Studies on the origin of faecal amino acids in cystic fibrosis¹

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SUMMARY Evidence is presented which demonstrates that excess faecal amino acids in cystic fibrosis are derived from unabsorbed dietary protein. Changes in the absorption of fat and nitrogen have little effect on the amino-acid patterns.

In an earlier paper (Gibbons, Seakins, and Ersser, 1967) it was shown that the faecal amino-acid patterns of children with cystic fibrosis were heavier than those of normal children and adults. Most of the amino acids found in proteins were involved, together with some of their decarboxylation products, amines and non-alpha amino acids. These findings were in contrast to normal faecal patterns found in an infant with Hartnup disease (Seakins and Ersser, 1967) and three children with cystinuria (unpublished observations).

Previously protein absorption in patients with cystic fibrosis and the effect of pancreatin have been studied by the balance technique (Harris, Norman, and Payne, 1955) or by the change in blood amino acids following loading tests (West, Wilson, and Eyles, 1946; Anfanger and Heavenrich, 1949). There is, however, little information on the composition of faeces and none on the possible interaction between unabsorbed constituents. In this paper, the composition of individual stools from different patients with cystic fibrosis are compared with those from normal children and adults. Further information was sought on the sources of faecal amino acids by varying the dietary protein and fat and by partial sterilization of the gut.

Methods

COLLECTION OF FAECES

Specimens were collected into containers, the time and presence or absence of marker noted. The specimens were either stored on solid carbon dioxide or at -20°C , which prevented further bacterial transformations, before analysis. Carmine or Edicol supra blue EG (ICI Ltd) were used as markers and were given with the first meal of the day.

PREPARATION OF SAMPLES

Preliminary investigations had shown that some faecal specimens possessed considerable proteolytic activity which resulted in a significant increase in the amino-acid pattern during the preparation of the sample for analysis. This was prevented by using dilute hydrochloric acid (3%) to make an accurate dilution (3:1 w/w) of the individual weighed faecal specimen in a Silverson homogenizer. The final pH was about 2, and the presence of the mineral acid did not interfere with the subsequent analyses.

CHROMATOGRAPHY OF AMINO ACIDS AND AMINES

A sample of the faecal homogenate (30 g) was diluted to 60 ml with acetone, shaken thoroughly, centrifuged, and the supernatant either stored at -20°C or processed immediately in the following way.

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A volume of the supernatant equivalent to 3 g faeces was diluted with an equal volume of aqueous acetone (1:1), filtered if necessary, and passed down a column of Zeo-Karb 225 (SRC 10 H⁺, 1 × 10 cm) washed successively with water, ethanol, and water to remove absorbed pigments, etc, and the amino acids and amines displaced with 5N-ammonia (25 ml). The ammoniacal effluent was taken to dryness in a rotary evaporator (a few millilitres of butanol were added to stop frothing) and the residue taken up in 10% isopropanol-water (0.3 ml).

All specimens were examined by one-way descending chromatography for 16 hours, and a length of run of 45 cm, on Whatman no. 3 paper with nBuOH-AcOH-H₂O (120:30:50) and by two-way electrochromatography in 25.5 cm square sheets of Whatman no. 3 paper, pH 2, followed by ascending chromatography in the above solvent system using an amount equivalent to 100 mg wet weight faeces (Seakins and Ersser, 1967). Amino acids and amines were visualized by ninhydrin/pyridine. The identity of the amino acids and amines was confirmed by their electrophoretic and chromatographic behaviour using authentic specimens, by their characteristic colours with the ninhydrin/pyridine and β -naphthaquinone-4-sulphonate (Folin's) reagents, and by specific location reagents (Table I); in addition diazotized sulphanilic acid (Pauly's reagent) was used to confirm the identity of tyrosine, tyramine, histidine, and histamine. Further confirmation where necessary was obtained by fractionation of the ammoniacal effluent on successive ion-exchange resins: (a) Zeo-Karb 226 (SRC 43, pH 7.0 pyridine acetate) which retained amines, basic amino acids, and basic peptides (Blau, 1961) which were eluted with acetic acid; (b) Zeo-Karb 225 (pyridine form) which retained the aromatic and non- α -

amino acids (Kakimoto and Armstrong, 1961); (c) De-Acidite FF (SRA 62, acetate) which retained aspartic and glutamic acids (Kakimoto and Armstrong, 1961).

