $^{51}Cr_2O_3$ the recovery was 94% or more. While the mean daily fecal concentration varied considerably from patient to patient, in each the variability from one collection period to another was relatively small, i.e., the coefficient of variation was less than 25% in all cases. A plateau of fecal Cr_2O_3 concentration was reached in 4–12 days, and the steady state after 6–18 days. Slowness in reach-

TABLE II

Pool Sizes, Turnover Data, and Recoveries of Cr_2O_3

All data are related to 4 indexes of bowel habits in 6 ideal patients.

	Patient						Mean of data (± SD) for 6 patients
	1	2	3	4	5	6	
Chromic oxide, basic data							
Days on chromic oxide	80	172	109	65	179	180	
Daily intake, *mg/day*	295	295	300	600	295	295	
Mean daily output,* *mg/day*	303	304	305	603	274	278	
Mean daily concentration,* *mg/g feces*	2.7	3.4	7.0	5.6‡	5.5	6.1	5.1 ± 1.7
± SD	0.2	0.4	0.9	0.9	0.9	1.4	
Coefficient of variation, %	7.1	11.8	12.8	15.9	16.7	22.9	
Days to attain							
A plateau in fecal Cr_2O_3 concentration	6	4	6	12	8	8	7.2 ± 2.4
The steady state	6	18	6	16	8	8	10.3 ± 5.3
Recovery data							
(Output ÷ intake) × 100*	103	103	102	101	93	94	
Plateau level of cumulative recovery, %	101	100	98	94	90	90	95.5 ± 4.9
Total % recovery of Cr_2O_3§	102.6	103.5	103.2	99.4	89.2	94.2	98.7 ± 5.8
Total % recovery of $^{51}Cr_2O_3$	110.7	96.6	99.9	—	—	93.9	97.8 ± 3.1
Pool sizes and turnovers							
Cr_2O_3 pool size, *mg*	303	1527	1073	654‡	490	1115	860 ± 457
Fecal pool size, *g*	110	453	154	116	89	183	184 ± 136
Daily Cr_2O_3 turnover, %	100.0	19.9	28.4	46.0	56.0	24.9	45.9 ± 29.8
Daily fecal turnover, %	100.6	19.9	28.1	49.3	58.9	27.9	47.4 ± 29.9
Indexes of bowel function							
Average fecal weight, *g*	110.8	90.2	43.4	57.4	52.4	51.1	67.6 ± 26.7
Bowel movement weight, *g*	166.2	59.5	43.4	49.9	58.9	56.7	72.4 ± 46.3
Regularity index, %	66.6	97.6	100.0	98.1	84.3	81.4	
Frequency index, %	1.4	40.0	0.0	9.2	4.6	8.7	

* Average of all data, except for those during the first 8 days of marker administration.

‡ Value normalized to an intake of 300 mg of Cr_2O_3 per day.

§ Total recovery of marker during its administration and after its discontinuance.

ing the steady state was perhaps due to the fact that residue-free formula feeding was employed in this study (although we have not tested the effect of added residue). Nevertheless, it must be stressed that the regimen per se did not preclude attainment of the steady state.

In 22 other patients, mean daily Cr_2O_3 excretion closely approximated mean daily intake (± 10%) over the many days (24–220) in which 4-day stool collections were continuously made. Although all the information recorded in Table II was not sought in those 22 patients, the demonstration of equal intake and output indicated that the excretion of Cr_2O_3 was ideal and that the steady state had been attained in these formula-fed patients.

Pool sizes and turnover rates (Table II). Table II shows that pool sizes varied over a 5-fold range in these 6 patients, and daily turnover varied from 20 to 100%. In other terms, the amount of unexcreted intestinal contents varied from 90 to 450 g, and the amount of unexcreted Cr_2O_3 varied from 1 to 5 daily doses of the internal standard. Despite large variations in colonic emptying, the excretion of Cr_2O_3 was ideal in all these patients; this indicated that balance data for products excreted in the feces could be corrected meaningfully for variations in fecal flow.

This conclusion would not necessarily have been reached by consideration of the usual clinical indexes of bowel function. As seen at the bottom of

Table II, patient 1 had a bowel movement only 2 days out of 3, while patient 2 had more than one bowel movement per day 40% of the time. Nor did mean daily fecal weights correlate directly with fecal pool sizes and daily fecal turnovers. Patient 2 had the largest pool of unexcreted intestinal contents, but each day eliminated only 20% of that pool. Patient 1, on the other hand, had single bowel movements on 67% of the 80 days of the study period, yet each evacuation represented complete colonic emptying.

