ABSORPTION AND ELIMINATION OF ^{15}N AFTER ADMINISTRATION OF ISOTOPICALLY LABELLED YEAST PROTEIN AND YEAST PROTEIN HYDROLYSATE TO ADULT PATIENTS WITH COELIAC DISEASE

II. ELIMINATION OF ISOTOPE IN THE URINE AND FAECES

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Digestion in the normal human subject and the abnormalities in the enzymic hydrolysis and absorption of protein which are found in adult coeliac disease, as shown by investigations using ^{15}N -labelled protein and protein hydrolysate, were discussed in two previous papers (Crane and Neuberger, 1960a, 1960b). This communication is concerned with the excretion of isotopic nitrogen in the urine, the origin of faecal nitrogen in normal man and in certain cases of steatorrhoea, and the possible effects on the general nutrition of the patient of increased nitrogen excretion in the faeces. Analyses of urine and faeces were carried out on the four patients investigated in the first paper of this series (Crane and Neuberger, 1960b), but we include the results of another patient who suffered from adult coeliac disease and who excreted an unusually large amount of nitrogen in his faeces. To this patient ^{15}N -labelled glycine was administered and the effect on faecal nitrogen of varying the protein intake of the diet was examined.

RESULTS

Excretion of ^{15}N in the Urine

In three experiments, A_2 , C_2 , and J_1 (Table I), in which normal subjects were fed yeast protein equivalent to about 0.9 mg. of ^{15}N /kg. body weight, and allowed diets of their own choosing, the total ^{15}N eliminated in the urine during the subsequent three days was remarkably constant. Values expressed as percentages of the amounts of ^{15}N fed were 26.3, 26.6, and 27.4%. When labelled yeast protein hydrolysate was fed, equivalent to 0.83 mg. ^{15}N /kg. body weight in a similar experiment (C_3), the excretion was 28.1%, but in a second experiment (A_3) only 23.5% of the ^{15}N was excreted in the urine in three days. However, this subject fasted overnight and for the first 12 hours of the experiment, and 250 ml. of blood was withdrawn; hence it is possible that this lower value reflects some degree of nitrogen retention. Excretion was higher when labelled yeast protein was fed to two subjects placed on measured diets, adequate in protein but deficient in calories, and values for three-day periods were 30.0 and 31.9% of the ^{15}N fed (experiments A_1 and C_1).

Compared with the findings in normal subjects, the ^{15}N excreted in the urine after three days was much more variable when either whole-yeast protein or protein

hydrolysate was fed to patients with coeliac disease. Table I shows the results of seven of these experiments. After receiving protein three patients (experiments W_1 , K_1 , and H_1) excreted 9, 32, and 43% respectively less ^{15}N in the urine at the end of three days than did normal subjects on the average. The tendency of patients with coeliac disease to excrete only a relatively small proportion of the administered ^{15}N in the urine is also apparent in two out of three hydrolysate experiments shown in Table I.

TABLE I.—Elimination of ^{15}N in Urine and Faeces During Three Days After Feeding Labelled Yeast Protein and Protein Hydrolysate (Equivalent to 0.4–0.9 mg. ^{15}N /kg. Body Weight) to Normal Subjects and Patients with Adult Coeliac Disease. Values are Expressed as Percentages of the ^{15}N Administered

	Experiment	Urine	Faeces	Total	Average
<i>Protein</i>					
Normal subjects	A ₁	30.0	5.4	35.4	33.1
	A ₂	26.3	6.3	32.6	
	C ₁	31.9	3.6	35.5	
	C ₂	26.6	—	—	
	J ₁	27.4	1.5	28.9	
	WK	—	6.7	—	
Patients with adult coeliac disease	G ₁	32.2	11.2	43.4	33.3
	W ₁	24.4	9.2	33.6	
	K ₁	18.1	12.4	30.5	
	H ₁	15.2	10.2	25.4	
<i>Hydrolysate</i>					
Normal subjects	A ₃	23.5	4.7	28.2	31.1
	C ₃	28.1	6.0	34.1	
Patients with adult coeliac disease	W ₂	28.3	8.6	36.9	31.6
	H ₂	17.2	11.8	29.0	
	G ₂	20.4	8.6	29.0	

The ^{15}N elimination in two further experiments with a coeliac patient suggests that nitrogen metabolism in its quantitative aspects may vary considerably over a relatively short period of time. When yeast protein was given in experiment G_1 (Table I), the ^{15}N excreted was about 12% higher than normal—that is, 32.2% of the amount fed—but when the hydrolysate was substituted 12 days later (experiment G_2), the amount of ^{15}N was diminished, being about 70% of that excreted by the normal subject.