Amino acids were quantitated by visual comparison with standards of 2, 5, 10, and 20 μ g, and where appropriate the samples were diluted so that the concentration fell within the lower part of this range. Table I summarizes the procedures employed. Satisfactory methods could not be found for lysine, threonine, serine, and arginine, though the last three were rarely noted. Recovery experiments on authentic amino acids and a casein hydrolysate indicated an overall accuracy of between 5 and 10%.

AMINO NITROGEN

The sample was prepared as described above for chromatography of amino acids and the dried ammoniacal effluent stored overnight over concentrated sulphuric acid in a vacuum dessicator to remove residual ammonia. The residue was dissolved in a suitable volume of 0.1M potassium tetraborate, pH 9.4, to give an amino-nitrogen concentration between 0.02 and 0.15 mM, using the chromatogram as a guide. The amino nitrogen was determined by a trinitrobenzene sulphonic acid method (Mokrasch, 1967) as modified by Prenton and London (1967) for use on the Technicon AutoAnalyzer, but omitting the dialyzer unit. Glycine (0.02-0.15 mM) was used as a standard.

Total nitrogen was determined by Kjeldahl's method using selenium dioxide as catalyst, after 'black ashing' faecal homogenates with concentrated sulphuric acid. The method of Fowweather and Anderson (1946) to evaluate faecal fat was slightly modified. Equal weights (10 g) of the acid faecal homogenate and plaster of paris were mixed, dried, and pulverized before extraction with petroleum spirit (boiling point, 60-80°C) in a Soxhlet apparatus. A sample of this acid homogenate (1 g) was dried at 105°C for 24 hr and reweighed to measure water content. Urinary phenolic acids were isolated and chromatographed according to the methods described by Smith, Seakins, and Dayman (1969).

Clinical Material

NORMAL SUBJECTS

The three normal adults are the authors of this paper. A total of 48 children (6 months-15 years) who were inpatients at the Hospital for Sick Children served as normal controls. They were either awaiting or recovering from minor surgical operations or were mentally retarded children admitted for assessment. All were receiving normal ward diets, had normal bowel motions at the time of collection, had no history of

Amino Compound	Separation	Location Reagent
Glu, Asp	Electrophoresis, pH 4.4 pyridine acetate	0.2% ninhydrin and 5% pyridine in acetone ¹
Tyr, Phe	Electrochromatography (see text)	0.2% ninhydrin and 5% pyridine in acetone ¹
Leu, Ileu, Val, Tyr NH ₂ , (Met)	Test-pentanol-methyl ethyl ketone-water (60:20:20) in atmosphere of diethylamine ²	0.2% ninhydrin and 5% pyridine in acetone ¹
Ala	n-butanol-acetic acid-water (120:30:50) ¹	0.2% ninhydrin and 5% pyridine in acetone ¹
Pro, Hypo	n-butanol-acetic acid-water (120:30:50) ¹	Isatin ¹
Met, (CyS ₂)	n-butanol-acetic acid-water (120:30:50) ¹	Iodoplatinate ⁴
Try, Try-NH ₂	n-butanol-acetic acid-water (120:30:50)	Ehrlich's reagent ¹
His, Gly	n-butanol-acetic acid-water (120:30:50)	o-Phthalaldehyde ⁴

Table I Separation methods for the semi-quantitation of faecal amino acids and amines

¹Smith (1969)

²Munier and Sarrazin (1964)

³0.5% solution in acetone, 10 min at 40°C.

malabsorption or metabolic disease, and were not receiving antibiotics. The son of one of the authors (J.S.) acted as a further control, and specimens were collected at home.

