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

I. RATE OF ABSORPTION OF ^{15}N YEAST PROTEIN AND YEAST PROTEIN HYDROLYSATE

BY

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Coeliac disease in the adult (idiopathic steatorrhoea; non-tropical sprue) characterized by persistent ill-health, diarrhoea with bulky fatty stools, loss of weight, fatigue, anaemia, and, on occasion, vitamin deficiencies and disturbances of calcium metabolism, is generally regarded as one of the forms of the "malabsorption syndrome." In the chronic untreated patient severe malnutrition may eventually result, with pronounced loss of weight up to 30–40% of normal, inanition, low plasma proteins, and oedema of the legs. This picture could arise from the continued loss of calories from the body in the form of large amounts of faecal fat and nitrogen combined with a low dietary intake, but many studies indicate that in the majority of such cases the appetite of the patients is often excessive and that a more than adequate dietary intake of foodstuffs is maintained. A large amount of work has been done on the excretion of fat in the faeces, but it has only recently been appreciated that in this disease large faecal losses of nitrogen may accompany the steatorrhoea. Thaysen (1932), who was among the first to report detailed investigations on a series of these patients, although aware of the magnitude of the nutritional disturbances which sometimes occurred, nevertheless thought that the faecal excretion of nitrogen was but seldom greater than in normal subjects. In 1944 Bockus wrote that there was "rarely any marked increase in faecal nitrogen excretion since there is no defect in the splitting of protein."

Cooke *et al.* (1953b) and Taylor *et al.* (1952), on the other hand, investigated a large number of patients and found that faecal nitrogen losses were often as high as those encountered in patients with pancreatic steatorrhoea. Though it is implied by many workers that this increased nitrogen is derived, at least in part, from dietary sources through failure of intestinal absorption, this has never been satisfactorily demonstrated. Tests designed to assess intestinal absorption of amino-acids or protein in patients with adult coeliac disease have not been widely used. Anfanger and Heavenrich (1949) used both gelatin and glycine to investigate absorption in children in the coeliac disease; Erf and Rhoads (1940) and Butterworth *et al.* (1958) used a glycine-absorption test in patients with sprue, while Saint and Weiden (1952) investigated several cases of steatorrhoea, including two of adult coeliac disease, by a similar technique.

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The rise in concentration of amino-nitrogen or that of a particular amino-acid in the venous plasma after the ingestion of amino-acids or protein depends upon many factors other than absorption from the intestine as discussed by Fisher (1954), and in the presence of considerable disturbances of nitrogen balance these measurements may not give reliable indications of the rates of absorption. In the case of amino-acids, moreover, the quantities which need to be administered in order to achieve a measurable change of concentration in the blood are often non-physiological and may represent several times the amount of that amino-acid normally absorbed by the body over a whole day. Hence the way in which this large amount is dealt with by the body may differ greatly from normal. Other tests involving the use of ^{35}S-methionine and ^{131}I-labelled albumin have not been reported in investigations of this condition. To overcome some of these difficulties, and especially in view of the possible risks entailed in the frequent use of proteins labelled with a radioactive isotope, it occurred to us that ^{15}N-labelled protein might be used with advantage in studying protein absorption from the gut. ^{15}N-labelled amino-acids, ammonia, and urea have all been used in man, particularly by workers in the United States, but there are only a few published reports of the metabolism of proteins labelled with a stable isotope—namely, White and Parson (1950) and Sharp *et al.* (1956).

In a previous paper (Crane and Neuberger, 1960) we have shown that the absorption of ^{15}N-labelled yeast protein and protein hydrolysate may be studied in normal human subjects most conveniently by measuring the changes in isotope content of both ammonia and urea present in samples of urine passed at frequent intervals after ingestion, and that estimations of ^{15}N in amino-nitrogen and urea obtained from samples of venous blood offered no great advantage in precision over the technically much simpler analysis of the urine. The time relation for both urinary ammonia ^{15}N and plasma amino-acid ^{15}N were similar although the values of the ammonia were higher, while the corresponding figures for urinary and blood urea were almost identical. We assume that the urinary ammonia is derived to some extent from all the amino-acids liberated from the protein whether by oxidative deamination in the kidney or by a preliminary transfer of the amino-nitrogen to the amide group of glutamine within the liver.

The present paper records our findings of the rates of absorption after feeding whole-yeast protein and hydrolysate labelled with ^{15}N to four adult patients suffering from coeliac disease, and we have assumed that metabolism proceeds along the same pathways and at the same rates as in normal subjects once the nitrogen is absorbed into the body.

Clinical Histories

Of the four patients investigated (Table I), three were untreated men; the fourth, a woman, was in relapse after a period of treatment with a gluten-free diet. Their ages ranged from 27 to 67 years. All gave histories of chronic ill-health over long periods of time, 17–36 years, beginning in the third or fourth decade in three patients, and associated with severe diarrhoea lasting some weeks with greasy, bulky, light-coloured stools and accompanied by diffuse abdominal pain or discomfort and marked fatigue. One patient (B. K.) dated her illness from early childhood, when it was diagnosed as tuberculous enteritis, for which she was treated in hospital over a greater part of her childhood. Patients B. W. and H. G. had been previously regarded as suffering from, and on several occasions treated for, tropical sprue, patient H. G. having been admitted to several military hospitals for investigation.

