FINAL REPORT

NASA CONTRACT NAS 9-12728

APOLLO GASTROINTESTINAL ANALYSIS

August 15, 1975

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I. Objective and General Description

During the Apollo 17 flight, the Command Module Pilot and the Lunar Module Pilot experienced one loose bowel movement each on the eleventh and twelfth days of flight, respectively. In each case, Lomotil was taken as a preventive measure and was effective. Nevertheless, the occurrence of this unpleasant complication led to questions concerning the etiology of this gastrointestinal problem in the flight. In the Spring of 1973 we reported that alterations in fecal bile acid (BA) pattern were observed in samples obtained during this period of space flight (In-flight study). Specifically, % dihydroxy BAs, such as deoxycholic (DOC) acid were significantly reduced during the diarrheal episode, despite the small number of analyses performed on BA. This observation is indicative of an alteration of BA metabolism associated with diarrhea of undetermined cause. In the summer of 1973, we started to evaluate a possible role of diet in the genesis of this Apollo 17 problem by feeding the identical diet to three normal volunteers under normal ground conditions. No significant changes in BA composition were observed in duodenal or fecal samples obtained during the "ground" test of the Apollo 17 diet. In addition, no abnormalities of intestinal bacterial flora occurred during the study. This clearly indicates that the diet was not the cause of the diarrhea or the altered BA metabolism. (See Technical Report, December 3, 1973).

The next phase of our investigation was a study of BA profiles of patients with acute diarrhea of known or unknown etiology. The comparison of these data with that of Apollo 17 space flight was undertaken to provide clues to the cause of diarrhea as it occurred in the Apollo mission. The following is a report on the results of the study on fecal BA pattern of subjects with acute shigellosis (SH) and travellers' diarrhea (TD) of non-specific nature. Based on the findings obtained from these two projects, we can re-examine the data from Apollo 17 pre- and in-flight periods and that from the relevant pair-fed "ground" test.

II. Shigella Diarrhea*

Volunteers used in this study were healthy adult male inmates at the Maryland House of Correction, Jessup, Md. Each subject was fed with 139 organisms of Shigella flexneri 2a (M42-43) strain contained in 45 ml of

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*Presented in American Chemical Society, 30th Southwest Regional Meeting at Houston, Texas, December 9-11, 1974.

milk. About 40% of the subjects developed dysentery within 3 days after receiving the organism. All the diarrheal stools collected had positive identification of Shigella flexneri without exception. Fecal BA and neutral steroids from five randomly selected subjects (before and during infection but before antibiotic treatment) were analyzed by gas-liquid chromatography. In comparison with controls, an increase in primary BAs by 4 - 5 fold, decrease in secondary BAs such as DOC and LC+by about two-fold each was observed (Table 1). Shigella diarrhea also induced the following changes in fecal neutral steroid profile: 1) An increase in unmodified cholesterol by 3.5 fold; 2) a decrease in bacterial metabolites such as coprostanol (CO), coprostanone (COO) and epicoprostanol by 4 - 7 fold (Table 2). These results are consistent with the hypothesis that shigella diarrhea is associated with a reduced bacterial modification of acidic and neutral sterols in the intestine, probably due to an increase in transit rate. This is further supported by our studies on the fecal BA profiles of children with acute shigellosis and the diarrhea associated with enteropathogenic E. coli and Salmonella. Whether this phenomenon extends to all types of specific diarrhea remains to be established.

III. Travellers' Diarrhea**

Five adult male volunteers attending the 5th World Congress of Gastroenterology, October 1974 in Mexico City contracted Travellers' Diarrhea of non-specific nature. Fecal samples collected during and four weeks after recovery from diarrhea were analyzed by gas-liquid chromatography. In comparison with controls, an increase in % DOC and decrease in % LC was observed. Percent CDC was also decreased in diarrhea, but the difference is not significant statistically ($p < 0.10$). Total bile acid concentration in µg/g % C and unidentified BA were not changed (Table 3). This pattern is strikingly different from shigella diarrhea in that bacterial activity on fecal sterols was not reduced in the diarrheal state. Instead, the shift in BA profile in Travellers' Diarrhea could be an indication of enhanced bacterial 7α-dehydroxylation. To confirm this, analysis of fecal neutral sterols (to check the degree of conversion of cholesterol into coprostanol) and/or direct incubation of feces with radioactive cholic acid-24-^{14}C (to check the conversion of cholic into DOC) should be performed. The observation that DOC was significantly increased in the case of TD raises the question concerning the role of BA in the genesis of TD of unknown cause. DOC is known to be the most potent of all BAs in the inhibition of water absorption in the human colon. It can also induce water secretion at higher concentrations. However, DOC is known to adsorb to dietary fiber during its transit through the intestinal tract[1]. The current concept is

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**To be presented at American Chemical Society 27th Southwest-31st Southwest Combined Regional Meeting at Memphis, Tennessee, October 29-31, 1975.

1.A. Norman, Brit. J. Nutr. 18:173 (1964)

+ Lithocholic

that only those BAs not adsorbed to solid materials are biologically active. In other words, DOC exerts its effects as a potent laxative only when it is in solution. To support this hypothesis, Hofmann[2] observed that BA contents in the supernatant fractions of stool specimens of patients with ileal resection (BA malabsorption) were considerably augmented relative to pellet fractions (obtained by ultracentrifugation at 100,000 x g) as compared to those of healthy normal controls. In order to check the physical state of BA in the case of TD, we adopted Hofmann's method for the fractionation of feces. The results are shown in Table 4. It is quite obvious that the relative BA contents of pellet to supernatant fractions of the TD subjects were comparable to those of non-diarrheal controls. In both groups, the BA contents in solution (supernatant) constitute only 7 - 14% of total BA in feces. This is in contrast to Hofmann's observation in ileal resection. On the other hand, % DOC in solution increases significantly in TD patients (49.0 ± 3.9 vs 28.8 ± 3.3 in controls, $p < 0.02$). Apparently, further study on the role of BA in TD is warranted. It should be mentioned that the sterol profiles of the control samples from the TD project were completely comparable to those of Shigella controls (see previous section). It is evident then that the differences in BA patterns between the diarrheal stools from these two independent studies can be attributed only to the diseases themselves.