PATIENTS

A total of 55 patients (3 months-15 years) with a proven diagnosis of cystic fibrosis of the pancreas were studied. Of these, eight patients (3 months-2 years) were newly diagnosed and had not received any therapy. Where possible at

least three separate specimens were examined. Pancreatin supplements were given as either Pancrex V powder or capsules, 0.5-2 g four times daily with meals, or as an enteric-coated preparation, Pancrex V Forte tablets, 2-6 tablets four times daily before meals (Paines and Byrne Ltd, Greenford).

Results

The detailed faecal analyses on normal subjects are presented in Table II. Figure 1 is a typical two-way electrochromatogram. A further 51 specimens from 34 children were examined by one-way chromatography and by two-way electrochromatography only. Qualitatively, all patterns were similar. Lysine was present in most specimens, but was not quantitated. No change in the pattern of amino acids was observed when two adults took methyl cellulose (10 g/day for four days) to increase faecal bulk, nor when a normal boy was given pancreatin.

Figure 2 is a typical two-way electrochromatogram from a patient with cystic fibrosis. The detailed analyses on the 15 patients with cystic fibrosis are summarized in Table III and in Figures 3 and 4. The variations in isoleucine and valine content approximately paralleled that of leucine; aspartic acid was always within the normal range, in contrast to glutamic acid; tryptophan, proline, and methionine were elevated in about one third of the patients; excess lysine was present in most patients. Additionally variable amounts of δ -aminovaleric, α - and γ -amino-n-butyric acids, β -alanine, ethanolamine, cadaverine, and less frequently histamine and putrescine were observed. One-way chromatograms and two-way electrochromatograms from the remaining 40 children with cystic fibrosis gave a similar range in faecal amino-acid patterns.

The water content of faeces from the 15 patients with cystic fibrosis was in the normal range (overall mean $75.1 \pm 3.7\%$; Fig. 5) with the exception of three specimens from three patients which had a very high fat content (25-30%) and a low water content (approximately 50%). These specimens were not included in the above mean value. For a given patient, it was noted that a higher fat content was associated with a lower water content (see also Table IV).

The faecal nitrogen (1.28 ± 0.33 g/100 g) was not significantly different from normal subjects, although the actual daily loss of nitrogen was significantly higher in most patients because of the increased faecal bulk. The fat content varied considerably (Fig. 5) and no relationship between faecal amino nitrogen, total nitrogen, fat, and bulk could be discerned. This is exemplified by comparing the detailed figures in two patients. In the first patient (no. 6) faecal fat (3.4 ± 0.8 g/100 g) and nitrogen (0.82 ± 0.08 g/100 g) were

Amino Acid (mg/100 g)	Adults ^a		Children ^a	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Gly	2.1 $\pm$ 1.5	0- 5	5.1 $\pm$ 3.1	2-14
Ala	3.4 $\pm$ 1.1	2- 5	6.3 $\pm$ 3.8	3-10
Val	1.1 $\pm$ 0.3	1- 2	3.4 $\pm$ 2.8	1-10
Ileu	1.2 $\pm$ 0.4	1- 2	3.8 $\pm$ 3.8	1-15
Leu	1.1 $\pm$ 0.3	1- 2	3.8 $\pm$ 3.6	1-15
Met	0 —	0	0.5 —	0- 2
Pro	1.8 $\pm$ 0.6	1- 3	2.8 $\pm$ 2.2	1- 8
Phe	0 —	0	1 —	0- 1
Tyr	0 —	0- 1	1 —	0- 1
Try	0 —	0- 1	1 —	0- 2
His	0 —	—	2 —	0- 2
Asp	7.0 $\pm$ 2.8	2-10	10.6 $\pm$ 6.7	5-35
Glu	25.1 $\pm$ 7.9	16-40	24.5 $\pm$ 10.4	12-30
Tyr NH ₂	0 —	0	1 —	0- 2
N-NH ₂	27.1 $\pm$ 8.5	13-50	29.5 $\pm$ 7.6	16-48
Total N(g/100 g)	1.39 $\pm$ 0.33	0.71-1.99	1.11 $\pm$ 0.34	0.71- 2.06
Fat (g/100 g)	4.69 $\pm$ 1.47	2.51-7.49	3.88 $\pm$ 2.43	1.02-10.5
Water (%)	74.3 $\pm$ 5.4	65-85	76.7 $\pm$ 6.9	60-86