Anomalous excretion of Cr_2O_3 in 7 nonideal patients

In 7 patients (patients 8–11, 17, 21, 22) certain peculiarities in excretion of Cr_2O_3 were noted. (None of them showed evidences of intestinal disease or greater variations in the usual indexes of bowel function than the much larger group in whom the excretion of marker was ideal.) First, in the 4 patients in whom the transit time of Cr_2O_3 was measured, it varied from 3 to 5 days, in contrast to 2 days in 9 ideal patients. In the second place, the 7 patients excreted the marker so slowly, and consistently retained so much of the daily intake, that the steady state was never reached; indeed, during the 43–302 days of study these patients excreted only 61–80% of the cumulative intake. Third, while the concentration of Cr_2O_3 per gram of feces was approximately the same as in the ideal group described in Table II, the variation in concentration of marker from one period to the next varied much more widely: in 3 patients the coefficient of variatoin was 38–41%, whereas in only 1 of 6 ideal patients (Table II) was the coefficient of variation greater than 20%.

Accumulation and sequestration. Fig. 2 illustrates the slow attainment of a stable fecal Cr_2O_3 concentration in a nonideal patient (patient 10), for 43 days; the concentration plateau was not reached for 25 days. The balance data show a persistent retention of the marker, with less Cr_2O_3 excreted than administered per day on 35 of the 43 stool collection days. Thus, the steady state was never reached, and the cumulative recovery never exceeded 72% for as long as the daily intake of marker continued. It was calculated that this patient had retained 7.6 g of Cr_2O_3 over a period of 43 days, equivalent to 13 days of intake at 600 mg/day; of this, 4.3 g was recovered in the last

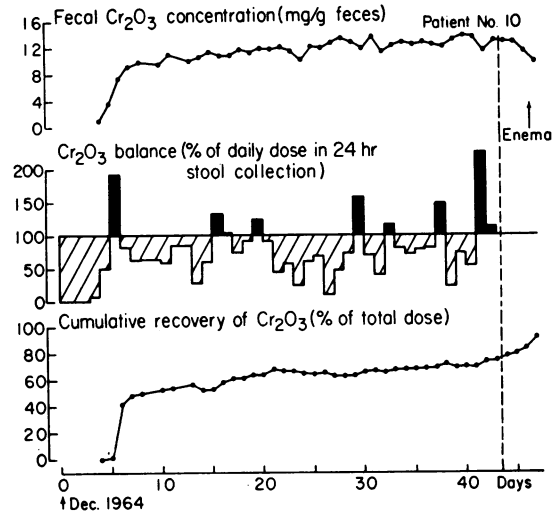

FIGURE 2 Excretion pattern of Cr_2O_3 administered daily in a "nonideal" patient. Patient 10, given 600 mg Cr_2O_3 per day, had a transit time of 4 days and slowly achieved a constant concentration of Cr_2O_3 in the feces (25 days). The balance data showed wide fluctuations in daily excretion, as well as net retention of marker. The cumulative recovery curve showed that a steady state of excretion was never achieved; at least 28% of the Cr_2O_3 administered had been retained. When the marker was discontinued and purgation resorted to, 57% of the retained marker was recovered in the next 4 days.

4 days of hospitalization, after the dosage of marker was discontinued, largely as the result of administering an enema on the final day. Yet on that day the fecal concentration of Cr_2O_3 fell only slightly, despite dilution with enema fluid, which suggests that marker had been sequestered in the colon in concentrated form. An X-ray flat plate of the abdomen, made on the final day of Cr_2O_3 dosage, showed faint homogeneous densities in the hepatic and splenic flexures and descending colon, compatible with the presence of retained Cr_2O_3. Several months later a barium enema showed marked redundancy of the transverse and descending colon. Patient 10 had an enormous pool of Cr_2O_3 (2146 g) and a daily turnover of Cr_2O_3 and of unexcreted colonic contents of only 11–12%; yet regularity and frequency indexes were similar to those of patient 4 whose daily turnover was 50%. (Similar findings were again obtained in patient 10 1 yr after an ileal exclusion operation, as described by Buchwald (12), was performed.)