IV. Analysis of Fecal Samples from Apollo 17 Mission*

Fecal samples from the following sources were analyzed by the combined thin-layer/gas-liquid chromatography: 1) paired-fed ground study; 2) Apollo 17 pre-flight period; 3) Apollo 17 in-flight period.

A. Paired-fed Ground Study

A possible role of dietary changes in the pathogenesis of diarrhea during the Apollo 17 spaceflight was ruled out by feeding the identical diet to three normal male volunteers under normal ground conditions. No significant changes in BA compositions were observed in fecal or duodenal samples obtained during the "ground" test of the Apollo 17 diet (Table 5). Furthermore, no abnormalities of intestinal micro-organisms occurred during the ground study (see Technical Report, Dec. 3, 1973). We also analyzed the neutral sterol (NS) fraction of the samples from this project by the chromatographic technique modified from that of Grundy et al[3] in order to confirm our findings on BA. No significant differences in neutral sterol composition were observed for all stool specimens from "early" and "late" periods of Apollo diet (Table 6). Thus, analysis of both BA and neutral

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2. A. F. Hofmann and J. R. Poley, Gastroenterology 62:918 (1972).
3. T. A. Miettinen, E. H. Ahrens and S. M. Grundy. J. Lipid Res. 6:411 (1965)

* A part of the data from this study was presented at the Western Hemisphere Nutrition Congress IV, Miami Beach, Fla., August 19-22, 1974.

sterol strongly indicated that no alteration on BA or NS metabolism was associated with dietary changes in the Apollo project. It is noteworthy to indicate that fecal sterol compositions of the paired-fed subjects were very similar to those of control subjects on regular mixed diets from both shigellosis and TD studies despite apparent differences in the fibre contents in their diets. These observations are interesting because dietary fibre has been theorized to play a role in the transit rate of the bowel contents in the binding of BAs and the alteration of colonic microflora.[4] However, the total BA and NS concentrations in mg/g of feces (wet weight) of the paired-fed subjects were indeed considerably lower (by 6 - 10 fold) than those on diets of higher fibre content (see Tables 11 - 12).

B. Fecal Samples from Apollo 17 Pre-flight Period

Fecal BA analyses on samples from three astronauts in the pre-flight period show some significant changes as compared with those of "ground" studies on the identical diet. A reduction in LC (27.2 $\pm$ 7.1 vs 41.7 + 8.4 in ground controls, $p < 0.005$) and in increase in unidentified BA (7.8 $\pm$ 4.9 vs 2.9 $\pm$ 1.6 in ground controls, $p < 0.02$) were observed, although total BA concentrations in mg/g of dry weight were similar for both groups (see Table 7). Mean value of % DOC was also lower in pre-flight (32.7 $\pm$ 4.9) than that of ground study (39.7 $\pm$ 4.9) but the difference is not significant ($p < 0.10$). Although it is tempting to postulate a reduction in bacteria activity in pre-flight period due to environmental stress and/or age differences (subjects used in ground study are younger adults), further studies are necessary to clarify this.

C. Fecal Samples from Apollo 17 Flight

Sterol profiles obtained for fecal samples with wet weight < 150 g/bowel movement (BM) were comparable with those of wet weight > 200 g/BM except that % DOC was reduced in samples of higher fecal mass ($p < 0.05$) Table 8). In fact, there was a negative correlation between % DOC of total BA and fecal wet weight in g/BM ($r = 0.65$, $p < 0.02$). The samples with wet weight of < 150 g/BM have significantly higher DOC than that of pre-flight period (46.2 $\pm$ 6.9 vs 32.7 $\pm$ 7.9, $p < 0.01$). Total BA concentration in mg/g of dry weight also increased by about 3 fold in the flight ($p < 0.01$). This is probably due to a significant reduction in both the frequency of bowel movement (from 1.1 $\pm$ 0.2 day^{-1} pre-flight to 0.4 $\pm$ 0.1 day^{-1} in flight, $p < 0.01$) and the mean fecal wet weight per day for each astronaut during the flight (Table 10). On the other hand, fecal BA of in-flight samples with > 200 g/BM were entirely comparable with those of pre-

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4. "The Role of Fibre in the Diet" in Daily Council Digest, Vol. 46, No. 1 Jan-Feb 1975. Published by National Dairy Council, Chicago, Ill.

flight period (Table 11). Tables 11 - 12 give a general survey of the data on BA and NS from the projects studied by us with regard to the gastrointestinal problems encountered during Apollo 17 flight. In conclusion, fecal sterol analysis of Apollo 17 mission gives no indication of an infectious diarrhea of specific (such as shigellosis) or non-specific (such as travellers' diarrhea) etiology occurring in the entire flight period. A possible role of dietary changes in the pathogenesis of diarrhea was ruled out. At this point, it seems reasonable to assume that gastrointestinal problems encountered in Apollo flight are the consequences of altered physiology, perhaps secondary to physical or emotional stress of flight.

V. Other Relevant Studies to be Done in This Project

1. Drug-induced diarrhea or constipation - to study fecal BA and neutral sterols profile as a function of intestinal motility and transit rate by the administration of castor oil, magnesium sulfate or Lomotil.

2. Effect of virus diarrhea on fecal sterols.

3. Completion of neutral sterol analysis on travellers' diarrhea and Apollo pre-flight and in-flight samples in order to confirm degree of bacterial modification on BAs and the large increase in NS excretion in flight (Table 9). This enhanced NS excretion in flight, if confirmed, may provide an answer to the 15% decrease in serum cholesterol as observed during the flight.