Table II Faecal amino acids in normal subjects

^aThree adults, 16 specimens.
^aFifteen children, 21 specimens.

Fig. 1 Two-way electrochromatogram from a normal subject.

Fig. 2 Two-way electrochromatogram from a patient with cystic fibrosis.

normal as were the corresponding daily faecal outputs, 4.9 g fat and 1.11 g nitrogen, but there was a heavy amino-acid pattern in all specimens. In the second patient (no. 15), the faecal nitrogen was normal (1.67 ± 0.10 g/100 g), the faecal fat was elevated (15.4 ± 7.7 g/100 g), and the corresponding daily outputs were grossly increased, being respectively 4.8 g and 47.7 g, but the amino nitrogen was just outside the normal range.

Over two-thirds of the patients studied gave an elevated urinary excretion of p-hydroxyphenylacetic acid, but there was no clear correlation between faecal tyramine and the excretion of its urinary metabolite.

CHANGE IN NATURE AND QUANTITY OF DIETARY FAT

Four variations in diet were investigated in patient no. 5 (Table III). As far as possible diets were isonitrogenous and isocaloric. Faecal analyses were performed for each period on six consecutive specimens collected at least one week after the change in diet. Table IV summarizes the effects of these changes.

The transit time remained unchanged throughout at approximately 24 hours, although the

	Case No.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age (yr)	0.3	1.5	3	3	3.8	4	4	8	9	10	11	11	12	12	16
Sex	M	F	M	F	F	M	M	F	M	F	F	M	M	M	M
Mean nitrogen (g/100 g)	0.96	—	1.86	1.52	2.07	0.82	1.19	1.16	1.04	1.45	—	1.32	1.54	1.41	1.67
Pancreatin ¹	P	O	O	P	O	P	P	P	P	P	O	P	P	P	P
Transit time (hr)	22	—	—	25	24	23	25	6	—	96	35	—	22	—	14
	26			32		25	29	26							45
No. of specimens	3	2	2	6	6	5	4	9	3	6	3	3	3	5	4

Table III Data on children with cystic fibrosis

¹P, receiving pancreatin; O, off pancreatin.

Amino Acid (mg/100 g)	Period ¹				Significance of Differences between Periods	
	I	II	III	IV		
Gly	87 ± 16 ^a	70 ± 21	69 ± 10	14 ± 13	(>0.05) I > II, III, >IV (<0.001)	
Ala	60 ± 12	60 ± 28	88 ± 10	104 ± 18	I, II <III, IV (0.001)	
Ileu	14 ± 5	18 ± 17	33 ± 12	30 ± 9	Not significant	
Leu	15 ± 5	23 ± 16	36 ± 15	36 ± 13	Not significant	
Val	17 ± 8	27 ± 13	35 ± 13	36 ± 9	Not significant	
Pro	3 ± 1	2 ± 1	4 ± 3	2 ± 1	Not significant	
Phe	5 ± 1	12 ± 7	11 ± 5	11 ± 8	Not significant	
Tyr	3 ± 1	2 ± 2	3 ± 3	5 ± 2	Not significant	
Try	1	1	1	7 ± 2	I, II, III, <IV (0.002)	
His	5 ± 1	5 ± 1	18 ± 13	11 ± 5	I, II, <III, IV (0.005)	
Asp	8 ± 1	8 ± 1	8 ± 2	8 ± 1	Not significant	
Glu	57 ± 14	33 ± 13	57 ± 7	24 ± 10	I, III, >II, IV (0.001)	
Tyr-NH ₂ ³	10 ± 5	6 ± 5	14 ± 6	32 ± 11	I, II, III, <IV (0.001)	
N-NH ₂ ⁴	48 ± 21	71 ± 44	94 ± 15	103 ± 23	I, <II, III, IV (0.005)	
N(g/100 g) ⁵	2.07 ± 0.06	2.33 ± 0.13	2.40 ± 0.26	1.16 ± 0.11	I, II, III, >IV (0.001)	
Fat (ng/100 g)	9.9 ± 5.1	6.5 ± 1.3	5.5 ± 1.4	7.1 ± 3	I, >II, III, IV (0.001)	
Water (%)	64 ± 6	74 ± 4	74 ± 5	82 ± 7	I, <II, III, <IV (0.01)	