Excretion of ^{15}N in the Faeces of Normal Subjects

Compared with the excretion of ^{15}N in the urine after feeding labelled yeast protein to normal subjects, the amount of ^{15}N eliminated in the faeces at the end of three-day periods was much more variable. In four experiments, A_1 , A_2 , C_1 , and WK (Table I), when protein was fed equivalent to 0.4–0.9 mg. ^{15}N /kg. body weight, values were 5.4, 6.3, 3.6, and 6.7% of the amount fed. In two of these experiments where stool collections were continued up to six days, a further 1% only of the isotope was excreted. Fig. 1 and Table II show the results in detail of two of these experiments. The bulk of the ^{15}N was eliminated during the first 48 hours after feeding and the maximum isotope concentration appeared during this time. In three out of six experiments in which yeast protein or hydrolysate were fed to normal subjects, the maximum output of labelled nitrogen in the faeces occurred in the first 24 hours, while in the other three experiments the maximum output of the isotope was in the second 24-hour period. After the first 48 hours the proportion of ^{15}N eliminated in the faeces diminished rapidly and fell to a value between 0.1 and 0.2% of the dose on the sixth day, the isotope content remaining at 0.006 atoms% excess after the third day.

Fig. 1 and Table II also show the amounts of faecal nitrogen soluble in 5% trichloroacetic acid (T.C.A.) and

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the percentage of T.C.A.-soluble ^{15}N . In three experiments about one-third of the total nitrogen and of the ^{15}N were in this form, and thus the ^{15}N content of the T.C.A.-soluble nitrogen (Table II) was similar throughout to that of the total faecal nitrogen. In another experiment (J_1) only 3.5% of the total ^{15}N fed appeared in the faeces after six days. During the first two days the total nitrogen excreted was 1.5

FIG. 1.—After an overnight fast, a normal patient aged 42 was given 75.3 mg. of ^{15}N in the form of yeast protein (experiment A_1). Faeces were collected for six days and a normal diet was allowed throughout the period.

TABLE II.—A Normal Subject, W. K., Aged 62, was Given, After Fasting Overnight, Yeast Protein Containing 30.5 mg. of ^{15}N (0.41 mg. ^{15}N /kg. body weight) in 250 ml. of Water. He Was Allowed a Normal Diet, and Faeces Were Collected for Six Days

Time (Days)	Total Nitrogen			T.C.A.-soluble Nitrogen		
	N (mg.) a	^{15}N Atoms % Excess b	% ^{15}N given Eliminated/ Day $a \times b$ 30.5	N (mg.) c	^{15}N Atoms % Excess d	% ^{15}N given Eliminated/ Day $c \times d$ 30.5
1	1,980	0.030	1.93	650	0.036	0.77
2	3,330	0.041	4.50	1,200	0.038	1.50
3	1,350	0.006	0.28	490	0.008	0.13
			6.71			2.40
4	2,180	0.006	0.45	898	0.005	0.16
5	1,605	0.006	0.34	732	0.006	0.15
6	1,082	0.006	0.22	450	0.006	0.09
	Total		7.72	Total		2.80

and 2.2 g., but the ^{15}N contents were only 0.004 and 0.002 atoms% excess and the percentages of isotope excreted only 0.1% and 0.08% of the amounts fed. The maximum ^{15}N content did not appear until the fifth day. Although the isotope contents of the T.C.A.-soluble nitrogen and total nitrogen were similar, a smaller percentage (20%) of the nitrogen was soluble in T.C.A.

We must comment briefly on the significance of the low ^{15}N value obtained in faecal specimens in some of the experiments reported here (Tables II-VI). The reproducibility of the figures for atoms% ^{15}N with the mass spectrometer used and the range of values under consideration is about ± 0.002 to ± 0.003 atoms% excess. It has been suggested (Sharp *et al.*, 1956) that the nitrogen obtained from biological material may be

enriched with respect to ^{15}N up to about 0.006 atoms% excess if compared with gaseous nitrogen obtained from a commercial source. Our blank values were obtained with analytically pure ammonium sulphate which was found by Sharp *et al.* to have on the average 0.0048% more ^{15}N than the commercial gaseous nitrogen. We have therefore assumed that values for ^{15}N of 0.004% or less are probably not significant, but that figures lying between 0.005 and 0.015 are significant but may be in error by a considerable amount, depending on the abundance of ^{15}N found.

After feeding the enzymic hydrolysate to two normal subjects, the amounts of ^{15}N excreted after three days were 4.7 and 6.0% (experiments A_3 and C_3 , Table I) of the amounts fed, and Table III gives the results in detail

TABLE III.—Labelled Yeast Protein Hydrolysate, Containing 50.4 mg. of ^{15}N and Equivalent to 0.6 mg. ^{15}N /kg. was Given to a Normal Male Subject Weighing 83 kg. After Fasting for 10 Hours (Experiment A_4). A Normal Diet was Given

Time (Days)	Total Nitrogen			T.C.A.-soluble Nitrogen		
	N (mg.) a	^{15}N Atoms % Excess b	% ^{15}N given Eliminated/ Day $a \times b$ 50.4	N (mg.) c	^{15}N Atoms % Excess d	% ^{15}N given Eliminated/ Day $c \times d$ 50.4
1	2,540	0.035	1.77	650	0.054	0.69
2	2,000	0.059	2.33	525	0.083	0.87
3	980	0.032	0.6	334	0.038	0.25
			4.70			1.81

of one of these experiments. As in the previous studies with whole-yeast protein, the bulk of the ^{15}N was eliminated in the first two days. A most significant finding from these two experiments was that the proportions of T.C.A.-soluble nitrogen and T.C.A.-soluble ^{15}N were, as for the protein experiments, about one-third of the total nitrogen and ^{15}N excreted. Thus it appears that the isotope is incorporated and eliminated to a very large extent as faecal protein, irrespective of whether insoluble whole-yeast protein or water-soluble protein hydrolysate consisting mainly of amino-acids is fed.