VI. List of Publications

A. Papers

New Solvent Systems for the Separation of Free and Conjugated Bile Acids. T. L. Huang and B. L. Nichols. J. of Chromatography, 101: 235-9, 1974.

The Role of Bile Acids in the Genesis of Skin Irritation about Enterostomies. J. T. Rodriguez, T. L. Huang, G. D. Ferry, W. J. Klish, F. J. Harberg and B. L. Nichols. J. Pediatrics. In Press.

New Solvent Systems for the Separation of Free and Conjugated Bile Acids II. Separation of Free Bile Acids as a Group. C. T. L. Huang and B. L. Nichols. J. Chromatography, 109:427-31, 1975.

Effects of Shigella Diarrhea on Steroid Metabolism. C. T. L. Huang, W. E. Woodward, R. B. Hornick and B. L. Nichols. Submitted for publication.

B. Abstracts

Fecal Bile Acids as an Indicator of Bacterial Colonization of Small Bowel in Chronic Diarrhea. J. T. Rodriguez, T. L. Huang, J. Alvarado, J. V. Ordonez and B. L. Nichols. Pediatric Research, 7:340, 1973.

Role of Free Bile Acids in Acquired Monosaccharide Intolerance. J. T. Rodriguez, T. L. Huang, J. Alvarado, W. J. Klish, W. E. Darby, N. Flores and B. L. Nichols. Pediatric Research, 8:385, 1974. Presented in Plenary Session of the Society for Pediatric Research, April, 1974.

Alteration of Intestinal Flora and Function in Acute Non-Specific Diarrhea of Infancy. J. T. Rodriguez, A. Mastromarino, W. E. Darby, N. Flores, J. V. Ordonez, T. L. Huang, J. Alvarado, R. Wilson, H. Soriano and B. L. Nichols. Amer. J. Clin. Nutrition, 27:436, 1974.

Patron de Acidos Biliares Intestinales en Ninos Guatemaltecos Sanos y con Diarrhea Cronica. J. T. Rodriguez, T. L. Huang, J. Alvarado, W. J. Klish and B. L. Nichols. Presented at the XIV World Congress of Pediatrics, Buenos Aires, Argentina, October 1974.

Tratamiento Medico de la Dermatitis Producida en Pacientes con Enterostomia. J. T. Rodriguez, T. L. Huang, G. D. Ferry and B. L. Nichols. Presented at the XIV World Congress of Pediatrics, Buenos Aires, Argentina, October 1974.

Patron de Acidos Biliares Intestinales en Ninos Guatemaltecos Sanos y con Sindrome Diarreico Agudo. T. L. Huang, J. T. Rodriguez, J. Alvarado, W. J. Klish, H. Soriano and B. L. Nichols. Presented at the XIV World Congress of Pediatrics, Buenos Aires, Argentina, October 1974.

Ileal Bypass for Hypercholesterolemia in Childhood Intrahepatic Cholestasis. G. D. Ferry, J. T. Rodriguez, T. L. Huang and B. L. Nichols. Gastroenterology, 66:692, 1974.

Morphologic Basis for Glucose Malabsorption in Infants with Acquired Monosaccharide Intolerance. W. J. Klish, J. T. Rodriguez, H. Soriano, T. L. Huang, G. D. Ferry and B. L. Nichols. Pediatric Research, 8:382, 1974.

Duodenal Microflora of Guatemalan Children with Acute Diarrhea. A. Mastromarino, J. T. Rodriguez, T. L. Huang, R. Wilson, B. L. Nichols and J. V. Ordonez. Presented at the Annual Session of Microbiology Annual Meeting, Chicago, Illinois, May 1974.

Duodenal Microecology of Guatemalan Children with Acute Diarrhea. A. Mastromarino, J. T. Rodriguez, T. L. Huang, R. Wilson, B. L. Nichols and J. V. Ordonez. Presented at the Annual Session of the Texas Medical Association, Houston, Texas 1974.

Altered Bile Acid Metabolism During Apollo 17 Flight. T. L. Huang, J. T. Rodriguez, J. V. Ordonez, A. Mastromarino, R. Wilson, J. Alvarado and B. L. Nichols. Presented at Western Hemisphere Nutrition Congress IV, Miami, Florida, August 1974.

Difference in Bile Acid Metabolism Between Adults and Children. C. T. L. Huang, J. T. Rodriguez, W. J. Klish and B. L. Nichols. Presented at 30th Annual Southwest Regional Meeting, American Chemical Society, Houston, Texas, December 1974.

Chemistry and Physiology of 3β-hydroxy-5-cholenoic acid. C. T. L. Huang, J. T. Rodriguez, W. J. Klish and B. L. Nichols. Presented at 30th Annual Southwest Regional Meeting, American Chemical Society, Houston, Texas, December 1974.

Effects of Shigella Diarrhea on Fecal Bile Acids and Neutral Steroids. C. T. L. Huang, W. E. Woodward, R. B. Hornick, H. DuPont and B. L. Nichols. Presented at the 30th Annual Southwest Regional Meeting, American Chemical Society, Houston, Texas, December 1974.

Fecal Bile Acid and Neutral Sterols in a Gnotobiotic Child. G. S. G. Krishna, C. T. L. Huang, J. T. Rodriguez, W. J. Klish, B. L. Nichols and R. Wilson. Pediatric Research, 9:306 (1975).

Effect of Travellers' Diarrhea on Fecal Bile Acids. C. T. L. Huang, J. Udall, M. Merson and B. L. Nichols. To be presented at the American Chemical Society 27th Southeast-31st Southwest Combined Regional Meeting, Memphis, Tennessee, October 1975.

Production of Coprostanol as an Index of Bacterial 7 α-Dehydroxlase Activity. C. T. L. Huang and B. L. Nichols. To be presented at the American Chemical Society 27th Southeast-31st Southwest Combined Regional Meeting, Memphis, Tennessee, October 1975.