Table IV Changes in faecal composition with dietary fat

¹Period I, normal diet; II, low long-chain triglyceride (20 g/day); III, low long-chain triglyceride (20 g/day) with medium-chain triglyceride (40 g/day); IV, same triglyceride intake as III with pancreatin added. ²Mean ± 1 SD. ³Tyr-NH₂, tyramine; ⁴N-NH₂, amino nitrogen (TNBS method); ⁵N, nitrogen.

Fig. 3 Faecal amino acids and tyramine in cystic fibrosis (mean $\pm$ 1 SD mg/100 ml). --- Upper limit of normal (mean $\pm$ 2 SD). For phenylalanine, tyrosine, and histidine the upper limit of normal is about 2 mg/100 g, but tyramine is negligible.

Fig. 4 Faecal amino nitrogen (mean $\pm$ 1 SD) in patients with cystic fibrosis.

Fig. 5 Water and fat content (mean $\pm$ 1 SD) in patients with cystic fibrosis.

frequency of defaecation diminished when low long-chain triglyceride (20 g/day) with medium-chain triglyceride (40 g/day) was introduced with added pancreatin. The daily fat output fell from 44.2 g to 11.0 g/24 hour when long-chain triglyceride was restricted and was further diminished to 6.0 g/24 hour when pancreatin supplements were added ($p < 0.02$). Gas-liquid chromatography of a saponified petroleum ether extract of acidified faeces when low long-chain triglyceride and medium-chain triglyceride were given demonstrated the absence of C_{10} and lower fatty acids (Leyland, Fosbrooke, Lloyd, Segall, Tamin, Tomkins, and Wolff, 1969).

In addition to the changes in amino acid composition noted in Table IV, δ -amino-valeric

acid was prominent on the electrochromatograms throughout the four periods, whilst lysine, which was virtually absent when the patient was on a normal diet or was given low long-chain triglyceride, or when she was given low long-chain triglyceride with medium-chain triglyceride, was very heavy when pancreatin was also given. This increase in lysine would account for the increase in amino nitrogen. The excretion of p-hydroxyphenylacetic acid varied from normal to grossly abnormal. Similar results were obtained in two other patients studied in less detail.

GELATIN DIET

In three patients with cystic fibrosis, one normal adult, and one infant with normal gastrointestinal function, first-class protein was replaced as far as practicable with gelatin for two days. The faecal amino-acid patterns of the two normal subjects did not change. In one patient (no. 12) who received 43 g gelatin plus 19 g first-class protein during the test period as against 60 g first-class protein in the control period there was a very significant and prompt change in the faecal amino-acid pattern, which quickly returned to the pattern observed in the control period when a normal diet was resumed. Table V summarizes the results in this patient. Unexpectedly, no hydroxyproline was detected in the test period, γ -amino-n-butyric acid and β -alanine decreased, whilst δ -amino-n-valeric acid increased. In the other two patients with cystic fibrosis the results were not as clear cut as in the first, although the changes in the amino-acid patterns of the first marked stools were of the same order as in the first patient, but subsequent faecal patterns showed greater variation. The following significant changes were noted. In the second patient