Excretion of ^{15}N in Faeces of Adult Patients with Coeliac Disease

As can be seen from Table I of the first paper of this series (Crane and Neuberger, 1960b) the average values for the daily nitrogen excretion in the faeces in all four patients were in excess of normal figures, such as those given by Cooke *et al.* (1953), but day-to-day variations in the same patient were considerable (Tables IV *et seq.*).

TABLE IV.—23.6 mg. of ^{15}N in the Form of Yeast Protein (0.4 mg./kg. Body Weight) was Given to a Patient Aged 67 Years With Adult Coeliac Disease, After Fasting Overnight (Experiment H.). Faeces were Collected for Six Days, and a Normal Ward Diet was Allowed throughout this Period

Time (Days)	Total Nitrogen			T.C.A.-soluble Nitrogen		
	Total N (mg.) a	^{15}N Atoms % Excess b	% ^{15}N given Eliminated/ Day $a \times b$ 23.6	Total N (mg.) c	^{15}N Atoms % Excess d	% ^{15}N given Eliminated/ Day $c \times d$ 23.6
1	3,480	0.030	4.35	1,300	0.036	2.00
2	3,140	0.032	4.30	1,090	0.033	1.50
3	1,770	0.020	1.50	1,040	0.019	0.85
			10.15			4.35
4	4,730	0.013	2.50	1,750	0.012	0.90
5	1,760	0.009	0.70	691	0.009	0.27
6	3,900	0.009	1.50	1,450	0.009	0.56
	Total		14.85	Total		6.08

When labelled yeast protein equivalent to 0.4 mg. ^{15}N /kg. body weight was fed to patients with adult coeliac disease, the amounts of isotope eliminated at the end of three- and six-day periods as percentages of the amounts given were much greater than for normal subjects (Table I). Table IV gives the results of one of these experiments. The percentage of ^{15}N excreted in three days was 10.2, and in three further experiments, W_1 , G_1 , and K_1 (Table I), values were 9.2, 11.2, and 12.4. In the experiment outlined in Table IV and in two other experiments, the stools were collected and analysed for a further three days, during which time a further 4.8, 2.4, and 9.4% of the administered dose were eliminated. As in the previous experiments, the bulk of the isotope appeared in the faeces collected during the first two days, but Table IV and Fig. 2 emphasize that the quantity of

TABLE V.—26 mg. of ^{15}N was Given in the Form of Yeast Protein Hydrolysate to a Patient, H. G., Who was Suffering from Coeliac Disease. Faeces were Collected for Six Days and a Normal Diet was Allowed

Time (Days)	Total Nitrogen			T.C.A.-soluble Nitrogen		
	Total N (mg.) a	^{15}N Atoms % Excess b	% ^{15}N given Eliminated/ Day $\frac{a \times b}{26}$	Total N (mg.) c	^{15}N Atoms % Excess d	% ^{15}N given Eliminated/ Day $\frac{c \times d}{26}$
1	6,350	0.031	7.60	2,580	0.046	4.50
2	3,660	0.025	3.40	1,045	0.030	1.20
3	1,039	0.020	0.80	356	0.018	0.25
			11.80			5.95
4	5,280	0.012	2.40	2,000	0.010	0.73
5	5,710	0.010	2.10	2,080	0.006	0.48
6	1,145	0.010	0.40	498	0.006	0.11
			Total 16.70			Total 7.27

FIG. 2.—A patient aged 61 and weighing 56 kg. was given yeast protein equivalent to 0.4 mg. of ^{15}N /kg. body weight (experiment W_1). Faeces were collected on the following six days and during this time a normal ward diet was allowed. No faeces were passed during the first day of this experiment.

isotope excreted after the third day of each experiment was considerable, and this is in marked contrast to the findings in most normal subjects. The ^{15}N contents were, however, slightly higher than those obtained in the experiments with normal subjects, but the proportions of T.C.A.-soluble nitrogen and ^{15}N were each about one-third respectively of the total nitrogen and ^{15}N excreted in two experiments, and thus the same as in the previous experiments with normals. In general, the pattern of the nitrogen excretion, and hence the ^{15}N , followed that of the fat, and this is shown in Fig. 2.

When the protein hydrolysate was fed to three patients in the same proportion of ^{15}N to body weight, the quantity of ^{15}N excreted in the faeces in the first three-day period was about the same as when the whole protein was given. Values were 8.6, 8.6, and 11.8% of the quantities fed (experiments W_2 , G_2 , and H_2), and, as in the previous experiments, the bulk of the isotope was eliminated during the first two days. In one experiment a further 4.9% was excreted when the collection was prolonged to six days, and details are given in Table V. In this experiment the proportions of T.C.A.-soluble nitrogen to total nitrogen and T.C.A.-soluble ^{15}N to total ^{15}N were slightly higher than in the previous

experiments, and were about 40%. These slightly higher proportions were also found in a second experiment where faecal collections were made for three days only.