C. Manuscripts in Preparation

Fecal Steroids in Diarrhea. II. Travellers' Diarrhea. C. T. L. Huang, J. Udall, M. Merson and B. L. Nichols.

Fecal Steroids in Diarrhea. III. Comparison of Compositions of Fecal Bile Acid and Neutral Sterol Between Children and Adults. C. T. L. Huang, J. T. Rodriguez and B. L. Nichols.

TABLE 1

BILE ACID COMPOSITION OF FECAL SAMPLES FROM SHIGELLA-CHALLENGED SUBJECTS[a]

	Non-Diarrhea						Diarrhea						
% Bile Acid (BA)	J.A.	C.B.	R.H.	W.M.	A.N.	Mean ± SD	J.A.	C.B.	R.H.	W.M.	A.N.	Mean ± SD	p[f]
Lithocholic	45.2	45.2	30.6	43.4	37.9	40.5 ± 6.3	16.5	27.5	24.7	39.7	31.4	28.0 ± 8.5	< 0.05
Isodeoxycholic	12.0	5.0	12.6	15.5	11.7	11.4 ± 3.9	23.5	4.5	16.6	7.9	16.4	13.8 ± 7.6	NS
Deoxycholic	29.0	35.3	48.8	25.0	32.2	34.1 ± 9.1	14.3	15.5	15.6	19.9	19.7	17.0 ± 2.6	< 0.005
Chenodeoxycholic	3.8	3.0	0.7	4.9	3.5	3.2 ± 1.6	3.5	20.9	9.5	3.8	4.8	8.5 ± 7.3	NS
Ursodeoxycholic	3.7	2.9	0.6	1.3	0.9	1.9 ± 1.3	2.9	4.3	0.8	10.0	1.4	3.9 ± 3.7	NS
Cholic	0.9	2.4	2.2	2.9	1.1	1.9 ± 0.9	12.9	18.8	7.4	7.3	14.2	12.1 ± 4.9	< 0.005
Keto Acid[b]	3.4	1.8	3.9	5.0	10.7	5.0 ± 3.4	9.4	3.4	12.8	5.5	1.7	6.6 ± 4.5	NS
Unidentified	2.0	4.4	0.6	2.0	2.0	2.2 ± 1.4	17.0	5.1	12.6	5.9	10.4	10.2 ± 4.9	< 0.01
Primary BA[c]	4.7	5.4	2.9	7.8	4.6	5.1 ± 1.8	16.4	39.7	16.9	11.1	19.0	20.6 ± 11.1	< 0.02
DiOH BA[d]	48.5	46.2	62.7	46.7	48.3	50.5 ± 6.9	44.2	45.2	42.5	41.6	42.3	43.2 ± 1.5	< 0.05
Total BA (mg/g)[e]	7.6	22.9	29.9	9.6	5.8	15.2 ± 10.6	1.5	3.4	3.1	0.6	1.9	2.1 ± 1.2	< 0.05

a. All values are expressed as % of total Bile acid unless otherwise indicated. NS = not significant.

b. Keto acid includes 7-ketolithocholic, 12-ketolithocholic, $\Delta^{9(11)}$12-ketolithocholic, 7-ketodeoxycholic, and 3,12-diketocholanoic acids.

c. Primary BA includes cholic and chenodeoxycholic acids.

d. DiOH BA includes chenodeoxycholic, deoxycholic, isodeoxycholic and ursodeoxycholic acids.

e. Concentrations are in mg/g of feces (wet weight).

f. Determined by Student t-test.

TABLE 2 NEUTRAL STEROID COMPOSITION OF FECAL SAMPLES FROM SHIGELLA-CHALLENGED SUBJECTS[a]

	Non-Diarrhea						Diarrhea					
Subject	C.B.	A.N.	W.M.	J.A.	R.H.	Mean ± SD	C.B.	A.N.	W.M.	J.A.	Mean ± SD	P
Animal Steroid (AS)												
Coprostanol	48.1	71.0	64.3	72.3	73.1	65.8 ± 10.5	1.8	36.2	1.2	9.7	12.2 ± 16.4	< 0.001
Epicholestanol	0.3	0	2.5	1.4	0.3	0.9 ± 1.0	0	0	0	0	0.0 ± 0.0	NS
Epicoprostanol	2.7	3.3	3.7	2.2	2.3	2.8 ± 0.6	0	1.5	0	0	0.4 ± 0.7	< 0.005
Cholesterol	43.5	20.5	24.9	17.6	18.4	25.0 ± 10.7	96.2	60.9	98.5	89.3	86.2 ± 17.3	< 0.001
Cholestanol	0	0	0	1.6	0	0.3 ± 0.7	0	0	0	0	0.0 ± 0.0	NS
Cholestanone	0.1	0	0	0.1	0.1	0.1 ± 0.1	0.1	0	0.1	0	0.1 ± 0.1	< NS
Coprostanone	4.7	2.4	2.4	2.2	4.3	3.2 ± 1.2	1.6	0.7	0.2	0.9	0.9 ± 0.6	< 0.01
Unidentified	0.6	2.8	2.2	2.6	1.5	1.9 ± 0.9	0.3	0.7	0	0.1	0.3 ± 0.3	< 0.01
Plant Steroid (PS)												
Demosterol	1.3	5.9	3.2	0.6	0.1	2.2 ± 2.4	1.5	15.0	9.0	6.9	8.1 ± 5.6	NS
Campesterol	11.8	18.0	12.1	25.5	8.9	15.3 ± 6.6	22.8	12.8	30.2	21.7	21.9 ± 7.1	NS
Stigmasterol	2.5	1.9	0	3.9	0	1.7 ± 1.7	2.2	3.3	5.3	0	2.7 ± 2.2	NS
Ergosterol	0	1.1	0	0	0	0.2 ± 0.5	0	0	0	12.0	3.0 ± 6.0	NS
β-Sitosterol	65.3	31.9	29.9	22.3	41.5	38.2 ± 16.6	61.3	57.4	47.6	54.4	55.2 ± 5.8	NS
Fucosterol	2.8	0.6	1.8	2.7	0.8	1.7 ± 1.0	5.5	5.1	2.6	2.0	3.8 ± 1.8	NS
Unidentified	16.3	40.6	53.0	45.0	48.7	40.7 ± 14.4	6.7	6.4	5.3	3.0	5.4 ± 1.7	< 0.005
Total AS (mg/g)[b]	5.32	6.42	10.39	13.46	7.11	8.54 ± 3.34	1.18	0.67	0.64	1.89	1.10 ± 0.59	< 0.005
Total PS (mg/g)[b]	1.41	0.50	0.71	0.64	0.53	0.76 ± 0.37	0.17	0.05	0.02	0.17	0.10 ± 0.08	< 0.02