Protein (g/100 g)		Amino Acid	Faeces (mg/100 g) (mean $\pm$ 1 SD)			
Casein	Gelatin		Normal Diet (n = 4)	Gelatin Diet (n = 5)	P	
2	27	Gly	28 $\pm$ 18	145 $\pm$ 37	<0.001	
3	10	Ala	38 $\pm$ 5	20 $\pm$ 10	<0.025	
6	2	Val	17 $\pm$ 5	2 $\pm$ 0.5	<0.001	
6	2	Ileu	12 $\pm$ 6	0.2 $\pm$ 0.6	<0.005	
9	4	Leu	18 $\pm$ 4	1 $\pm$ 0.7	<0.001	
10	14	Pro	4	1	Not significant	
5	2	Phe	10 $\pm$ 7	1 $\pm$ 1	0.05	
6	1	Tyr	11 $\pm$ 8	1 $\pm$ 1	0.05	
3	1	His	9 $\pm$ 3	6 $\pm$ 2	Not significant	
6	7	Asp	3 $\pm$ 1	1	Not significant	
20	11	Glu	23 $\pm$ 6	4 $\pm$ 1	<0.001	
0	13	Hyp	0	0	—	
1	0	Try	0	0	—	
3	1	Met	0	0	—	
		Tyr-NH ₂	23 $\pm$ 5	3 $\pm$ 3	<0.001	
		N-NH ₂	71 $\pm$ 8	135 $\pm$ 8	<0.001	
N (g/100 g)			1.32 $\pm$ 0.26	1.32 $\pm$ 0.18	Not significant	
Fat (g/100 g)			12.6 $\pm$ 3.0	12.9 $\pm$ 2.2	Not significant	
Water (%)			76 $\pm$ 1	74 $\pm$ 4	Not significant	

Table V Composition of casein and gelatin and the effect of gelatin diet on faecal amino acids

(no. 14) the glycine content increased from 40 ± 13 to 100 ± 5 mg/100 g (P, 0.001) while glutamic acid fell from 83 ± 28 to 15 ± 5 (P, 0.005), alanine from 47 ± 22 to 15 ± 5 (P, 0.05), and tryptophan was no longer detectable. In the third patient (no. 13) there was an immediate increase in glycine from 18 ± 12 mg/100 g (5-30) to 200 ± 70 (150-300) (P, 0.02). As with the first patient, no hydroxyproline was detected in these two test periods. The effect of pancreatin supplements was also investigated in these two patients: no consistent changes in the pattern were observed, except that hydroxyproline was still not detected.

PROTEIN HYDROLYSATE

The effect of substitution of an enzymic hydrolysate of lactalbumin (Nesmida¹) for whole (milk) protein was studied in five infants with cystic fibrosis, chosen because of failure to thrive. There was an immediate improvement in the faecal amino-acid pattern. Figure 6 shows a serial one-way chromatogram in one patient and the corresponding amino-nitrogen values. The excretion of p-hydroxyphenylacetic acid also diminished considerably.

¹Nesmida, Nestlé SA, Vevey, Switzerland.

THE USE OF ANTIBACTERIAL AGENTS

Faecal specimens from two patients with cystic fibrosis (aged eight and 13), receiving phthalylsulphathiazole (800 mg tds) and streptomycin (400 mg tds) for five days, gave heavy bacterial cultures throughout the period. Those faecal amino acids, which were abnormal in the control period, remained abnormal in the test period. The excretion of p-hydroxyphenylacetic acid fluctuated but was still abnormal throughout.

Discussion

Although visual comparison of amino acids separated by paper chromatography is less accurate than ion-exchange resin chromatography, paper chromatography has the advantages of specificity and speed, and in this study enabled a large number of specimens to be examined. Some of the variations observed may be attributed to this.