Patient 11 (Fig. 3), after 172 days on Cr_2O_3 at 300 mg/day, excreted much less Cr_2O_3 each day

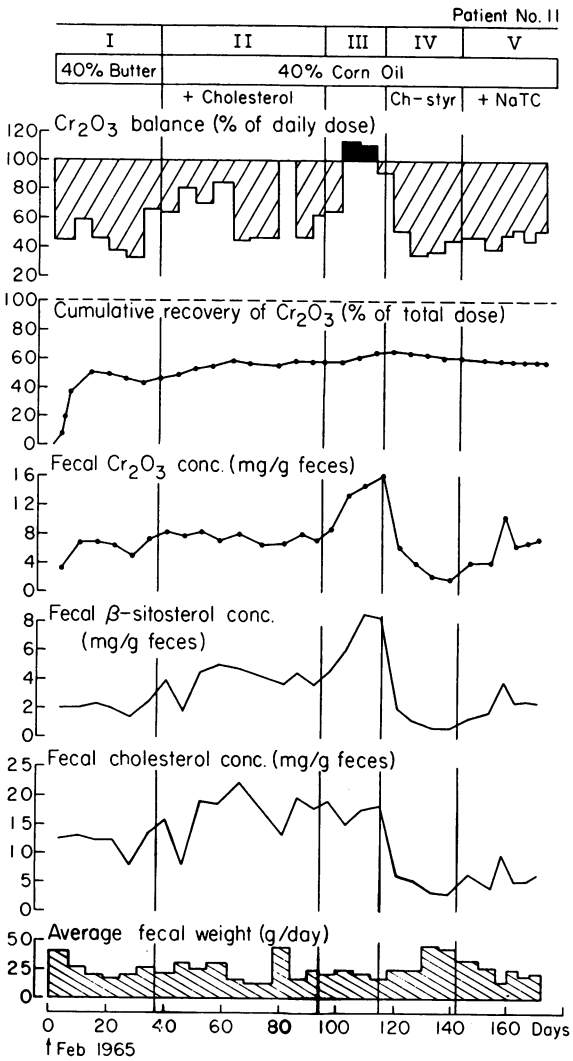

FIGURE 3 Anomalous accumulation and sequestration of Cr_2O_3 and fecal neutral steroids. Patient 11 retained 39% of the Cr_2O_3 and fecal neutral steroids. Patient 11 retained 39% of the Cr_2O_3 administered over a 172 day study period. In period III the concentrations of Cr_2O_3 and of β-sitosterol* in the feces were doubled without any change in average daily fecal weight. The excretion of cholesterol* in period III continued at the same concentration level as in period II, despite the withdrawal of cholesterol from the diet. These findings indicated excretion of feces previously accumulated and concentrated in the colon. (* The data displayed included the parent sterols as well as their neutral steroid bacterial conversion products (8); Ch-styr, cholestyramine, increasing from 4 to 24 g/day, given orally; NaTC, sodium taurocholate, 500 mg/day, by mouth.)

than was administered, and indeed retained 19.4 g, equivalent to 64 days' intake. This Cr_2O_3 pool size, considered with a mean fecal Cr_2O_3 concentration of 7.5 mg/g of feces, indicates a pool size of unexcreted colonic contents of 2.6 kg. In a 15 yr old girl, free of intestinal disease and weighing only 33.3 kg, this estimate of colon contents seems implausible; a more likely explanation for the discrepancy is that the sequestered Cr_2O_3 was more highly concentrated than that excreted in feces.

Not only was the internal standard sequestered in this patient; in period III (Fig. 3) feces highly concentrated in Cr_2O_3 *and* in β-sitosterol were excreted as the Cr_2O_3 balance became temporarily positive for the only two stool collection periods in this long study. This finding has led us to the conclusion that, in patients who fail to reach a steady state for excretion of Cr_2O_3, balance data for other fecal constituents must be considered to be unreliable. This sequestration of marker plus β-sitosterol was demonstrated about a year later in patient 11, and also in a second patient (patient 17) in whom the excretion of Cr_2O_3 was nonideal.

Use of Cr_2O_3 to correct fecal flow

Uniform dispersion in feces. For Cr_2O_3 to be useful in correcting for variations in fecal flow, it must be shown to be uniformly dispersed and not absorbed. Indirect but strong evidence of the uniform mixing of Cr_2O_3 in feces was afforded by kinetic analysis of the excretion of $^{51}Cr_2O_3$ in 6 patients. If the elimination of orally administered $^{51}Cr_2O_3$ follows first-order kinetics, plotting

$$\left[-\log\left(1-\frac{u_t}{u_{max}}\right)\right]$$

as a function of time should give a straight line. In 5 out of 6 patients such a relationship was obtained, which suggests that the basic assumptions of first-order kinetics were fulfilled—namely, that the substance was evenly distributed in a homogeneous pool, and that pool size did not change during the period of observation.