TABLE I.—*Data Relating to Four Patients Suffering from Adult Coeliac Disease*

Patient:	B.W.	G.H.	H.G.	B K.
Sex	M	M	M	F
Age (years)	61	67	52	27
Duration of illness (years)	36	17	20	25
Weight (kg.)	56	64·4	57·5	40·4
Av. day over 6 days:				
Faecal fat (g.)	16·6	20·9	34·4	13·1
,, nitrogen (g.)	4·3	3·9	3·0	2·3
Water-load: % excreted (4 hours)	90	57	17	22
Hb (g./100 ml.)	11·2	10·4	8·9	11·9
Total serum protein (g./100 ml.)	6·6	4·8	5·1	5·6
Serum albumin (g./100 ml.)	3·6	2·3	2·6	2·5
Blood urea (mg./100 ml.)	41	46	35	26
Plasma electrolytes:				
Na^+ (mEq/l.)	137	138	140	140
K^+	4·5	3·0	3·7	3·9
Cl^- ,,	97	108	110	111
TCO_2 (mMol/l.)	29·2	22·5	18·9	21
Plasma calcium (mg./100 ml.)	9·4	8·2	7·3	8·6
,, phosphorus ,, ,,	3·8	2·6	2·1	—
Serum cholesterol ,, ,,	134	101	158	—

Apart from H. G., all patients enjoyed good appetites; three complained of swelling of the ankles during attacks of diarrhoea and two of persistent nocturia. Only one patient (B. K.) complained of thirst, but all the patients were aware of sore tongues during or after the diarrhoea. H. G. gave a history of frequent cramps in both legs, while B. K. had been admitted to hospital on two previous occasions with tetany, and on the present admission Chvostek's sign was positive. B. W. was admitted with subacute obstruction, abdominal pain, distension, and constipation following severe diarrhoea.

On examination all the patients were thin, wasted, with decreased body fat and cold extremities, and all were noticeably slow in their movements. Estimated loss of weight varied between 20 and 30% compared with average weights for age and height computed from standard tables (Sunderman and Boerner, 1949). All had "hatchet"-shaped faces with prominent zygomatic arches and red, smooth tongues. No patient showed obvious signs of anaemia. Meteorism was a prominent feature in each case, and active peristalsis was visible on the abdominal wall in three cases. H. G. and B. K. had slight oedema of the ankles, but the blood-pressure in each patient was within normal limits. There were no neurological abnormalities apart from two patients (B. W. and G. H.) who had some loss of vibration sense below both knees. Two patients, however, had histories and physical signs of cardiovascular disease, complicating their steatorrhoea. For some 20 years G. H. had terminated occasional episodes of irregular tachycardia by lying quietly for 30 minutes, and two years before the present admission to hospital had undergone a left lumbar sympathectomy for incipient gangrene of his left foot, after which intermittent claudication handicapped his walking after some 200 yards. No pulse could be detected below either knee. While in hospital he had two episodes of slow atrial fibrillation with absence of raised external jugular pressure and basal rales but with marked peripheral oedema. Sinus rhythm was restored after digitalization followed by quinidine.

H. G. had a coronary occlusion four years previous to his admission, followed after convalescence by intermittent claudication in both legs on walking about 50 yards, and this remained unaffected after bilateral sympathectomy two years later. He also complained of angina of effort. B. K. had been treated for two years with antituberculous drugs after a left-sided pleural effusion and a haemoptysis. There was some residual pleural thickening.

Clinical Investigations

All patients showed the features typical of adult coeliac disease on *x*-ray examination of the small intestine, with dilatation and loss of outline. In addition, two patients (B. W. and B. K.) showed the typical flocculation pattern when simple barium-sulphate suspensions were used. B. W. had multiple fluid levels and H. G. an extremely dilated colon. Jejunal biopsy (Dr. Margot Shiner) of each patient showed atrophy or clubbing of the mucosal villi, and results of estimations of amylase and trypsin carried out on the duodenal juice from fasting patients at the same time were in all cases within normal limits. Values for 24-hour excretions of faecal fat and nitrogen (averaged over six days while patients were on ward diets containing between 50 and 100 g. fat/day) were abnormally high and are given in Table I. The water-excretion test (urine collected for four hours after a loading of 20 ml./kg. following a 10-hour fast) was normal in B. W., but the remaining patients showed impairment of excretion. G. H. and B. W. excreted less than 3 and 2 g. respectively of D-xylose in urine collected for five hours after a loading dose of 25 g. (Benson *et al.*, 1957), while the remaining two patients showed flat blood-glucose curves after administration of 50 g. of glucose.