These results show that patients with coeliac disease excrete in the faeces a much larger proportion of the administered ^{15}N compared with normal subjects, and the figures are similar when either the whole-yeast protein or the protein hydrolysate is fed. Moreover, in the four experiments where the stools were analysed for T.C.A.-soluble nitrogen it was found that, irrespective of the type of material fed, 60% or more of the isotope was found to be eliminated in a form insoluble in T.C.A.

Table I also shows that the total amount of ^{15}N eliminated, expressed as a percentage of the ^{15}N of labelled protein or hydrolysate administered, is more or less the same in patients with steatorrhoea and in normal persons. But the latter excrete during the three days after the administration of the labelled material four to ten times as much of the ^{15}N in the urine as in the faeces; in coeliac patients the ratio varies between 1.5 and 3.5.

Excretion of Isotope from Feeding ^{15}N -labelled Protein to a Coeliac Patient in Remission After a Gluten-free Diet

In Part I of this investigation the absorption of ^{15}N -labelled yeast protein was found to be normal in a patient, B. K., who made a rapid clinical improvement 14 days after recommencing a gluten-free diet. She had abandoned the diet some months prior to admission, in consequence of which the fatty diarrhoea returned; there was rapid loss of weight, hypoalbuminaemia, and

TABLE VI.—In Both Experiments Patient B. K. Received 18 mg. of ^{15}N in the Form of Isotopically Labelled Yeast Protein After an Overnight Fast. Further Details are Given in the Text

Day	Total Fat (g.)	Total N (g.) a	^{15}N Atoms % Excess b	% ^{15}N given Eliminated/Day $\frac{a \times b}{18.00}$
A. Findings during Remission				
1	0	0	0	0
2	11.2	2.15	0.016	1.90
3	7.3	1.31	0.006	0.40
4	13.6	2.46	0.033	4.50
				6.80
B. Findings during Relapse				
1	3.7	1.24	0.077	1.15
2	28.0	4.52	0.039	9.70
3	13.8	2.04	0.013	1.47
				12.32

peripheral oedema. Stools were collected for four days after the patient took the labelled yeast protein since she was constipated during the first day, but up to this time she had her bowels open once or twice a day. The results of the faecal analyses from the experiment are set out in Table VI A, and the findings from a similar experiment carried out some eight months previously, when the patient was in relapse and was taking a normal diet, are set out in Table VI B. Protein was supplied equivalent to 0.4 mg. ^{15}N /kg. body weight. It appears that over these short periods of time there was but slight reduction in the quantities of fat and nitrogen excreted when the patient was put on to a gluten-free diet, but the ^{15}N contents of the nitrogen were lowered during the gluten-free period, and thus the quantity of isotope eliminated was less and within the limits found for normal subjects.

Results Obtained on Administering Diets of Varying Nitrogen Content and ^{15}N Glycine to a Patient Suffering from Coeliac Disease

We wish to record the results of an earlier investigation of a patient with coeliac disease who had an abnormally high excretion of nitrogen in the faeces.

Case History

R., a man aged 45, a presumptive coeliac in childhood, had a sudden attack of diarrhoea with bulky fatty stools in 1940, when 30 years old and while serving in the Army. The diarrhoea occurred about once a year until 1951, when he was admitted to hospital with a myocardial infarct, after which the diarrhoea became persistent and was followed by severe loss of weight, ankle oedema, glossitis, anaemia, and tetany.

From 1952 until his final admission in 1955 he was treated in hospital on several occasions with folic acid, vitamins, and low-fat and gluten-free diet, but relapses were frequent. On admission in December, 1955, he was wasted, pale, apathetic, with a large protuberant abdomen. His fingers were clubbed and his tongue was smooth. His blood-pressure was 105/60, and there was oedema of the legs and ascites. A barium-meal examination showed loss of normal pattern of the small intestine, and faecal fat was 30% by weight of a dried 24-hour specimen. The haemoglobin was 65%, serum proteins 3.8 g./100 ml. (albumin 1.0 g./100 ml.), and serum calcium was 8.3 mg./100 ml. On duodenal intubation and stimulation of pancreatic secretion with secretin, aspirated juice gave normal values for diastase and lipase, and values near the lower limit of normal for trypsin. He improved slightly on reconstituting the gluten-free regime and cortisone, and, later, attempts were made to improve his nutrition with high-protein feeding (see below).

After a period of steady deterioration his diarrhoea suddenly became very much worse and was accompanied by a swinging temperature and severe abdominal pain; later there was marked abdominal distension. He became electrolyte-depleted, with plasma Na^+ 121 and Cl^- 82 mEq/l., and 24 hours before death his blood-pressure fell to 75/40.