The plots of ^{51}Cr elimination in the two patients with the largest and smallest fecal pool sizes of the group so tested (patients 2 and 5) are presented in Fig. 4. There was good agreement between the fecal Cr_2O_3 turnover rate calculated from the Cr_2O_3 balance data and that derived from the value of k in the $^{51}Cr_2O_3$ experiments. For patient 5 these values were 56 and 54%, and for patient 2 they were 20 and 22%, respectively. Regrettably,

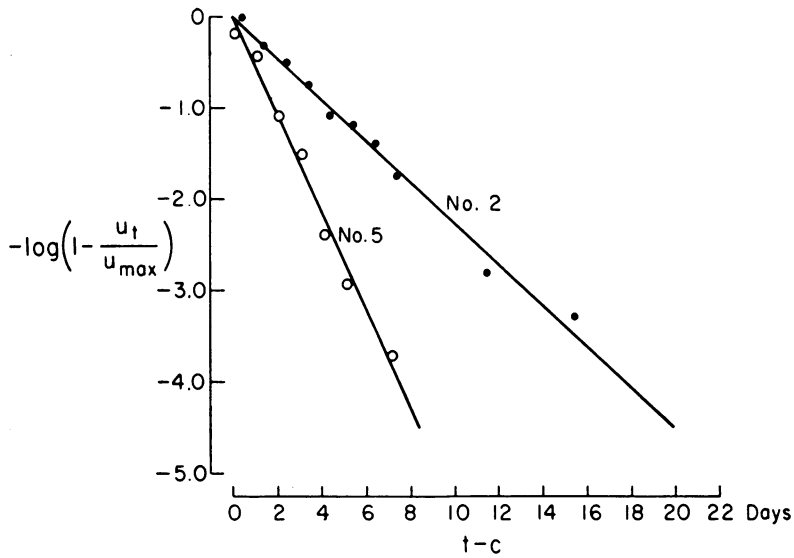

FIGURE 4 First-order kinetics of $^{51}Cr_2O_3$ excretion in 2 patients with low and high fecal Cr_2O_3 turnover rates. Patients 2 and 5 were given 10 μc of $^{51}Cr_2O_3$ by mouth, 19 and 96 days respectively after the start of daily administration of 300 mg of unlabeled Cr_2O_3. After correction of t for the transit time c, the plotted times intersected the zero ordinate. The turnover rates calculated from these kinetic data agreed well with those calculated from Cr_2O_3 balance data (see Table II).

$^{51}Cr_2O_3$ studies were not carried out in any of the nonideal patients.

Nonabsorbability. Table II shows that in 6 patients the recovery of Cr_2O_3 administered daily for 65–180 days was 90% or greater. Indeed, in patients 1–4 the recovery was essentially complete.

As a further test of the nonabsorption of Cr_2O_3, five experiments with $^{51}Cr_2O_3$ were undertaken. The labeled compound (10 μc) was given in a single day to patients who were already receiving 300 mg of unlabeled Cr_2O_3 per day; measurements were made of radioactivity in feces until no further activity was detectable, and in blood and urine for comparable periods in 2 patients. Table II shows that in 3 of 5 patients the recovery in the feces was 100%; since no radioactivity was detectable in any of the blood or urine samples, it seems unlikely that a soluble form of ^{51}Cr had been absorbed from the intestine.

Fecal flow correction. Patients given 300 mg of Cr_2O_3 per day in divided doses with each formula feeding are, in effect, ingesting 1 mg/2.08 min. Thus, if the steady state is reached and the marker is dispersed evenly in the feces, each milligram of marker excreted represents that portion of feces formed every 2.08 min. If the amount of Cr_2O_3 excreted per unit time is known, the measured daily output of feces (or of fecal constituents) can be corrected for irregularities of elimination of feces from the large bowel. Thus, the daily Cr_2O_3 intake (mg) divided by the fecal Cr_2O_3 concentration (mg/g of feces) gives a corrected daily fecal weight. After this correction, the day-to-day variation of fecal weights (or of fecal constituents) should be decreased, providing the diet is constant and the patient is in a metabolic steady state.