All patients were in fact anaemic, but in only one case (H. G.) was the anaemia macrocytic with an M.C.H.C. of 28%, and M.C.V. of 105 cubic microns, and a reticulocytosis of 7.7%. The other patients showed a mild iron-deficiency type of anaemia. The serum proteins, plasma electrolytes, urea, calcium, and phosphorus were normal in B. W. In the remainder, serum proteins were low, with albumin values less than 3 g./100 ml. Blood ureas were normal, but there was some slight degree of acidosis, associated in H. G. with a urinary ammonia output of between 40 and 80 mg./hour. This patient had a slight albuminuria, and numerous hyaline casts were seen in the spun urinary deposit. Plasma calcium values were also low and associated with tetany in B. K., with some depression of the phosphate value in the other two patients. *X*-ray examination of the skeleton, however, did not reveal

evidence of osteomalacia in any patient. In all patients results of routine liver-function tests were within normal limits, and routine analyses of specimens of urine, apart from the findings in patient H. G. already discussed, were normal. Serum cholesterol values for three patients were low.

Investigations with ^{15}N-labelled Yeast Protein and ^{15}N-labelled Yeast Protein Enzymic Hydrolysate

These test substances were prepared from a strain of yeast supplied by the Distillers Company, as outlined by Crane and Neuberger (1960).

Normal Controls.—A man aged 64 was given 1 g. of yeast protein (0.4 mg. ^{15}N/kg.) in 250 ml. of water after a fast of 12 hours. Blood samples were taken at intervals up to two hours and analysed for α-amino-nitrogen as described previously. Faeces were collected for six days and analysed for total and trichloroacetic acid (T.C.A.) soluble nitrogen.

Adult Coeliac Patients.—Each patient was fasted overnight but allowed a free diet during the experiments, and at 8 a.m. on the day of the test was given some tap-water to drink, 20 ml./kg. Two hours later the bladder was emptied, and either the protein or the hydrolysate was given in a volume of 250 ml. of water, in amounts equivalent to 0.4 mg. ^{15}N/kg. body weight. Further quantities of 250 ml. of water were given hourly for two hours and specimens of urine were collected at 30-minute intervals for the first three hours, and then at convenient times up to six hours. Lunch was provided three hours after the beginning of the experiment. Urine was collected up to three days and stored under toluene at 4° C. Faeces were collected daily for six days in three protein experiments, and for three days in one protein and three hydrolysate experiments, and immediately frozen. The patients were weighed daily. Six days were allowed between the end of a protein experiment and the beginning of a hydrolysate experiment.

In order to assess the effect of the water-load in coeliac patients, a further experiment with yeast protein (0.4 mg. ^{15}N/kg.) was carried out with patient B. W. under the previous conditions but without the administration of water before the protein; samples of blood were withdrawn at suitable intervals over the first three hours of the experiment and were analysed for plasma amino-nitrogen.

An opportunity to investigate the uptake of ^{15}N yeast protein after a rapid response to a gluten-free diet was made with patient B. K. Within two weeks of commencing the diet the diarrhoea ceased, peripheral oedema disappeared, and she felt very much better. 0.4 mg. ^{15}N/kg. body weight was given and blood samples were taken at suitable intervals throughout the first three hours of the experiment.

Analyses of Urine, Faeces, and Plasma.—Urine samples were analysed for urinary urea, ammonia, and 3-, 6-, and 24-hour aliquots for total nitrogen; the blood samples for urea and plasma α-amino-nitrogen and the faeces for total and T.C.A. soluble nitrogen by methods published in a previous paper (Crane and Neuberger, 1960). Nitrogen was assayed for ^{15}N content in a mass spectrometer by the Rittenberg hypobromite technique.

RESULTS

Table II shows the results of a typical experiment after feeding both whole protein and protein hydrolysate.

TABLE II.—*Amounts of ^{15}N-labelled yeast protein and protein hydrolysate equivalent to 0.4 mg. ^{15}N/kg. body weight were given to a fasting male patient (B. W.), aged 61, with adult coeliac disease, 2 hours after a water-load of 20 ml./kg. (Experiments W_1 and W_2.)*

Time (Hour)	Protein			Hydrolysate		
	Ammonia		Urea (Atoms % Excess)	Ammonia		Urea (Atoms % Excess)
	N (mg.)	^{15}N (Atoms % Excess)		N (mg.)	^{15}N (Atoms % Excess)	
0–0·5	8·6	0·011	—	4·0	0·028	—
0·5–1	6·3	0·047	0·006	5·3	0·081	0·013
1–1·5	5·6	0·127	—	6·0	0·152	—
1·5–2	4·5	0·177	0·031	4·7	0·164	0·049
2–3	5·3	0·215	0·046	8·3	0·147	0·057
3–3·5	6·5	0·134	—	6·6	0·095	—
4·5–5	4·3	0·067	0·049	4·8	0·055	0·055

Rate of Absorption of ^{15}N-labelled Yeast Protein

^{15}N Content of Urinary Ammonia

Fig. 1 shows the ^{15}N content of urinary NH_3 from two such experiments K_1 and W_1, compared with a normal subject (experiment A_4) who received protein equivalent to 0.44 mg. ^{15}N/kg. after fasting and in whom 20-minute collections of urine were made during the first hour of the experiment.