a. All values are in % of total animal steroid (AS) or plant steroid (PS) unless otherwise indicated. NS = not significant.
b. Concentrations are in mg/g of feces (wet weight).

TABLE 3

BILE ACID COMPOSITION OF FECAL SAMPLES FROM PATIENTS WITH TRAVELLERS' DIARRHEA IN MEXICO CITY

Bile Acid (BA)	Traveller's Diarrhea (n = 5)							Controls (n = 9)		
	M.M.	H.S.	D.W.	L.S.	G.M.	Mean ±	SEM	Mean ±	SEM	p[a]
C	29.1	33.9	28.0	16.4	21.8	25.8 ±	3.0	38.9 ±	2.0	< 0.005
ISODOC	[illegible]	11.8	12.9	8.4	5.2	9.9 ±	1.4	10.0 ±	1.2	NS
DC	33.9	47.6	53.9	53.0	45.6	46.8 ±	3.6	34.3 ±	2.2	< 0.01
DC	2.9	1.5	0.7	0.8	2.4	1.7 ±	0.4	3.4 ±	0.6	< 0.10
RSODOC	1.6	0.4	0.5	6.3	5.9	2.9 ±	1.3	2.1 ±	0.5	NS
	1.6	1.3	1.0	9.1	4.8	3.6 ±	1.5	2.4 ±	0.5	NS
ETO A	15.3	3.0	1.9	5.0	5.1	6.1 ±	2.4	6.0 ±	1.0	NS
NIDENTIFIED	4.4	0.5	1.1	1.0	9.2	3.2 ±	1.6	3.0 ±	0.6	NS
RIMARY BA	4.5	2.8	1.7	9.9	7.2	5.2 ±	1.5	5.8 ±	0.8	NS
IOH BA	49.6	61.3	68.0	68.5	59.1	61.3 ±	3.5	49.7 ±	1.8	< 0.01
OTAL BA[b] (mg/ g)	0.294	1.378	0.795	0.804	0.256	0.705 ± 0.205		8.743 ± 0.354		NS

. Significant differences as determined by Student's t-test.

. Concentrations of BA in mg per g of homogenized feces (wet weight).

Abbreviations used: LC = Lithocholic; ISODOC = Isodeoxycholic; DOC = Deoxycholic; CDC = Chenodeoxycholic; URSODOC = Ursodeoxycholic; C = Cholic; KETO A = KETO Acid (see footnotes under Table I); Primary BA and DIOH BA (see footnotes under Table I).

TABLE 4

COMPOSITION OF BILE ACIDS IN THE PELLET AND SUPERNATANT FRACTIONS OF TRAVELLERS' DIARRHEA STOOLS OBTAINED BY ULTRACENTRIFUGATION METHOD[a,b]

% Bile Acid	Traveller's Diarrhea			Controls		
	Pellet (n=5)	Supernatant (n=5)	P	Pellet (n=3)	Supernatant (n=3)	P
LC	28.9 ± 2.8*	4.2 ± 0.6*	< 0.001	40.5 ± 3.5	9.7 ± 2.4	< 0.005
IsoDOC	9.3 ± 1.6	14.2 ± 2.1	< 0.10	8.3 ± 1.9	6.8 ± 2.1	NS
DOC	47.0 ± 4.2	49.0 ± 3.9†	NS	34.6 ± 2.4	28.8 ± 3.3	NS
CDC	1.7 ± 0.5	1.3 ± 0.4	NS	2.9 ± 0.9	2.0 ± 0.9	NS
UrsoDOC	2.3 ± 1.0	5.6 ± 2.4	NS	2.7 ± 1.0	1.9 ± 1.3	NS
C	2.1 ± 0.8	10.5 ± 3.9	< 0.10	3.2 ± 1.3	3.1 ± 1.1	NS
Keto A	5.0 ± 2.4	12.2 ± 2.5	< 0.10	6.6 ± 1.1	16.6 ± 8.7	NS
Unidentified	3.6 ± 2.1	2.9 ± 1.3*	NS	1.2 ± 0.3	31.2 ± 13.6	< 0.10
Primary BA	3.9 ± 0.9	11.8 ± 3.8	< 0.10	6.1 ± 1.9	5.1 ± 1.8	NS
DiOH BA	60.3 ± 4.1	70.2 ± 4.4δ	NS	48.5 ± 2.4	39.4 ± 2.6	< 0.10
Total BA %	86.4 ± 3.2	13.6 ± 3.2	< 0.001	92.9 ± 2.8	7.1 ± 2.8	< 0.001

a. Centrifugation of homogenized feces at 100,000 x g at 4°C for one hour. All values are expressed as (Mean ± SEM) % of total BA.

b. Significant differences as compared to the corresponding fractions of the controls are denoted by: *p < 0.05; †p < 0.02; δp < 0.005.