Table III presents comparative data for uncorrected and corrected daily fecal weights and daily excretion of total fecal steroids in 5 ideal patients. The means were not significantly different in the two pairs of columns in any of the 10 study periods, while in 18 of 20 comparisons the standard deviations were greatly reduced by correcting for fecal flow. This reduction in variation simply means that stool collections made on a strictly calendar basis are usually misleadingly timed, because intestinal contents are held up in the colon for unpredictable periods.

TABLE III

Use of Cr_2O_3 for Correction of Fecal Flow Rates

First 8 days of data in each diet period were eliminated in these calculations.

Patient	Diet*	Stool collections		Daily fecal weight‡		Stool collections		Total fecal steroids‡	
		No.	Period	Raw data	Corrected by Cr_2O_3	No.	Period	Raw data	Corrected by Cr_2O_3
		n	*days*	*g/day*		*n*	*days*	*mg/day*	
1	I	8	4	53 ± 19	52 ± 5	8	4	730 ± 260	700 ± 70
	II	17	4	110 ± 19	108 ± 10	18	4	1707 ± 404	1707 ± 339
2	I	16	4	82 ± 24	82 ± 7	17	4	608 ± 297	600 ± 87
	II	22	4	93 ± 38	91 ± 8	22	4	1477 ± 695	1423 ± 267
4		54	1	57 ± 24	54 ± 8	10	2	781 ± 285	682 ± 122
5	I	24	4	56 ± 30	57 ± 10	10	8	763 ± 171	873 ± 132
	II	18	4	58 ± 19	59 ± 10	4	8	1051 ± 177	1161 ± 81
6	I	17	4	52 ± 13	55 ± 14	9	2–4	560 ± 226	609 ± 48
	II	16	4	60 ± 28	61 ± 30	10	2–4	870 ± 432	817 ± 174
	III	7	4	47 ± 11	44 ± 4	7	4	773 ± 200	735 ± 97

* I–III refer simply to different dietary regimens in each patient and do not imply the same regimens in different patients. The compositions of the diets are not relevant to the objectives of the present study.

‡ Values are given as means ± SD.

DISCUSSION

The ideal marker. The need for an ideal marker for metabolic studies has long been recognized, and it is generally agreed that the criteria for ideality are: nonabsorbability, nontoxicity, uniform dispersibility relative to the fecal constituent of interest in the particular investigation, rapid attainment of the steady state (in which, on the average, excretion of the marker equals its intake), and ease of measurement.

Cr_2O_3 was introduced as an intestinal marker in veterinary work by Edin, Kihlén, and Nordfeldt (13) in 1944; it was first extensively evaluated in clinical studies by Irwin and Crampton (14) in 1951. Stanley and Cheng (15) applied it in their ingenious studies of cholesterol metabolism in 1956; in 1957 they discussed the advantages of the inert indicator method in detail (16), and in 1960 Whitby and Lang (17) reported their own extensive experience with Cr_2O_3. These three clinical studies demonstrated that Cr_2O_3 met all five of the criteria listed above, although Whitby and Lang warned of the possibility that "streamlining" of intestinal contents may occur and that steady states may not always be attained; indeed, in their hands 15 of 52 calcium balance studies carried out on solid food diets were judged not to be acceptable for these reasons. It is noteworthy that, in previous studies of Cr_2O_3 steady states, solid food diets were administered. While the three groups of investigators referred to used large daily dosages (1–12 g of Cr_2O_3), we found 300 mg/day to be entirely adequate.

In the present study of 35 patients fed liquid formula diets exclusively, the five criteria of ideality were met in all but 7 patients. Thus, in 2 years' experience with this internal standard (5000 patient-days on the marker) we have seen no evidence of toxicity; but we have purposely not studied patients with gastrointestinal disease and cannot state whether this particulate material may be irritating to an inflamed gut wall, even though it is chemically unreactive. Its nonabsorbability seems to have been established within the limits of accuracy of our methods for measuring chromium salts and radioactive ^{51}Cr. Cr_2O_3 can mix uniformly with other fecal constituents, if one is to judge from the work of previous investigators (14, 15, 17); our own kinetic studies of $^{51}Cr_2O_3$ excretion established that labeled chromium was eliminated from a single homogeneous pool in 5 of 6 ideal patients. Finally, the simple and accurate

measurement of Cr_2O_3 in feces has been well established by all previous workers and confirmed in the present study.

