Warner and Brinkhous[3] noted a similar phenomenon during an episode of canine distemper which complicated the convalescence in one of their dogs following chloroform-induced hepatic damage.

The galactose tolerance test was within normal limits in all of the individuals studied. There was a wide variation in the results recorded for the bromsulphalein dye test.

Conclusion. In the human subjects here studied, without biliary obstruction of biliary fistulae, the quantitative levels of plasma prothrombin and the amounts of hippuric acid excreted following the ingestion of a known quantity of sodium benzoate would seem to have reflected most sensitively and consistently the degree of liver damage existing. There was no such suggestive correlation or relationship observed between these tests and the plasma fibrinogen levels, the bromsulphalein dye clearance or galactose utilization, either singly or collectively, when all were studied in the same patient.

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Determination of Ascorbic Acid in Feces. Its Excretion in Health and Disease.

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In studying the absorption of ascorbic acid from the gastrointestinal tract, we found it necessary to develop a method for the estimation of ascorbic acid in feces. Although reference to ascorbic acid in feces has been made by 2 authors,[1, 2] no description of the method used was given in one case[1] and in the second,[2] only an abstract was available which referred to a colorimetric procedure. In the method to be described, the total indophenol reducing substances of the feces are estimated, an aliquot is then treated with an ascorbic acid oxidase, after which the reducing value is again determined. The difference represents the ascorbic acid present.

Feces upon excretion are immediately weighed to the nearest gram and transferred to a mortar in which they are rubbed to a homogeneous sludge with a minimum volume of freshly prepared 5%

[1] van Eeckelen, M., *Biochem. J.*, 1936, **30**, 2291.

[2] Ishibashi, T., *Acta Pædiat. japon*, 1937, **43**, 187. Abstracted by *Am. J. Dis. Child.*, 1937, **54**, 1101.

metaphosphoric acid solution. The sludge is transferred quantitatively to a suitable sized stoppered graduated cylinder (500 ml for 100 g stool) with sufficient 5% HPO_3 to give a suspension (Suspension A) in which 5 ml contain 1 g of original feces. In making a 24-hour assay, feces should be suitably marked with string. Stools passed at night are rubbed up with a known weight (usually 10 g) of powdered HPO_3 in an ointment jar, and then placed in a refrigerator for analysis in the morning.

Approximately 100 ml of suspension A (divided between two 50 ml centrifuge tubes) are centrifuged at moderate speed for 20 to 30 minutes. From the tubes 50 ml of supernatant fluid are pipetted into a 200 ml volumetric flask. Through this liquid is passed a vigorous stream of N_2 or CO_2 for 20 minutes to remove any trace of H_2S. (We do not depend upon a later treatment with lead acetate alone to remove all traces of H_2S.) At the end of this time, the aeration tube is rinsed and the solution diluted to the mark with 5% HPO_3. Twenty ml of the resulting solution (Fluid B) are now equivalent to 1 g of the original feces.

Two or more 5 ml aliquots (for checks) of Fluid B are pipetted into 15 ml centrifuge tubes. To each are added 1 ml of glacial acetic acid and 5 ml of 12.5% lead acetate solution. (62.5 g anhydrous neutral $Pb(C_2H_3O_2)_2$, C.P. are dissolved in 350 ml of distilled water to which sufficient glacial acetic acid (5 to 15 ml) to cause solution is added. This is diluted to a volume of 500 ml.) The tubes are mixed well and centrifuged for 2 to 5 minutes. The meta-phosphates of lead adsorb most of the color. Any remaining turbidity can be dispelled by the further addition of 2-3 drops of 10% HPO_3. Two ml aliquots are now transferred to small test tubes, and titrated by daylight to a distinct pink with a standardized 2:6-dichlorphenol-indophenol[3] solution* delivered from a 5 ml micro burette (div. 0.01 ml). A No. 20 hypodermic needle fitted to a ground burette tip permits delivery of drops of not over 0.015 ml. The data from this titration represents *total indophenol reducing substances* (expressed as ascorbic acid).

To determine *non-ascorbic acid reducing substances,* pipette 2 or more 5 ml aliquots of Fluid B into 15 ml centrifuge tubes. To each, add sufficient 10% NaOH to bring the reaction to pH 5.5 to 6.0 as tested with nitrazine paper. Add to each tube 1 ml of cauliflower oxidase prepared according to the method of Hopkins and Morgan.[4]

[3] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625.

* Hereinafter referred to as "standardized dye."

[4] Hopkins, F. G., and Morgan, E. J., *Biochem. J.*, 1936, **30**, 1446.