Post-mortem examination showed severe atheroma, narrowing of the anterior descending branch of the left coronary artery, and ulcerated plaques in the abdominal aorta. There were pleural adhesions at the right apex of the lung, with some tuberculous cavitation, and at the left base an area of tuberculous bronchopneumonia. The pleural cavity contained a quantity of turbid exudate. The small intestine was grossly distended and contained altered blood, and there were a number of shallow ulcers scattered throughout the small gut. The pancreas was normal.

Nitrogen Balance and Faecal Nitrogen.—As Table VII shows, during a six-day period, when the daily calculated dietary intake of nitrogen was 12.5 g. (supplied as normal

TABLE VII.—Patient R, With Adult Coeliac Disease, Received Protein Equivalent to 78 and 175 g./day for Two Periods of Six Days, During Which Time all Urine and Faeces were Collected. A Three-day Equilibrium Period was Allowed in Each Experiment. A Further Experiment was Carried Out in Which the Nitrogen was Supplied as 26 g. of Protein and 59 g. of Protein Hydrolysate

Period (3 Days)	N Intake (g./Day)	Average Daily N Output (g./Day)			Balance (g. N/Day)
		Urine	Faeces	Total	
78 g. of Protein Fed Daily					
1	12.5	11.3	5.6	16.9	-4.4
2	12.5	10.5	5.6	16.1	-3.6
175 g. of Protein Fed Daily					
1	28.0	16.1	12.6	28.7	-0.7
2	28.0	16.8	12.6	29.4	-1.4
26 g. of Protein and 59 g. of Hydrolysate Fed Daily					
1	11.0	9.3	4.1	13.4	-
2	11.0	9.5	4.1	13.6	-2.6

food), the patient was in negative nitrogen balance to the extent of 4 g./day and his average daily faecal nitrogen excretion amounted to 5.6 g., which was equivalent to 34% of the total nitrogen excreted. When the nitrogen intake was increased to 28 g./day his output also increased, but the nitrogen balance improved and, allowing for analytical errors, approached zero. However, the proportion of nitrogen excreted in the faeces increased to between 42 and 43% of the total output, and during the six-day period the average faecal nitrogen excretion was 12.6 g.; this is approximately the urinary nitrogen output of a normal subject. During the third six-day period the patient was given a diet consisting of 26 g. of protein and 59 g. of protein hydrolysate (as "nesmeida"), making a total daily nitrogen intake of 11 g. During this period the patient excreted in his faeces an average of 30% of the total nitrogen eliminated from the body, and the nitrogen balance became negative but not so markedly as during the first feeding period.

Administration of ^{15}N Glycine.—The patient was given 475 mg. of ^{15}N -labelled glycine containing 31.44 atoms% excess ^{15}N immediately before breakfast and after an overnight fast. Urine was collected for 48 hours and faeces for three days, and a diet of his own choosing was allowed throughout this period. The isotope contents of the total nitrogen, urea, and ammonia in the urine were measured by methods already referred to. The results are not given in detail, but the following points may be mentioned. The patient excreted in the urine during the first 48 hours 26.7% of the total isotope administered, and the maximum ^{15}N concentration was found in the urea during the second three-hour period (three to six hours) after administration, and was 0.080 atoms% excess, while the isotope concentration of the ammonia was higher in the first three-hour period and was 0.121 atoms% excess. The isotope results relating to the faeces are given in Table VIII. The ^{15}N concentrations found in the faeces were lower than those found later in experiments when labelled protein was fed. For reasons which have been mentioned above, values for ^{15}N less than 0.004 atoms% excess are of doubtful value; the figures given in Table VIII are between two and four times this value,

TABLE VIII.—475 mg. of Labelled Glycine (0.6 mg. ^{15}N /kg.) was Given to a Fasting Coeliac Patient, Who had Breakfast Immediately Afterwards. The Patient was Allowed a Normal Ward Diet, and Faeces were Collected for Three Days

Time (Days)	Total Faecal Nitrogen			T.C.A.-soluble Faecal Nitrogen		
	Total N (mg.) a	^{15}N Atoms % Excess b	% ^{15}N given Eliminated/Day $a \times b$ 28.4	Total N (mg.) c	^{15}N Atoms % Excess d	% ^{15}N given Eliminated/Day $c \times d$ 28.4
1	2,680	0.008	0.75	700	0.011	0.27
2	3,680	0.015	1.94	1,190	0.011	0.46
3	8,640	0.013	3.96	2,380	0.015	1.26
			6.65			1.99

but the accuracy is not very high. However, it is concluded that some labelled nitrogen was excreted in the faeces.

Again we found, as in the protein experiments described above, that the ^{15}N content of the T.C.A.-soluble fraction of the faeces was approximately the same as that of the total nitrogen of the faeces. As the total proportion of the T.C.A.-insoluble protein was again about two-thirds of the total faecal nitrogen, it would appear that two-thirds of the labelled nitrogen which was ingested in the form of free glycine was incorporated into more complex structures, probably protein.