For abbreviations used in this table see footnotes under Tables 1 and 3.

TABLE 5

BILE ACID COMPOSITION OF FECAL SAMPLES FROM PAIRED-FED VOLUNTEERS[a]

% Bile Acid (BA)	Early (n = 4)	Late (n = 4)	P
LC	38.1 ± 3.2	45.3 ± 4.7	NS
$\Delta^5,3\beta$	0.0 ± 0.0	0.1 ± 0.1	NS
IsoDOC	7.2 ± 1.7	4.2 ± 2.1	NS
DOC	39.1 ± 2.0	40.2 ± 3.1	NS
CDC	2.4 ± 0.8	2.7 ± 0.2	NS
UrsoDOC	2.8 ± 0.6	1.4 ± 0.4	NS
C	1.5 ± 0.8	0.7 ± 0.1	NS
Keto A	5.0 ± 0.7	3.8 ± 0.9	NS
Unidentified	4.0 ± 0.6	1.7 ± 0.4	<0.025
Primary BA	3.9 ± 1.0	3.4 ± 0.2	NS
DiOH BA	51.5 ± 3.9	48.5 ± 4.5	NS
Total BA			
(mg/g)[b]	1.745 ± 0.778	1.518 ± 0.451	NS
(mg/g)[c]	0.349 ± 0.156	0.304 ± 0.090	NS
24 hr excretion			
(mg/kg/day)	0.89 ± 0.35	0.72 ± 0.16	NS
(mg/day)	61.5 ±21.0	51.0 ±11.5	NS

a. Early = Day #4-5. Late = Day #8-10. All values are expressed as (mean ± SEM) % of total BA unless otherwise indicated. For abbreviations and explanatory notes, see footnotes under Tables 1 and 3.
b. Calculated on the basis of dry weight of feces.
c. Calculated on the basis of wet weight of feces, assuming 80% H_2O content for all stool specimens.

TABLE 6

NEUTRAL STEROL COMPOSITION OF FECAL SAMPLES FROM PAIRED-FED VOLUNTEERS[a]

Neutral Sterol	Early (n = 3)	Late (n = 5)	P
Animal Sterols (AS)			
Coprostanol	63.5 ± 1.2	62.9 ± 2.3	NS
Epicholestonol	0.0 ± 0.0	0.0 ± 0.0	NS
Epicoprostanol	3.1 ± 0.8	1.9 ± 0.5	NS
Cholesterol	20.7 ± 0.8	28.2 ± 1.4	NS
Cholestanol	1.5 ± 0.2	1.1 ± 0.4	NS
Unidentified	2.7 ± 0.8	4.1 ± 1.7	NS
Coprostanone	2.5 ± 0.3	1.7 ± 0.6	NS
Cholestanone	0.0 ± 0.01	0.04 ± 0.04	NS
Plant Sterols (PS)			
Desmosterol	3.4 ± 0.8	2.0 ± 0.8	NS
Campesterol	16.7 ± 1.8	12.4 ± 3.4	NS
Stigmasterol	4.8 ± 1.1	3.3 ± 1.3	NS
Ergosterol	4.0 ± 0.4	2.7 ± 1.3	NS
β-Sitosterol	37.4 ± 4.2	44.4 ± 1.3	<0.10
Fucosterol	5.8 ± 1.4	7.7 ± 1.2	NS
Unidentified	27.9 ± 5.3	27.6 ± 5.0	NS
Total AS (mg/g)[b]	6.582 ± 0.946	7.386 ± 1.307	NS
Total PS (mg/g)[b]	0.442 ± 0.065	0.537 ± 0.054	NS
Total AS (mg/g)[c]	1.316 ± 0.189	1.477 ± 0.261	NS
Total PS (mg/g)[c]	0.088 ± 0.013	0.107 ± 0.011	NS
24 Hr AS excretion (mg/kg/day)	3.48 ± 0.50	6.67 ± 2.22	NS
(mg/day)	241.9 ± 12.3	446.2 ± 142.2	NS
24 hr PS excretion (mg/kg/day)	0.233 ± 0.033	0.466 ± 0.120	NS
(mg/day)	16.2 ± 0.8	32.1 ± 8.2	NS

a. Early = Day #4. Late = Day #8-11. All values are expressed as (mean ± SEM) % of total animal or plant sterol.
b. Calculated on the basis of dry weight of feces.
c. Calculated on the basis of wet weight of feces, assuming 80% H_2O content for all stool specimens.

TABLE 7

BILE ACID COMPOSITION OF FECAL SAMPLES FROM APOLLO 17 PRE-FLIGHT PERIOD AS COMPARED WITH THOSE OF PERIOD-FED GROUND STUDIES[a]

% Bile Acid (BA)	Pre-Flight (n=8)	Ground Early & Late (n=8)	P
Lithocholic	27.2 ± 2.5	41.7 ± 3.0	<0.005
$\Delta^5,3\beta$	1.5 ± 1.5	0.0 ± 0.0	NS
Isodeoxycholic	18.3 ± 2.3	5.7 ± 1.4	<0.001
Deoxycholic	32.7 ± 2.8	39.7 ± 1.7	<0.10
Chenodeoxycholic	3.0 ± 0.6	2.6 ± 0.4	NS
Ursodeoxycholic	1.7 ± 0.4	2.1 ± 0.4	NS
Cholic	2.2 ± 0.7	1.1 ± 0.4	NS
Keto Acid	5.7 ± 1.2	4.4 ± 0.6	NS
Unidentified	7.8 ± 1.7	2.9 ± 0.6	<0.02
Primary BA	5.2 ± 1.0	3.7 ± 0.5	NS
DiOH BA	55.7 ± 3.9	50.0 ± 2.8	NS
Total BA (mg/g)[b]	1.542 ± 0.240	1.631 ± 0.419	NS
(mg/g)[c]	0.429 ± 0.089	0.326 ± 0.084	NS
24 hr excretion (mg/kg/day)	0.63 ± 0.14	0.81 ± 0.18	NS
(mg/day)	49.2 ± 10.8	56.2 ± 11.3	NS

a. All values are expressed as (mean ± SEM) % of total BA unless otherwise indicated.
b. Calculated on the basis of dry weight of feces.
c. Calculated on the basis of wet weight of feces.
d. $\Delta^5,3\beta$ = 3β-hydroxy-5-cholenoic acid.