In the normal subject the ^{15}N content of the ammonia rose rapidly to a maximum and there was already an appreciable concentration present in the first 20-minute sample of urine, equivalent to 15% of the maximum value. The maximum concentration was reached in the 40–60-minute interval at about 50 minutes, and values then declined more gradually, falling after three to three and a half hours to about 20% of the maximum value. Similar results were obtained from two further experiments with normal subjects who received protein equivalent to 0.9 mg. ^{15}N/kg. body weight. In contrast, the rise of ammonia ^{15}N in all the coeliac patients was much slower. The percentages of the maximum value obtained during the first 30 minutes of each experiment were 5, 6, 4.5, and 7.5 for experiments W_1, K_1, G_1, and H_1 respectively, and during the next interval, 30–60 minutes, values were raised to 20, 28, 39, and 22% only. Maximum values were delayed until about two hours in experiment K_1 and two and a half hours in experiments W_1 and H_1. In a further experiment, G_1, a plateau was obtained so that it was impossible to assign a time for the peak ^{15}N concentration, but it was likely that a maximum value was reached later than one and a half hours. The fall of ^{15}N concentration from the maximum values was also slower than in the normal, and values at five hours (two and a half to three hours from the peak concentration) ranged from 28 to 38% of the maxima reached. It appears, therefore, that in three patients the ^{15}N ammonia curves are displaced by about 60–90 minutes; we can assume that this condition imposes a time delay of this order on the absorption of the yeast protein from the gut.

The shapes of the ammonia curves compared with the normal are thus slightly broader with rounded peaks; but it is of interest to note that, in spite of the displacement in time, the maximum values of 0.195, 0.184, and 0.215 ^{15}N atoms % excess for three experiments H_1, K_1, and W_1 respectively are not greatly dissimilar from the value of 0.246 found for the normal. The amount of ^{15}N yeast protein administered for all subjects was proportional to total rather than fat-free body weight. Since in adult coeliac disease it is the loss of body fat which contributes in the main to fall in body weight,

normal subjects with adequate fat deposits therefore received a slightly higher amount of ^{15}N than the patients. As the ^{15}N administered almost certainly does not interact to any appreciable extent with the fat deposits, we should expect the labelling of the urea, ammonia, and amino-acids to be slightly greater in the normal subject; if this factor is taken into account little difference probably exists between the maximum ^{15}N value of the ammonia in the normal subjects and that of the coeliac patients.

^{15}N Content of Urinary Urea

Fig. 2 shows the ^{15}N-labelling of urinary urea in two experiments, G_1 and H_1, described in the previous section compared with a normal experiment, C_2. The experiment in the normal was carried out as described above for the urinary ammonia except that a larger amount of yeast protein, equivalent to 0.9 mg. ^{15}N/kg. body weight, was fed. The values obtained for the ^{15}N content of the urea were thus larger and have been adjusted to 0.4 mg. ^{15}N/kg. body weight. In the normal curve, C_2, a maximum value of ^{15}N was reached at about 75 minutes and remained constant thereafter for about three to four hours, the values then falling slowly. Similar values have been obtained in two other experiments. In the interval, 30–60 minutes, the urea was labelled to the extent of 70% of the maximum figure, and during the interval of one and a quarter to five hours, over which the ^{15}N was constant, the rate of entry of labelled urea to the total body urea pool was balanced by the excretion into the urine. Inspection of Figs. 1 and 2 shows two further features: the ratio of ^{15}N of urinary NH_3 to ^{15}N urinary urea reached unity between three and four hours, and the maximum ^{15}N content of the urea was achieved at about 20 minutes after the ^{15}N maximum of the urinary ammonia.

The urinary urea curves, G_1 and H_1, for two of the coeliac patients (Fig. 2), on the other hand, show, as for urinary ammonia (Fig. 1), delays in reaching maximum values. In all the experiments the highest labelling occurred in the interval of five to six hours, but differences from the two- to three-hour periods were slight and were within the experimental error of the ^{15}N determinations. The maximum ^{15}N values were therefore probably reached during this interval—that is, at about two and a half hours—displacing the curves by about one and a half hours from the normal.

The ^{15}N values during the period 30–60 minutes were more variable, being 13–30% of the maximum value reached in three experiments, W_1, K_1, and G_1, and 50% in experiment H_1. Apart from experiment H_1 all the curves lie close to one another, and the maximum values of 0.049, 0.057, and 0.053 ^{15}N atoms % excess for experiments W_1, K_1, and G_1 respectively did not differ too greatly from the maximum value of 0.063% for the normal C_2. The low isotope contents of the urea in experiment H_1 deserve a brief discussion. Shortly after starting the experiment, patient G. H. began a slow atrial fibrillation (pulse=78/min.). There was some slight oedema of the legs but no palpable liver, raised external jugular pressure, or oedema of the lungs. He put on about 3 lb. (1,360 g.) in weight during the next three days, and 36 hours after beginning the experiment his blood urea was 47 mg./100 ml. It is difficult to reconcile these low ^{15}N urea values with either such a small expansion of the E.C.F. or an increased urea pool, and, in the absence of a palpable liver, with a decreased capacity of the liver to make urea. We conclude that these low values are probably due to nitrogen retention, less being available for katabolic processes, of which urea formation is one. In an experiment 12 days later with yeast protein hydrolysate, urea isotope values of this patient were still low and, as is shown below, his total ^{15}N excretion in the urine was only about 50% of that normally obtained.