A careful examination (for details see Ersser, 1969) of the solvent extraction method previously employed (Seakins and Ersser, 1967) showed that the extraction of faecal amino acids was incomplete, and furthermore the same sample

Fig. 6 One-way descending chromatogram of faecal extracts from an infant with cystic fibrosis receiving high protein (A), high protein + pancreatin (B), enzymic hydrolysate (C), enzymic hydrolysate + pancreatin (D). The corresponding amino nitrogen values are: A, 116; B, —, 80, 68; C, 67, 46, 33; D, 34 mg/100 g.

could not be used for other analyses. Aqueous extraction methods (Bickel, 1964; Hooft, Carton, Snoeck, Timmermans, Antener, van den Hende, and Oyaert, 1968) gave variable increases in amino acids, traced to continued enzymic and bacterial proteolytic activity during homogenization. Homogenization with phosphate buffer followed by extraction into butanol (Yarbro and Anderson, 1966) gave a very poor recovery of amino acids. By using dilute hydrochloric acid, as detailed in the methods section, proteolytic activity was stopped, and an excellent extraction of faecal amino acids was achieved, with the exception of taurine and cysteic acid which are not retained on Zeo-Karb 225 (H^+). This method of extraction does not distinguish between intracellular and extracellular amino acids. These observations in part explain the higher values reported by Hooft *et al* (1968) and the lower α -amino-nitrogen values given by Asatoor, Chamberlain, Emmerson, Johnson, Levi, and Milne (1967).

The trinitrobenzenesulphonic-acid method for amino nitrogen (Mokrasch, 1967) was preferred to the specific α -amino-nitrogen method of van Slyke, since it was readily automated, is less sensitive to ammonia, and gives a better measure of amino acids (both α and non- α) and amines than the latter, although oligopeptides retained on the resin and present as a streak with low mobility and R_f on the electrochromatograms will also contribute to the colour yield.

The gravimetric method of Fowweather and Anderson (1946) for faecal fats was chosen rather than van der Kamer's since some children were receiving medium-chain triglyceride (*cf* Leyland *et al*, 1969).

The results in normal subjects when the dietary protein was radically altered, earlier work on protein amino-acid loads (Milne, Crawford, Girao, and Loughridge, 1960; Milne, Asatoor, Edwards, and Loughridge, 1961; Seakins and Ersser, 1967), and the study of the fate of cyclo-leucine (Christensen and Clifford, 1962) show that faecal amino acids in normal subjects are predominantly of bacterial origin or arise through the bacterial action on unabsorbed endogenous protein (Nasset, 1964) rather than by secretion into the large bowel. Likewise, Sheffner, Kirsner, and Palmer (1948) found that the composition of normal faecal protein was little changed when dietary protein was varied.

In contrast to the consistent patterns given by normal subjects, the majority of children with cystic fibrosis gave heavy patterns which varied somewhat from sample to sample in an individual and from patient to patient. It is therefore unlikely that a defect in amino-acid transport analogous to that found in Hartnup disease (Milne *et al*, 1960) or cystinuria (Milne *et al*, 1961) operates in cystic fibrosis or excess secretion of amino acids into the large bowel occurs.

In spite of the increased faecal bulk and

reduced transit time observed in cystic fibrosis, the water content of faeces was in the same range observed in normal subjects, indicating efficient water absorption from the large bowel.

The total faecal nitrogen comprises proteins, substances such as ammonia and volatile amines which are lost in the column preparation, and amino compounds which are measured by the TNBS method. In view of the high faecal amino-nitrogen content found in cystic fibrosis, the finding of the same nitrogen content as in normal subjects was unexpected. This implies that the protein and/or volatile amine content is lower in cases of cystic fibrosis, which is consistent with the observation that these faecal specimens have the same water content but higher fat content than normal specimens. Furthermore, the longer transit time in normal subjects would allow more extensive degradation of any amino acid present to substances such as ammonia. No relationship between total nitrogen, amino-nitrogen, fat, bulk, or water content and the amino-acid pattern of individual specimens was noted in patients receiving a free-choice diet.