However, the fact that not all patients excrete the marker in an ideal manner and our finding that a few patients may sequester large amounts of Cr_2O_3 and of fecal neutral steroids serve as warnings that the steady state must first be demonstrated in each patient if balance data for fecal constituents are to be reliably corrected through use of Cr_2O_3. The demonstration need not be laborious: it is sufficient to show that in any given period of study the mean daily output of marker is 90% or more of the daily intake. If this *cannot* be shown, there would be reason to believe that stool collections were incomplete, that the steady state for Cr_2O_3 excretion had not yet been attained, or that some of the marker was being accumulated within the intestinal tract. In the latter case, the burden of proof would rest on the investigator to prove whether or not the fecal constituents under study were being sequestered along with the marker. Whatever the explanation for nonideality, uncorrected raw balance data for fecal constituents would be suspect; thus, the use of Cr_2O_3 can indicate which balance study periods are technically reliable. Indeed, it has been our practice for the last 2 yr to administer Cr_2O_3 to all patients involved in balance studies in which fecal constituents are to be analyzed. We apply corrections for fecal flow and for completeness of stool collections only when the recovery of the marker is greater than 90%, and we disregard all balance data in patients who fail to reach the Cr_2O_3 steady state.

Indexes of colonic emptying. In this study we have derived a method for calculating the pool sizes and turnovers of Cr_2O_3 and of the unexcreted contents of the large intestine, and have shown that these estimates provide information not indicated by the usual clinical descriptions of bowel habits. It is clear from our data on pool size and turnover that in some patients colonic contents may remain unexcreted for surprisingly long periods. This type of subject may be unsuitable for balance studies for two reasons. First, quantification of excretion rates will be misleading because fecal composition does not reflect current colon contents. Second, when balance studies involve the measurement of fecal constituents that may be altered by bacterial (or other enzymatic) action within the gut lumen, it becomes critically important to recognize those patients in whom such alterations are likely to occur.

TABLE IV

Inverse Relationship between Fecal Turnover and Transformations of Fecal Neutral Steroids

All patients except patient 4 were fed a formula containing 40% of calories as commercial corn oil.

Patient No.	Pool sizes		Daily fecal turnover	Formation of neutral steroid conversion products		Degradative losses of dietary β-sitosterol
	Cr_2O_3	Feces		From cholesterol	From β-sitosterol	
	*days**	*g*	%	%	%	%
1	1.0	110	100.0	1.0	0.0	7.6
5	1.6	89	56.0	36.7	28.6	25.0
4‡	2.2	116	46.0	3.3	4.6	12.0
3	3.6	154	28.4	5.6	2.9	22.8
6	3.8	183	24.9	33.9	13.8	42.9
2	5.2	453	19.9	77.7	69.5	24.6

* Cr_2O_3 pool size expressed in terms of days of intake, rather than in milligrams as in Table II.
‡ 40% of calories as corn oil molecularly distilled to remove unesterified plant sterols.

Table IV illustrates this latter point. The data are arranged in decreasing order of daily fecal turnover in 6 patients. Two effects of bacterial action on the sterols within the intestinal lumen were measured in each patient: (*a*) the degree of bacterial conversion of cholesterol to the 5β,3α-OH- and 5β,3-keto-derivatives (coprostanol and coprostanone) and similar products derived from β-sitosterol; and (*b*) the losses of dietary β-sitosterol due to degradation of the sterol ring structure (9–11). There is a significant ($P = 0.05$) inverse correlation (18) between daily fecal turnover and formation of neutral steroid conversion products from cholesterol. Stated in another way, the longer the colonic contents remained unexcreted, the greater the chance for bacteria to modify the *trans* A/B ring structure and 3β-OH configuration of cholesterol. That the correlation is not perfect is probably a result of differences in bacterial flora in the 6 patients.

An inverse correlation is seen also between daily fecal turnover and degradative losses of large amounts of dietary β-sitosterol. Grundy, Ahrens,

and Salen [1] have recently amassed considerable evidence that the amount of β-sitosterol absorbed in man is trivial, and that losses of this sterol are due to degradation of the ring structure into products no longer recognizable as steroids; moreover, they have found that the percentage losses of cholesterol and of β-sitosterol in any one patient are exactly equal. The data in Table IV show that the degree of this degradation is correlated with the residence time of contents within the colon.