Rate of Absorption of Yeast Protein Hydrolysate

^{15}N Content of Urinary Ammonia

Fig. 3 shows a delay in the absorption of yeast protein hydrolysate in two experiments, G_2 and H_2, similar but less marked than the delay observed in the protein

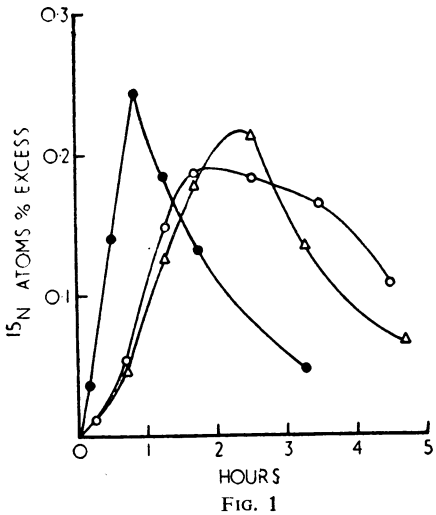

FIG. 1

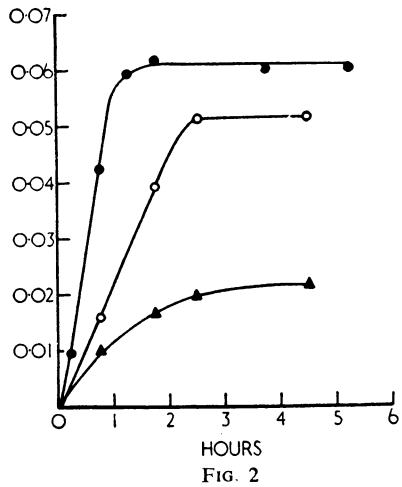

FIG. 2

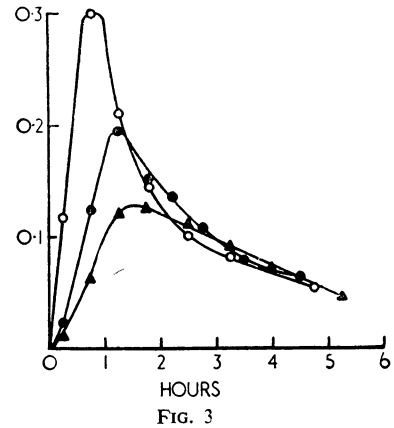

FIG. 3

FIG. 1.—Rate of change of isotope content of urinary ammonia is shown in two experiments, K_1 (—○—) and W_1 (—△—), after feeding ^{15}N-labelled yeast protein to two patients with coeliac disease. Results from a similar experiment, A_1 (—●—), with a normal subject are given for comparison. Values of ^{15}N content are plotted at the mid-point of the urine-collection-time intervals, and further details are given in text. FIG. 2.—Rate of change of ^{15}N of urinary urea is plotted for two experiments, G_1 (—○—) and H_1 (—▲—) after feeding ^{15}N-labelled protein to two coeliac patients. The results from a normal experiment, C_2 (—●—), have been adjusted (see text) to 0.4 mg. ^{15}N/kg. body weight for comparison. Values for ^{15}N content are plotted as for Fig. 1. FIG. 3.—Comparison betwen the changes with time of the ^{15}N content of urinary ammonia in an experiment C_3 (—○—) after giving protein hydrolysate to a normal subject, with values obtained in two similar experiments, H_2 (—●—) and G_2 (—▲—), when the same material was fed to two patients with coeliac disease. Further details of the experiments are given in the text, and the ^{15}N values are plotted at the mid-points of the time intervals.

experiments described above. The normal curve was obtained from an experiment, C_3, in which the hydrolysate equivalent to 0.83 mg. of ^{15}N/kg. was fed, and the ^{15}N values have been adjusted to 0.4 mg./kg. for comparison. As in the protein experiment (Fig. 1), the ammonia ^{15}N content rose sharply and attained a maximum value at almost 45 minutes of 0.3 atoms % excess, a value some 10% higher than when the whole protein was fed. The ^{15}N value decreased in a similar fashion, and the value in the two- to three-hour interval was close to that in the first 30-minute specimen. The time of the maximum ^{15}N reached has been confirmed in a further experiment in which blood samples were analysed simultaneously for amino-acid ^{15}N.