DiOH includes chenodeoxycholic, deoxycholic, isodeoxycholic and ursodeoxycholic acids.

TABLE 8

BILE ACID COMPOSITION OF FECAL SAMPLES FROM APOLLO 17 IN-FLIGHT PERIOD[a]

% Bile Acid (BA)	Non-Diarrhea <150 g/BM (n=5)	Diarrhea > 200 g/BM (n=2)	P
Lithocholic	26.1 ± 1.8	24.9 ± 1.0	NS
$\Delta^5,3\beta$	0.0 ± 0.0	3.8 ± 3.8	NS
Isodeoxycholic	10.6 ± 1.3	12.6 ± 7.2	NS
Deoxycholic	46.2 ± 3.1	31.6 ± 4.0	<0.05
Chenodeoxycholic	2.0 ± 0.3	2.4 ± 2.1	NS
Ursodeoxycholic	1.7 ± 1.1	0.1 ± 0.1	NS
Cholic	1.4 ± 0.7	2.7 ± 0.6	NS
Keto Acid	5.2 ± 1.2	7.9 ± 2.6	NS
Unidentified	7.0 ± 1.3	14.1 ± 12.4	NS
Primary BA	3.3 ± 0.9	5.1 ± 1.5	NS
DiOH BA	60.4 ± 1.7	46.7 ± 13.3	NS
Total BA (mg/g)[b]	4.719 ± 1.121	2.571 ± 0.400	NS
(mg/g)[c]	1.076 ± 0.274	0.454 ± 0.020	NS
Total BA excretion (mg/kg/BM)	1.079 ± 0.268	1.437 ± 0.042	NS
(mg/BM)	85.0 ± 21.9	108.1 ± 2.5	NS

a. BM = Bowel movement. All values are expressed as (mean ± SEM) % of total BA unless otherwise indicated.
b. Calculated on the basis of dry weight of feces.
c. Calculated on the basis of wet weight of feces.
d. DiOH includes chenodeoxycholic, deoxycholic, isodeoxycholic and ursodeoxycholic acids. $\Delta^5,3\beta$ = 3β-hydroxy-5-cholenoic acid.

TABLE 9

NEUTRAL STEROL COMPOSITION OF FECAL SAMPLES FROM APOLLO 17 IN-FLIGHT PERIOD[a]

Neutral Sterols	Non-Diarrhea < 150 g/BM (n=2)			Diarrhea > 200 g/BM (n=2)			P
Animal Sterols (AS)							
Coprostanol	77.2	±	2.5	75.1	±	4.4	NS
Epicholestanol		0			0		NS
Epicoprostanol	2.2	±	0.4	2.2	±	0.1	NS
Cholesterol	18.1	±	2.0	17.6	±	2.9	NS
Cholestanol		0			0		NS
Unidentified	1.6	±	0.3	1.7	±	0.3	NS
Coprostanone	1.1	±	0.0	3.3	±	1.1	NS
Cholestanone	0.0	±	0.0	0.2	±	0.1	< 0.10
Plant Sterols (PS)							
Desmosterol	5.2	±	4.0	5.4	±	1.6	NS
Campesterol	10.9	±	3.3	4.7	±	3.0	NS
Stigmasterol	1.9	±	0.5	2.4	±	2.0	NS
Ergosterol	1.2	±	1.2	0.0	±	0.0	NS
β-Sitosterol	34.2	±	3.5	39.0	±	2.6	NS
Fucosterol	7.1	±	1.9	5.8	±	3.1	NS
Unidentified	39.6	±	5.4	42.9	±	9.1	NS
Total AS (mg/g)[b]	34.18	±	0.17	24.67	±	8.57	NS
Total PS (mg/g)[b]	1.45	±	0.33	1.35	±	0.03	NS
Total AS (mg/g)[c]	8.067	±	0.110	4.798	±	2.447	NS
Total PS (mg/g)[c]	0.341	±	0.052	0.247	±	0.052	NS
Total AS excretion							
(mg/kg/BM)	10.5	±	3.5	14.8	±	6.8	NS
(mg/BM)	826.9	±	299.8	1,107.1	±	508.0	NS
Total PS excretion							
(mg/kg/BM)	0.421	±	0.067	0.777	±	0.115	NS
(mg/BM)	33.0	±	7.0	58.1	±	8.5	NS

a. BM = bowel movement. All values are expressed as (mean ± SEM)% of total animal or plant sterol.
b. Calculated on the basis of dry weight of feces.
c. Calculated on the basis of wet weight of feces.

TABLE 10

SURVEY OF BOWEL MOVEMENTS FOR THREE ASTRONAUTS DURING APOLLO 17 MISSION[a]

	Pre-Flight		In-Flight	
	# Stool/Day	Fecal Mass (g/day)	# Stool/Day	Fecal Mass (g/day)
Evans	1.00	200.0	0.46	92.3
Cernan	1.00	87.6	0.46	44.8
Schmitt	1.43	77.0	0.31	51.1

a. Average values for 7 days and 13 days for pre-flight and in-flight periods, respectively.