Pancreatin was not the major source of faecal amino acids, for heavy patterns were observed in eight newly diagnosed patients before any therapy had been instituted, although there was some evidence to suggest that pancreatin given with ordinary protein further increased the abnormal pattern.

Controlled dietary studies indicated that steatorrhoea was not the cause of the amino-acidorrhoea, although reduction in faecal fat by replacing long-chain triglyceride with medium-chain triglyceride and subsequently adding pancreatin did produce significant changes in some amino acids, which may reflect altered bacterial metabolism.

Direct evidence for the dietary origin of faecal amino acids in cystic fibrosis was obtained from investigations in which first-class protein was replaced by second class. Previous workers (Anfanger and Heavenrich, 1949; Christensen and Schwachman, 1949; Gould and Shwachman, 1956) showed that gelatin, in common with other proteins, is poorly absorbed in untreated cystic fibrosis. With the exception of alanine, proline, and hydroxyproline, the prompt changes in faecal amino acids were in the direction anticipated from the difference in composition of first-class protein (eg, casein) and gelatin. It is known that proline, hydroxyproline, and alanine occur in gelatin (collagen) in sequences such as Gly-Pro-Ala, Gly-Pro-Hyp, Gly-Pro-Gly, which are more resistant to degradation by the usual proteolytic enzymes (Grassmann, Hannig, and Schleyer, 1960; Grassman, Nordwig, and Hörmann, 1961) and this is the most likely explanation for the low values found for alanine and proline and the absence of hydroxyproline, rather than degradation of these amino acids.

The improvement in amino-acid pattern follow-

ing the introduction of an enzymic protein hydrolysate indicated that absorption of (free) amino acids in cystic fibrosis is not seriously impaired, and this is in agreement with earlier observations on the blood amino-acid response following loading tests of casein hydrolysates or glycine (West *et al.*, 1946; Anfanger and Heavenrich, 1949; Rossi and Menano, 1953).

Although the tryptic and chymotryptic activity of faeces from patients with cystic fibrosis not receiving pancreatin is normally negligible (Haverback, Dyce, Gutentag, and Montgomery, 1963) the evidence presented points to bacterial action on unabsorbed (partially digested) dietary protein as an important source of faecal amino acids in these patients. Many bacteria are capable of forming peptidases and proteinases which are more active against denatured proteins than against native proteins (Sokatch, 1969). However, attempts to sterilize the gut were not successful, so that direct evidence for bacterial proteolysis could not be obtained; neomycin cannot be used since it itself produces a malabsorption syndrome (Asatoor *et al.*, 1967).

Non-protein amino acids found in abnormal amounts in faecal specimens from children with cystic fibrosis are most likely derived by bacterial action from protein amino-acid precursors (Meister, 1965); β -alanine from aspartic acid (also found in babies by Bickel, 1964); γ -amino-n-butyric acid from glutamic acid (in babies, Bickel, 1964; in adults, Asatoor *et al.*, 1967) and first tentatively identified by (Ross 1951), and δ -amino-n-valeric acid (not previously reported) from lysine and proline (Rodwell, 1969). The identity of this last amino acid was confirmed by the procedures outlined in the section on methods. The diamines cadaverine (from lysine) and putrescine (from ornithine) were found in a number of specimens from patients with cystic fibrosis; cadaverine in trace amounts was found in a few normal adults and children in agreement with Abraham, Radonich, and Jones (1968), but putrescine was absent in all normal specimens.

Tyramine was a very common constituent in faecal specimens from children with cystic fibrosis, and is the source of urinary p-hydroxyphenylacetic acid in these patients (Gibbons *et al.*, 1967; Gjessing and Lindeman, 1967), but unexpectedly phenylethylamine and tryptamine were never found in any specimen, although many contained abnormal amounts of phenylalanine and tryptophan, and the tyrosine decarboxylase of *S. faecalis* has considerable phenylalanine decarboxylase activity (Meister, 1965).

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