The maximum ^{15}N values for the coeliac patients presented a more variable picture, ranging from 75 minutes in experiments K_2 and H_2 to 105 to 120 minutes in experiments G_2 and W_2. But the shapes of the curves after the maximum values were reached were similar to those in the whole-protein experiments. The ^{15}N content was higher in the early specimens of urine compared with whole protein, and ranged from 40% of the maximum in experiment G_2 to 86% in experiment K_2 in the 30–60-minute specimens. There is therefore a delay in the absorption of yeast protein hydrolysate, and the values found exceed the normal by 30–75 minutes. The maximum values in three experiments were about 25% lower than when the whole protein was fed. These findings are in contrast with experiments in normal subjects, where the maximum value was 10% higher after feeding the hydrolysate.

^{15}N Content of Urinary Urea

Fig. 4 compares the ^{15}N contents of the urinary urea of two patients with adult coeliac disease (experiments H_2 and K_2) with the blood urea from a normal subject (experiment C_3) after feeding the hydrolysate. In the normal, maximum ^{15}N values were achieved at about one hour and remained constant for a further three and a half hours, and in the first 20 minutes the urea was labelled to about 50% of the maximum value, the early values being higher than when whole protein was fed.

In the experiments with the coeliac patients the results were more variable. For example, in experiment K_2 a value corresponding to 85% of the maximum was reached in about 45 minutes, and the maximum value itself between 90 and 120 minutes. In the remaining three experiments the rise of ^{15}N content was slower, reaching peak values between two and a half and three hours, as was found when the whole protein was fed (Fig. 2). For experiments G_2 and W_2 the time to reach maximum values of ^{15}N were in agreement with the findings for the urinary ammonia (Fig. 3), but in experiment H_2, where the maximum ammonia value was reached in about 75 minutes, the ^{15}N urea maximum was not reached until two and a half to three hours. As in the protein experiment H_1, the ^{15}N content of the urea in experiment H_2 was low, and it is presumed that during this time the anabolism of dietary nitrogen was continuing.

These results show, therefore, that in the coeliac patient there is a delay in absorption of yeast protein amounting to a difference from the normal of about 90 minutes, while the corresponding difference for the absorption of the hydrolysate, amounting to 30 to 75 minutes, is significantly smaller. The ^{15}N labelling of the blood urea after ingestion of protein and hydrolysate in two separate experiments carried out with a normal subject showed that the enzymic hydrolysis of the protein delayed its absorption by at the most 10 minutes, and the two curves of ^{15}N content of urinary ammonia (Fig. 5) after feeding with protein and hydrolysate are superimposable. On the other hand, in the adult coeliac patients these two curves become dissociated (Fig. 6).

Effect of a Water-load on the Absorption of Labelled Yeast Protein

Fig. 7 shows the ^{15}N content of urinary ammonia and plasma amino-nitrogen after giving a water-load of 500 ml. 30 minutes before whole-yeast protein to a normal subject, and the plasma amino-nitrogen after