TABLE 11

FECAL BILE ACID AND NEUTRAL STEROL PROFILES FOR ADULT MALE SUBJECTS[a]

	Shigella Diarrhea (n=5)	Travellers' Diarrhea (n=5)	Regular Mixed Diet Controls (n=9)	Paired-Fed Ground Controls (n=8)	Apollo 17 Pre-Flight (n=8)	Apollo 17 In-Flight <150g/BM (n=5)	Apollo 17 In-Flight >200g/BM (n=2)
% Bile Acid (BA)							
Lithocholic	28.0 ± 3.8	25.8 ± 3.0	38.9 ± 2.0	41.7 ± 3.0	27.2 ± 2.5	26.1 ± 1.8	24.3 ± 1.0
Isodeoxycholic	13.8 ± 3.4	9.9 ± 1.4	10.0 ± 1.2	5.7 ± 1.4	18.3 ± 2.3	10.6 ± 1.3	12.6 ± 7.2
Deoxycholic	17.0 ± 1.2	46.8 ± 3.6	34.3 ± 2.2	39.7 ± 1.7	32.7 ± 2.8	46.2 ± 3.1	31.6 ± 4.0
Chenodeoxycholic	8.5 ± 3.3	1.7 ± 0.4	3.4 ± 0.6	2.6 ± 0.4	3.0 ± 0.6	2.0 ± 0.3	2.4 ± 2.1
Ursodeoxycholic	3.9 ± 1.6	2.9 ± 1.3	2.1 ± 0.5	2.1 ± 0.4	1.7 ± 0.4	1.7 ± 1.1	0.1 ± 0.1
Cholic	12.1 ± 2.2	3.6 ± 1.5	2.4 ± 0.5	1.1 ± 0.4	2.2 ± 0.7	1.4 ± 0.7	2.7 ± 0.6
Keto Acid	6.6 ± 2.0	6.1 ± 2.4	6.0 ± 1.0	4.4 ± 0.6	5.7 ± 1.2	5.2 ± 1.2	7.9 ± 2.6
Unidentified	8.1 ± 1.4	3.2 ± 1.6	3.0 ± 0.6	2.9 ± 0.6	7.8 ± 1.7	7.0 ± 1.3	14.1 ± 12.4
Δ^5,3β	2.1 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 1.5	0.0 ± 0.0	3.8 ± 3.8
Total BA (mg/g)	1.063 ± 0.605	0.705 ± 0.205	8.743 ± 3.561	(1.631 ± 0.419)[b] 0.326 ± 0.084	(1.542 ± 0.240)[b] 0.429 ± 0.089	(4.719 ± 1.721)[b] 1.076 ± 0.274	(2.571 ± 0.400)[b] 0.454 ± 0.020
% Neutral Sterol (NS)			(n=5)	(n=8)		(n=2)	(n=2)
Coprostanol	12.2 ± 8.2		65.8 ± 4.7	63.1 ± 1.4		77.2 ± 2.5	75.1 ± 4.4
Cholesterol	86.2 ± 8.7		25.0 ± 4.8	27.6 ± 0.9		18.1 ± 2.0	17.6 ± 2.9
Coprostanone	0.8 ± 0.3		3.2 ± 0.5	2.0 ± 0.4		1.1 ± 0.0	3.3 ± 1.1
Total Animal Sterol (AS) (mg/g)	1.10 ± 0.30		8.54 ± 1.49	(7.08 ± 0.85)[b] 1.417 ± 0.170		(34.2 ± 0.2)[b] 8.067 ± 0.110	(24.7 ± 8.6)[b] 4.798 ± 2.447
Total Plant Sterol (PS) (mg/g)	0.10 ± 0.04		0.76 ± 0.17	(0.50 ± 0.04)[b] 0.100 ± 0.009		(1.5 ± 0.2)[b] 0.341 ± 0.052	(1.4 ± 0.0)[b] 0.247 ± 0.052

a. All values are given in (mean ± SEM)% of total bile acid or neutral sterol. Concentrations are expressed as mg/g of feces.

b. Values in parentheses are calculated based upon dry weight of feces.

c. Δ^5,3β = 3β-hydroxy-5-cholenoic; BM = Bowel movement.

TABLE 12

EFFECT OF DIARRHEA ON FECAL STEROID PROFILE IN ADULTS AND CHILDREN[a]

% Bile Acid (BA) or Neutral Sterol (NS)	Shigella Diarrhea (n=5)[d]	Chronic Non-Specific Diarrhea-Children (n=8)[g]	Travellers' Diarrhea (n=5)[d]	Paired-Fed Ground Controls (n=8)[d]	Apollo 17 Pre-Flight (n=8)[e]	Apollo 17 In-Flight <150g/BM (n=5)[f]	Apollo 17 In-Flight >200g/BM (n=2)[f]
Lithocholic	↓	ns	↓	ns	↓	ns	ns
Deoxycholic	↓	ns	↑	ns	↓ or ns[b]	↑	ns
Chenodeoxycholic	ns	ns	↓ or ns[b]	ns	ns	ns	ns
Cholic	↑	ns	ns	ns	ns	↓ or ns[b]	ns
Unidentified	↑	ns	ns	ns	↑	ns	ns
Total BA (mg/g)	↓ or ns[b]	↓	ns	↓	ns	↑	ns
Correlation of deoxycholic with fecal mass (g/day)	-	ns	-	-	ns	negative correlation r = 0.65 p<0.02	
Bacterial activity on cholesterol[c]	↓	ns	-	ns	-	-	-
Total Animal Sterol (mg/g)	↓	ns	-	ns	-	-	-
Total Plant Sterol (mg/g)	↓	ns	-	↓	-	-	-

a. For explanatory notes and abbreviations, see footnotes under Table 4. Significant differences as determined by Student's t-test are those $p < 0.05$. ns = not significant. BM = bowel movement.

b. $p < 0.10$.

c. Determined by the degree of conversion of cholesterol to coprostanol.

d. As compared to nine normal male adults on regular mixed diet, (n=9).

e. As compared to thre paired-fed ground controls, (n=8).

f. As compared to Apollo 17 pre-flight samples, (n=8).

g. As compared to children controls of same age, (n=4).