Mix thoroughly and incubate in a water bath at 45-50°C for 30 minutes. At the end of this period add to each tube 1 ml of glacial acetic acid and 5 ml of lead acetate solution. Mix thoroughly and centrifuge. Two ml aliquots from respective tubes are titrated to the same end point as that obtained above. From these data are calculated the *non-ascorbic acid reducing substances* of the feces, expressed as milligrams of ascorbic acid.

Example of Procedure. 132 g of feces were made up to 660 ml with 5% HPO_3 (Suspension A). Fifty ml of supernatant liquid from suspension A were diluted to 200 ml with 5%HPO_3 (Fluid B). To each of three 5 ml aliquots taken for analysis, were added 1 ml glacial acetic acid, and 5 ml 12.5% lead acetate solution. After centrifugation, 2 ml portions of respective supernatant liquids were titrated, requiring an average of 0.66 ml "standardized dye". From this is calculated the total indophenol reduction.

To each of the 3 additional samples of Fluid B, 0.50 ml of 10% NaOH were required to adjust to pH 5.5-6.0. One ml oxidase was then added. After incubation, and clarifying with acetic acid and lead acetate, 2 ml aliquots required an average of 0.20 ml "standardized dye" for titration. From this is calculated the non-ascorbic acid reduction. One ml of "standardized dye" was equivalent to 0.020 mg ascorbic acid.

Calculations. To simplify calculations, the following equations are used.

(1) Total reduction $= 4.40 \times A \times B \times E$.

(2) Non-ascorbic acid reduction $= 0.40 \times B \times C \times D \times E$.

(3) Ascorbic acid (mg) = total reduction − non-ascorbic acid reduction.

where

A = ml dye used for 2 ml aliquot before oxidase treatment.

B = total volume of suspension A in ml.

C = ml dye used for 2 ml aliquots after oxidase treatment.

D = total volume to which 5 ml of Fluid B is diluted after addition of NaOH, oxidase, acetic acid and lead acetate. It will vary depending upon the amount of NaOH required to adjust the pH.

E = ascorbic acid equivalent of 1 ml of dye.

In the above example: A = 0.66 ml; B = 660 ml; C = 0.20 ml; D = 12.5 ml; and E = 0.020.

Therefore:

Total reduction $= 4.40 \times 0.66 \times 660 \times 0.020 = 38.33$ mg.

Non-ascorbic acid reduction $= 0.40 \times 660 \times 0.20 \times 12.5 \times 0.20$ $= 13.20$ mg.

Ascorbic acid = 38.33 — 13.20 = 25.13 mg.

Recoveries of Added Ascorbic Acid: In checking the method the following procedure was adopted: To known quantities of fecal suspension A, ascorbic acid† was added in varying amounts. Five ml samples were then carried through the procedure. Data from a series of analyses as given in Table I, indicate a recovery of 98.2%.

In a similar series in which the 1 ml glacial acetic acid was omitted, the recovery dropped to 92.3%. It should also be mentioned that complete destruction of ascorbic acid was obtained within 30 minutes by the cauliflower oxidase between pH 5.0 and 8.2. We, however, suggest adjustment to pH 5.5 to 6.0 because it was found that a destruction of the non-ascorbic acid reducing substances occurs above pH 6.5. As would be expected from the wealth of reducing bacteria in the large intestine, all of the ascorbic acid in the feces was found to be in the reduced form, thus eliminating a preliminary treatment with H_2S.

Effect of Diet upon Ascorbic Acid Excretion. With a method available for the determination of ascorbic acid in feces, it was possible for the first time to follow simultaneously the blood level and the urinary and fecal excretions after ingestion of varying amounts of ascorbic acid. Finger blood was removed from the fasting individual, and the plasma ascorbic acid content determined by the micromethod of Farmer and Abt.[5] Urine samples collected during the day

TABLE I.
Recoveries of Added Ascorbic Acid.

Blank* as mg Ascorbic Acid	Ascorbic Acid Added, mg	Total Present, mg	Recovery, mg	% Recovery
.0456	.0252	.0708	.0690	94.6
.0154	.0348	.0402	.0474	94.4
.0456	.0378	.0834	.0854	102.4
.0310	.0378	.0688	.0676	98.3
.0176	.0382	.0558	.0520	93.2
.0384	.0482	.0866	.0852	98.4
.0456	.0504	.0960	.0976	101.7
.0154	.0524	.0678	.0680	100.3
.0342	.0544	.0886	.0904	102.0
.0374	.0690	.1084	.1036	95.6
.0154	.0698	.0852	.0870	102.4
.0176	.0764	.0940	.0900	95.8
.0176	.0954	.1130	.1104	97.7
				98.2 ± 0.6

* Blank = indophenol reducing substances other than ascorbic acid.