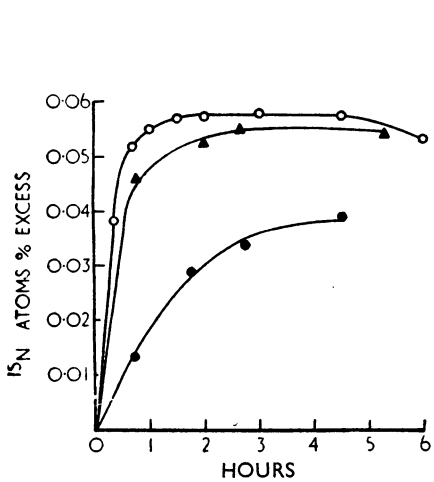

FIG. 4

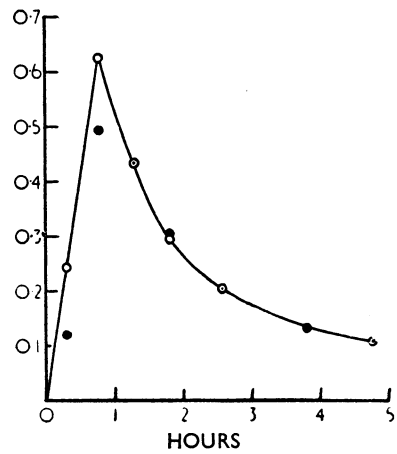

FIG. 5

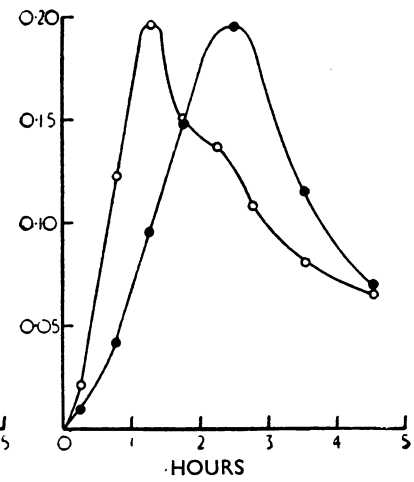

FIG. 6

FIG. 4.—Changes of ^{15}N content of urinary urea with time from experiment H_2 (—●—) (see Fig. 3 and text) are plotted with values from a similar experiment K_2 (—▲—) obtained from another patient with coeliac disease. The isotope values are plotted at the mid-points of the time intervals. For comparison, the ^{15}N content of blood urea is plotted from a control experiment C_3 (—○—) with a normal subject. Hydrolysate equivalent to 0.83 mg. ^{15}N/kg. body weight was given. The figures have therefore been adjusted to 0.4 mg. ^{15}N/kg. body weight, and are plotted at the times of collection of the blood samples. FIG. 5.—Changes with time of isotope labelling of urinary ammonia are compared in two separate experiments, C_2 and C_3, in which ^{15}N-labelled yeast protein (—●—) and hydrolysate (—○—) were fed to a normal subject. Values are plotted as for Fig. 1. FIG. 6.—Comparison between the changes with time of ^{15}N contents of urinary ammonia in two separate experiments, H_1 and H_2, after feeding whole protein (—●—) and protein hydrolysate (—○—) respectively to a patient with coeliac disease. Values are plotted as for Fig. 5.

feeding the protein without a water-load to another subject. Maximum values in all three cases were achieved at about 40 to 45 minutes, showing that a water-load under the conditions of the experiment did not affect the rate of absorption of the protein. Fig. 8 compares the isotope labelling of urinary ammonia with that of plasma amino-nitrogen in two separate experiments on a patient (B. W.) with coeliac disease, with and without a water-load.. The maximum concentrations were reached at about two and a half hours in both experiments.

It is concluded that a water-load, given two hours before the protein, is not responsible for the delay in the rate of absorption of protein found in patients with coeliac disease.

Rate of Absorption of ^{15}N-labelled Protein in a Patient with Adult Coeliac Disease in Remission After a Gluten-free Diet

After a relapse, and a return of the diarrhoea, peripheral oedema, and some loss of weight, patient B. K. was admitted to hospital, where she improved rapidly after reinstitution of a gluten-free diet. Fourteen days later she was given ^{15}N yeast protein (0.4 mg./kg.) and samples of blood were withdrawn at suitable intervals up to three and a half hours and analysed for amino-acid nitrogen. Faeces were collected over the following four days.

Fig. 9 shows a comparison between the ^{15}N content of plasma amino-acids during the remission (experiment K_3) and the ^{15}N content of urinary ammonia during a relapse (experiment K_1). The time at which maximum concentration of ^{15}N occurred returned from two hours to normal at about 45 minutes, and the curve showed a rapid rise, a sharp peak, and fall of ^{15}N content similar to the urinary ammonia curve of a normal subject (A_4 in Fig. 1). The maximum value of ^{15}N in the blood urea was achieved between 75 and 105 minutes, compared with the previous value of about two and a half hours for the urinary urea.

DISCUSSION

Clinical Findings

The clinical symptoms, chronicity, physical and *x*-ray signs, and chemical findings (Cooke *et al.*, 1953a; Bossak *et al.*, 1957), together with peroral jejunal biopsy (Shiner and Doniach, 1959) and satisfactory response in three of the patients to a gluten-free diet (French *et al.*, 1957; Schwartz *et al.*, 1957), are compatible with the diagnosis of adult coeliac disease.

The coexistence of severe cardiovascular disease, with low serum-cholesterol concentration in two patients (G. H., aged 67, and H. G., aged 52 years, with values of 101 and 158 mg./100 ml. respectively (Table I) at the time of investigation) was of some interest, since both had had steatorrhoea for about 20 years. In view of the interest in the relationship between cardiovascular disease and the concentration of cholesterol in the serum, it would appear that chronic adult coeliac disease with low serum cholesterol concentrations may offer no protection. A further severe case of steatorrhoea, a presumptive adult coeliac patient aged 45 with a 16-year history who had a myocardial infarction nine years after the onset of his diarrhoea, was found at post-mortem examination to have severe atheroma of the coronary arteries and extensive atheroma elsewhere in the arterial vessels.

Rate of Absorption of ^{15}N Yeast Protein and Hydrolysate

The findings from the experiments with adult coeliac patients indicate that, compared with normal subjects, there is a marked delay in the uptake of yeast protein from the gut of between 60 and 90 minutes and a