DISCUSSION

Origin of Faecal Nitrogen in Normal Man

Daily faecal nitrogen values in normal man on diets consumed in western countries are reported to average between 1 and 2 g./day (Cooke *et al.*, 1953; Wollaeger *et al.*, 1947), but the amount eliminated varies to a large extent with the diet (Lusk, 1928). A large part of this nitrogen must be derived from sources other than the diet. This follows from the fact that on prolonged starvation or complete deprivation of dietary protein, faecal nitrogen is never reduced to zero, and values varying between 0.1 and 0.8 g./day have been reported under such conditions (Mendel and Fine, 1912; Lusk, 1928). F. Voit (1892) and C. Voit (1894) were probably the first to suggest that the bulk of the faecal nitrogen was derived from endogenous sources on the basis of starvation experiments in dogs and also of investigations using a closed isolated loop of the intestine. The suggestion that large amounts of protein, calculated to be equivalent to about one-third of the dietary daily nitrogen intake, are excreted into the small intestine was made by Mosenthal (1911), who used dogs prepared with Thiery-Vella fistulae.

More recent work allows us to be more specific about the origin of faecal nitrogen. There are no reliable quantitative figures available of the amounts of protein which enter the alimentary canal every day in the form of saliva, gastric juice, pancreatic secretion, and succus entericus. Reference has already been made to the calculations of Mosenthal about the protein production of the gut wall, and the work of Borgström *et al.* (1957) has indicated surprisingly high concentrations of trypsin, chymotrypsin, and amylase in the small intestine. In addition, desquamation of the epithelial cells of the villi occurs at a rapid rate (Leblond and Stevens, 1948; McMinn, 1954), and in the species investigated these cells are renewed on the average every one and a half days. It has also been found (Walser and Bodenlos, 1959) that a considerable amount of urea, equivalent to at least 25% of that synthesized per day, is excreted into the gut. Amino-acids may also pass back into the lumen of the intestine, but, so far as we know, this has not been demonstrated.

Cope and Goadby (1935) suggested that in some cases of hypoalbuminaemia without demonstrable causes there may be an increased rate of destruction of albumin. Quantitative studies on the metabolism of plasma proteins in idiopathic hypoproteinaemia were later carried out by Albright *et al.* (1950) and by Schwartz and Thomsen (1957), the latter using ^{131}I -labelled albumin. Hypoalbuminaemia appears to be associated in some cases with a "hypertrophic" gastritis (Citrin *et al.*, 1957), and Gordon (1959), using ^{131}I -labelled polyvinylpyrrolidone, produced strong suggestive evidence that the increased loss of albumin was caused by extensive leakage of the protein into the gut. It is still uncertain whether this represents an exaggeration of a normal

process, but Holman *et al.* (1959) demonstrated by an immunological technique (Ouchterlony) the presence of albumin and γ -globulin in the intestinal contents of normal persons. It is thus possible that, as suggested by Holman *et al.*, digestion by intestinal enzymes is a mechanism of plasma protein katabolism in the normal subject.

Nasset (for review, see Nasset, 1957) has carried out extensive investigations on digestion in animals, and concluded that the nitrogen content of the chyme is largely independent of dietary protein intake. Although his quantitative data have been criticized by Geiger *et al.* (1958), the general thesis that nitrogenous compounds in the intestine represent a mixture of endogenous and dietary protein is well established. It seems likely that the amount of nitrogen in the form of protein or compounds of low molecular weight delivered into the lumen of the gut is equal to or even greater than that represented by the dietary intake. The two components blend, and in the normal subject about 95% of the total nitrogenous material is hydrolysed and absorbed or reabsorbed by the mucosa.

The results obtained in the present work are in accordance with this concept. In almost all our normal subjects receiving ^{15}N -labelled protein, very little of the label was excreted in the faeces after the third day. If the faecal nitrogen were derived exclusively from dietary protein and originated to an equal extent from all nitrogenous substances of the food, we should be able to predict the average isotope concentration of the faecal nitrogen by dividing the amount of ^{15}N given by the total nitrogen ingested over the period in question. For example, in one experiment a normal subject was given 75 mg. of ^{15}N (isotope excess), and during the next three days the total nitrogen intake was 47 g. The expected average isotope content in the faecal nitrogen is thus 0.157 atoms% excess. The total faecal nitrogen over this period was 3.8 g. and the total ^{15}N excreted in the stools was 4.02 mg. (^{15}N excess). The average observed isotope content was therefore 0.106 atoms% excess—that is, about 65% of the expected value. Calculations for three-day periods in two similar experiments were 0.180% (calculated), 0.094% (observed), and 0.145% (calculated) and 0.079% (observed). When similar calculations were made for shorter or longer periods than three days, the differences between observed and calculated values were much greater. While these calculations are somewhat crude, it is nevertheless, we believe, permissible to conclude that a source other than the diet makes a large contribution to the faecal nitrogen.