† We are indebted to Merck and Co., Inc., Rahway, N. J., for supplying the ascorbic acid (Cebione) used in this investigation.

[5] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 146.

were acidified with metaphosphoric acid and titrated immediately. Night samples were preserved in the refrigerator in large bottles containing 10 g of metaphosphoric acid and titrated early the following morning. Fecal samples were analyzed immediately after excretion.

The subject consumed a normal diet which was relatively high in vitamin C. In addition, varying amounts of l-ascorbic acid were

TABLE II.
Comparative Studies of Blood Plasma Level, Urinary and Fecal Excretions, on Various Levels of Ascorbic Acid Intake.
Subject H.C.

Date	Dietary Intake* mg Ascorbic Acid	Plasma Ascorbic Acid, mg %	Urinary Excretion		Fecal Excretion	
			Volume, ml	Excreted Ascorbic Acid, mg	Moist wt, g	Ascorbic Acid, mg
8/18	75				109.2	6.92
8/19	70	.88	1047	15.36	54.7	5.56
8/20	90	.68	810	16.63	31.4	4.35
8/21	35	.92	884	33.50	41.3	2.56
8/22	80	.92	1141	17.93	31.2	3.90
8/23	100	.96	847	30.34		
8/24	60	.76	602	16.78	125.2	15.44
Avg	73	.85	888	21.67	56.1	5.53
8/25	250	1.04	806	74.33	62.6	10.00
8/26	260	1.04	656	125.44	177.9	16.40
8/27	275	1.28	831	133.90	93.5	12.68
8/28	230	1.48	684	87.75		
8/29	260	1.76	825	155.53		
8/30	250	1.64	915	133.39		
8/31	250	1.76	829	123.94	169.2	29.60
Avg	255	1.43	792	119.18	71.9	9.81
9/1	540	2.04	867	252.61	70.2	12.70
9/2	570		810	180.45		
9/3	530	1.60	833	270.77	160.6	20.90
9/4	530	1.44	848	234.71		
9/5	550	1.44	1004	286.53	361.2	48.0
9/6	580	1.84	1012	454.91		
9/7	530	1.88	828	523.73	150.8	15.0
Avg	547	1.71	886	315.10	106.1	13.80
9/8	1050	1.68	1139	373.98	75.0	11.80
9/9	1070	1.60	932	379.86	140.0	16.16
9/10	1070	1.82	602	348.25		
9/11	1030	1.48	821	380.91		
9/12	1090	1.52	1266	482.14	149.9	33.31
9/13	1050	1.68	1212	497.79	149.0	18.12
9/14	1030	1.48	1367	375.90		
Avg	1054	1.61	1048	405.55	73.4	11.34

* Mg ascorbic acid in basal diet estimated from known food content, to which amount taken as supplement is added.

ingested. The daily supplement was taken in a single dose. To mark each day's feces, respectively, strings of a characteristic color were swallowed with each meal. Table II summarizes the results obtained on one of us (H.C.) during 28 consecutive days.

A relatively small but definite excretion into the feces can be noted in a normal individual, irrespective of intake. This is increased but slightly upon ingestion of large amounts of ascorbic acid. On the other hand, the blood plasma level rises with the intake until a maximum is reached, paralleled by a corresponding rise in the urinary excretion.

An average daily fecal excretion of 4.92 mg ascorbic acid was obtained in a study of 12 normal young male medical students subsisting on their usual mixed diet. A study of fecal excretion of patients suffering from various gastro-intestinal disorders showed much variation. Thus, an alcoholic following gastric resection excreted 233 mg when receiving 300 mg orally and 1000 mg intravenously per day. In a case of esophageal stricture, where 1000 mg were given intravenously, the daily fecal excretion averaged 1.5 mg over a period of 14 days. The excretion in colitis varied with the number and type of stool. One case receiving 450 mg ascorbic acid daily by mouth, excreted 34 mg into the feces while having 12 to 15 liquid stools per day. Another case in remission on a similar intake excreted 18 mg daily in 2 to 3 formed stools.

Conclusions. 1. A method for the estimation of ascorbic acid in feces is described, and by its use, the fecal content of the normal individual on an adequate but unsupplemented diet is shown to average about 5 mg daily. 2. The plasma, urinary and fecal ascorbic acid contents have been followed in a normal individual after administration of varying amounts of l-ascorbic acid by mouth. Large variations in the dietary intake were shown to affect the fecal excretion only slightly. 3. Patients suffering from certain gastro-intestinal disorders excreted larger quantities of ascorbic acid in the feces than normal individuals.