Table IV illustrates the value of knowing the fecal turnover rate when studies of sterol balance are undertaken. But it seems clear that balance studies involving any fecal constituent that can be acted upon by intestinal bacteria will be more accurate and more meaningfully interpreted when daily fecal turnover is high and pool size is small. Balance studies for fat, protein, and carbohydrates (including D-xylose) come to mind in this regard.

ACKNOWLEDGMENTS

We are indebted to Dr. Bruce Barron of this University for very helpful advice on the statistical aspects of this study. Also, we wish to express our appreciation to Mrs. Marilyn Aguirre and Miss Stephanie Knapp for valuable technical assistance.

This study was supported in part by U. S. Public Health Service grant HE-06222 from the National Heart Institute, by U. S. Public Health Service grant FR-00102 from the General Clinical Research Centers Branch of the Division of Research Facilities and Resources, and by a Commonwealth Fund Fellowship to W. J. Simmonds.

REFERENCES

1. Kreula, M. S. 1947. Absorption of carotene from carrots in man and the use of the quantitative chromic oxide indicator method in absorption experiments. *Biochem. J.* **41**: 269.
2. Fredrickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins. An integrated approach to mechanisms and disorders. *New Engl. J. Med.* **276**: 34, 94, 148, 215, 273.
3. Ahrens, E. H., Jr., V. P. Dole, and D. H. Blankenhorn. 1954. The use of orally-fed liquid formulas in metabolic studies. *Am. J. Clin. Nutr.* **2**: 336.
4. Grundy, S. M., E. H. Ahrens, Jr., and T. A. Miettinen. 1965. Quantitative isolation and gas–liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* **6**: 397.
5. Bolin, D. W., R. P. King, and E. W. Klosterman. 1952. A simplified method for the determination of chromic oxide (Cr_2O_3) when used as an index substance. *Science.* **116**: 634.
6. Roche, M., M. E. Perez-Gimenez, M. Layrisse, and E. Di Prisco. 1957. Study of urinary and fecal excretion of radioactive chromium-Cr^{51} in man. Its use in the measurement of intestinal blood loss associated with hookworm infection. *J. Clin. Invest.* **36**: 1183.
7. Lindstedt, S., and A. Norman. 1956. The turnover of bile acids in the rat. *Acta Physiol. Scand.* **38**: 121.
8. Miettinen, T. A., E. H. Ahrens, Jr., and S. M. Grundy. 1965. Quantitative isolation and gas-chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6**: 411.
9. Ahrens, E. H., Jr. 1966. Degradation of the sterol ring structure in the intestine, and the significance of this for sterol balance studies. *Gastroenterology.* **51**: 596. (Abstr.)
10. Grundy, S. M., and E. H. Ahrens, Jr. 1966. An evaluation of the relative merits of two methods for measuring the balance of sterols in man: Isotopic balance versus chromatographic analysis. *J. Clin. Invest.* **45**: 1503.
11. Ahrens, E. H., Jr. Studies of cholesterol metabolism in intact organisms. *Progr. Biochem. Pharmacol.* **4**.
12. Buchwald, H. 1964. Lowering of cholesterol absorption and blood levels by ileal exclusion. *Circulation.* **29**: 713.
13. Edin, H., G. Kihlén, and S. Nordfeldt. 1944. A summarized description of "Edin's indicator method" for the determination of the digestibility of feeds and feed mixture. *Lantbruks-Högskol. Ann.* **12**: 166.
14. Irwin, M. I., and E. W. Crampton. 1951. The use of chromic oxide as an index material in digestion trials with human subjects. *J. Nutr.* **43**: 77.
15. Stanley, M. M., and S. H. Cheng. 1956. Cholesterol exchange in the gastrointestinal tract in normal and abnormal subjects. *Gastroenterology.* **30**: 62.
16. Stanley, M. M., and S. H. Cheng. 1957. Excretion from the gut and gastrointestinal exchange studied by means of the inert indicator method. *Am. J. Digest Diseases.* **2** (N.S.): 628.
17. Whitby, L. G., and D. Lang. 1960. Experience with the chromic oxide method of fecal marking in metabolic balance investigations on humans. *J. Clin. Invest.* **39**: 854.
18. Griffin, H. D. 1958. Graphic computation of tau as a coefficient of disarray. *J. Am. Statistics Assoc.* **53**: 441.

[1] Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. Unpublished data.