Similar results were obtained in experiments where protein hydrolysate was given to two normal subjects. Thus in one experiment 63.6 mg. of ^{15}N (atoms excess) was given to a subject with a nitrogen intake of 14 g./day. The faecal excretion of total nitrogen in the following three-day period amounted to 5.22 g. and the ^{15}N to 4.1 mg. The calculated value on the basis of the assumptions stated above was 0.163 atoms% excess, while the observed value was 0.078—a difference similar to those observed for whole protein. If incomplete hydrolysis of ingested whole protein had been mainly responsible for the faecal nitrogen one would have expected that administration of ^{15}N in the form of soluble amino-acids would result in a much lower labelling of the faecal nitrogen. It is therefore assumed that, in addition to the contribution made by dietary nitrogen, part of the labelling of faecal nitrogen is the result of the sequence of processes consisting of absorption of labelled

amino-acids and peptides by the mucosa, the participation of those substances in anabolic and katabolic processes within the body, followed by secretion or elimination of urea or proteins and other substances into the gut, as discussed above.

Further support for the concept that faecal nitrogen is derived both from endogenous and from exogenous sources comes from the demonstration that the concentrations of ^{15}N in the T.C.A.-soluble fraction and T.C.A.-insoluble fraction are identical (see Tables, III, IV, and V). In fact, the identity of ^{15}N contents was observed in experiments in which whole protein and labelled hydrolysate were used. This result would not be expected if faecal nitrogen represented mainly unhydrolysed, unabsorbed dietary protein. The similarity of the labelling of total faecal nitrogen and T.C.A.-soluble nitrogen was also observed after administration of [^{15}N] glycine to a coeliac patient (Table VIII). While incorporation of amino-acids into protein may arise from microbial action, it would seem more likely that this result is explicable by an anabolic process occurring in tissues outside the lumen of the intestinal tract.

Source of Faecal Nitrogen in Adult Coeliac Disease

Since the patients with coeliac disease were not on measured diets during the experiments, the approximate nitrogen intake was calculated from the sum of the urinary and faecal nitrogen. For two three-day periods in two different experiments, the isotope contents expected on the basis of an exclusive dietary origin of faecal nitrogen were 0.084 and 0.058 atoms% excess when whole protein was fed, while the dilutions observed in the faeces were 0.029 and 0.019% respectively. In a further experiment when the hydrolysate was fed, the corresponding figures were 0.058 (calculated) and 0.019% (observed).

It would seem clear, therefore, that the greatly increased faecal nitrogen is, as in the normal, largely of endogenous origin. This conclusion was reached many years ago by McCrudden and Fales (1912, 1913) on the basis of the finding that the amount of nitrogen excreted in the faeces of children with coeliac disease was largely independent of dietary intake. In one experiment recorded here (Table VII) a patient was fed 175 g. of protein per day over six days, and excreted in the faeces on the average 12.6 g. of nitrogen per day. The nature of this nitrogen was not determined, but the suggestion is that in this patient faecal nitrogen was not completely independent of dietary intake.

The question arises regarding the cause of the increased faecal nitrogen found in most patients with adult coeliac disease. One possibility is that absorption of nitrogen in coeliac disease is deficient, as already shown (Crane and Neuberger, 1960b). As the amount of faecal nitrogen is determined by the rates of two normally balanced opposing reactions which are (a) the addition of protein and other nitrogenous constituents to the intestinal "pool," and (b) the absorption or reabsorption of nitrogenous compounds by the mucosa, it follows that defective absorption will automatically lead to an increased elimination of nitrogen in the faeces. The other possibility is that, owing to the disorganization of the mucosal surface, increased leakage of urea, amino-acids, or proteins into the lumen of the gut takes place and a reduced rate of absorption is unable to cope with this situation. We have no evidence on this point, but it is quite possible, and indeed it has been suggested by Schwartz and Jarnum (1959), that the rate of destruction

of plasma protein in the gut in steatorrhoea may be greatly increased. If this hypothesis is correct this would explain the low plasma-protein values found in many cases of steatorrhoea.

In one experiment the amino-acid composition of the faecal protein was examined chromatographically, and it could be shown that essential amino-acids were present in quantities characteristic of protein of high nutritive value. The continuous protein loss occurring in the faeces, especially of protein of high biological value, may be an important factor in causing the protein deficiency observed in these patients, particularly when it is remembered that, apart from nitrogen, there may be a loss of other foodstuffs such as non-absorbed or excreted faecal fat, deficient absorption of mineral elements such as potassium, calcium, and iron, and of vitamins such as folic acid, fat-soluble vitamins, and vitamin B₁₂. A normal person can adapt himself to a daily dietary protein allowance of 50 g., and the effective intake of a patient suffering from coeliac disease, excreting relatively large amounts of nitrogen in his faeces, is generally not smaller than this figure. It is likely that it is the combination of protein loss with a caloric deficiency and defective absorption of essential nutrients which causes the inanition and reduction in body protein which are so often found in the chronic stages of coeliac disease. It is also possible that reduced caloric intake, together with the more specific dietary deficiencies, prevents the adaptation to low nitrogen intake to be made which can be performed by the normal subject.

SUMMARY

Yeast protein labelled with the heavy nitrogen isotope ^{15}N was fed to normal individuals, and the total excretion of labelled nitrogen in the urine and in the faeces was measured over varying periods of time. The faecal nitrogen was also partitioned into protein N (T.C.A.-insoluble) and non-protein N (T.C.A.-soluble). Enzymic hydrolysates consisting mainly of amino-acids of the labelled protein were also given. The labelling of the faecal nitrogen was found to be less than would have been expected if it were to consist entirely of unhydrolysed dietary protein. Moreover, the extent of labelling and the distribution of the labelled nitrogen between T.C.A.-soluble and T.C.A.-insoluble fractions were identical in the experiments in which labelled protein and labelled protein hydrolysate were given. It is concluded that a large part, possibly more than half, of the faecal nitrogen is derived from endogenous sources such as gastric juice, pancreatic secretion, succus entericus, urea, and other compounds, possibly even including plasma proteins which have leaked out from the systemic circulation through the gut wall.

The total nitrogen in the faeces was increased in all the patients with coeliac disease examined, and the value for the isotope eliminated through faeces was also greatly enhanced. The general pattern of labelling suggested that, as in the normal, a large part of the faecal nitrogen was endogenous. In one patient faecal nitrogen was particularly high on a normal diet, and on increasing protein intake an average daily nitrogen excretion of over 12 g. was recorded. The possibility is discussed that in this and other cases the abnormally high nitrogen excretion may be due to a combination of deficient absorption or reabsorption and a leakage of endogenous constituents ultimately derived from the plasma. It is suggested that this loss of nitrogen through the faeces

adds considerably to the nutritional deficiency caused by loss of calories and defective absorption of mineral elements and vitamins, which are known to be associated with steatorrhoea of long standing.

We wish to express our gratitude to Sir Charles Harington, F.R.S., and to Dr. A. S. McFarlane for permission to use the mass spectrometer at the National Institute for Medical Research, Mill Hill, and to Mr. G. Dickenson for valuable co-operation in carrying out the very large number of determinations of ^{15}N . We thank Dr. Margot Shiner, of the Gastroenterological Unit, the Central Middlesex Hospital, for performing jejunal biopsies on the patients and for the estimation of duodenal enzymes; Dr. E. Miller of the Distillers Co. Ltd., Great Burgh, Epsom, Surrey, for gifts of yeast cultures; and Miss Sheila Wilkie, late dietitian of St. Mary's Hospital, for supervising the diets of one patient. Our thanks are also due to Professor W. S. Peart, Dr. W. D. W. Brooks, and Dr. T. A. Kemp, of St. Mary's Hospital, and Dr. F. Avery Jones, of the Gastroenterological Unit, the Central Middlesex Hospital, for permission to study patients under their care. We are also indebted to the Board of Governors of St. Mary's Hospital for a grant from endowment funds for the purchase of ^{15}N . Finally, one of us (C.W.C.) wishes to thank the British Medical Association for the award of a scientific scholarship during 1957-8 when a part of this work was carried out.

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Dr. F. TROSS, leader of the World Health Organization's yaws control team in the South Pacific, has made a three-month visit to the Cook Islands, where he conducted a survey and trained staff in the eradication of yaws. Dr. Tross has been working since 1954 in co-operation with local governments in South Pacific territories in a campaign against this disease. It involved the mass treatment of the entire indigenous population of these territories with penicillin, followed by several extensive surveys. As a result the incidence of yaws in Fiji, Western Samoa, British Solomon Islands, Gilbert and Ellice Islands, and the New Hebrides has dropped from an average of 15% to a few residual cases. (N.Z. Release No. A26/60.)

CORRELATION OF RADIOACTIVE AND CHEMICAL FAECAL FAT IN DIFFERENT MALABSORPTION SYNDROMES*

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Many methods of studying fat-absorption from the gastro-intestinal tract have been used. The most accurate method of quantitatively determining faulty assimilation of fat and protein is the balance technique accurately calculating the components in ingested food and chemically determining the residue of the stools (Cooke *et al.*, 1946, 1953; Wollaeger *et al.*, 1947; Annegers *et al.*, 1948; Comfort *et al.*, 1953; Weijers and van de Kamer, 1953; Frazer, 1955; French, 1955; Crowe and Blackburn, 1956; Comfort, 1957; Kalser, 1957). This method has certain disadvantages as a routine clinical diagnostic aid in that it is time-consuming, laborious, and expensive, requiring a metabolic ward and three to five days for the collection of stools. Such facilities are seldom available in the average hospital.

Since the advent of radioisotopes, particularly the ^{131}I labelled fats, the absorption tests in humans are becoming much more common. These tests are relatively simple to perform and may be carried out in any radioisotope laboratory. They do not require the special facilities needed for faecal chemical balance study.

In view of the simplicity of ^{131}I labelled fat studies as against the time-consuming, expensive, and laborious technique of faecal chemical-balance studies, it is important that controlled studies to correlate these two types of tests be done in the same subjects. No such studies to our knowledge were published until those of Grossman and Jordan (1958).

It is the purpose of this project to compare in a large series the accuracy of radioactive ^{131}I labelled triolein studies with faecal chemical intake-excretion studies as a diagnostic aid in the various malabsorption syndromes.

Methods

Twenty-four normal volunteers consisting of members of the house staff and laboratory personnel were studied as normal controls.

*This study was supported in part by U.S. Public Health Service Research Grant A2574, by the Julia Kveim Fund of the University of Pennsylvania, and by the Bockus Research Fund.