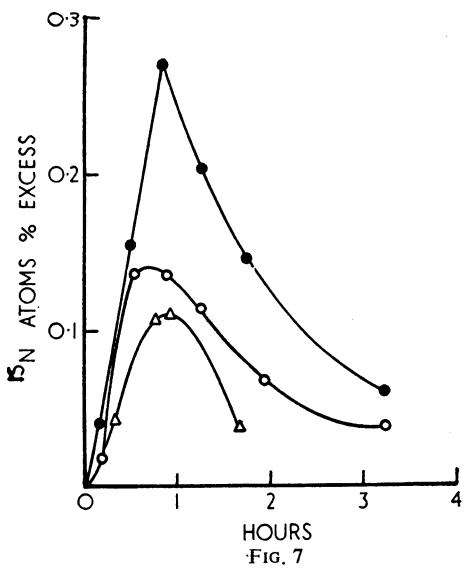

FIG. 7

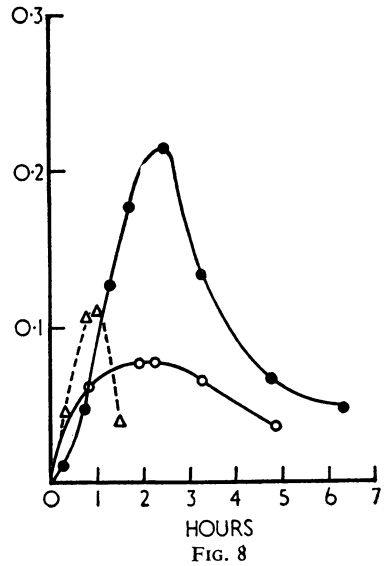

FIG. 8

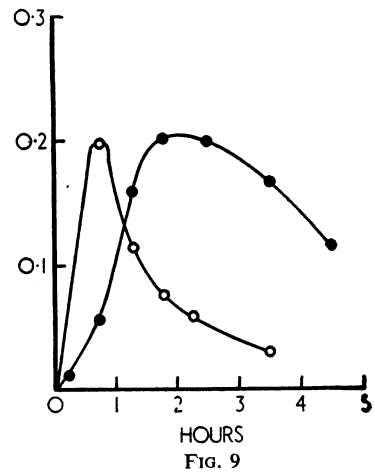

FIG. 9

FIG. 7.—Comparison of changes of ^{15}N content with time of urinary ammonia (—●—) and venous plasma amino-acids (—○—) on feeding yeast protein to a normal subject (experiment A_4) after a water load. In another experiment (WK_1), on a normal subject, the isotope content of the plasma amino-acids was measured (—△—) but no water load was given. Values for the isotope content of the ammonia are plotted as in Fig. 1 and the values for the amino-acids are plotted at time of collection of blood.

FIG. 8.—Comparison of changes of ^{15}N content of urinary ammonia (experiment W_1, —●—) and venous plasma amino-acids (experiment W_3, —○—) with time in two separate experiments after yeast protein was given to a patient with adult coeliac disease. A water-load was administered two hours before the protein in experiment W_1, but this was omitted in experiment W_3. Results are plotted as for Fig. 7 (—△— plasma α-amino-N, experiment WK_1, Fig. 7).

FIG. 9.—Changes of ^{15}N content with time of urinary ammonia in an experiment K_1 (—●—) after feeding whole-yeast protein to a coeliac patient are compared with isotope changes of plasma amino-acids (—○—) in a similar experiment (K_3) with the patient in remission 14 days after recommencing a gluten-free diet. No water load was given in the second experiment and the isotope values are plotted as for Fig. 7.

smaller delay of between 30 and 75 minutes when the the hydrolysate was fed; but this was more variable from patient to patient. Fig. 5 shows that in a normal subject the labelling of urinary ammonia after feeding either yeast protein or hydrolysate was similar and the two curves are superimposable, but that in adult coeliac patients (Fig. 6) the rate of labelling differed widely. It is necessary, therefore, to examine the various factors which might influence the slowing down of intestinal absorption of yeast protein in idiopathic steatorrhoea, and the possible influence of excess fat and water in the small intestine must now be considered.

Our patients were studied after a 12-hour fast and food was not given until three hours after administration of the protein or the hydrolysate. It is therefore very unlikely that there was an excess of dietary fat present in the small intestine during the first three hours of each experiment. Even if large amounts of endogenous fat were excreted into the intestine during this time, it is improbable that absorption would be influenced solely by mechanical interference between the enzymes present in the small gut and the ingested protein, since Atkinson *et al.* (1956) and Gross *et al.* (1950) have shown that the steatorrhoea arising in patients from biliary or liver disease is not accompanied by any disturbance in the excretion of faecal nitrogen. Investigations of patients suffering from other types of steatorrhoea will form a separate study, but it is of some significance that we have found little delay compared with normal in the absorption of labelled yeast protein in two other patients suffering from gross steatorrhoea, due to severe pancreatic disease in one and subsequent to a partial gastrectomy in the other. However, the possibility cannot be overlooked that the presence of small-chain fatty acids, which may be produced by fermentation in the small gut in adult coeliac disease could lower the *p*H of the intestinal contents below the optimum for both trypsin and chymotrypsin, and hence cause some slowing of hydrolysis of the yeast protein.

It is well known that patients with adult coeliac disease show impaired excretion of water, and Higgins *et al.* (1957) have compared the uptake of deuterium oxide from the small intestine in fasting adult coeliac patients with values obtained from normal controls. The mean times for 50% and 67% disappearance from the gut of the amount of isotopically labelled water given were about threefold above the normal, but this meant delays of no more than four and eight minutes respectively. Large amounts of water, if retained in the stomach or gut, might prolong these times, but the overall effect on the labelling of both urinary ammonia and urea and plasma amino-acids would not be expected to be great. In a normal subject (Fig. 5) the administration of about 500 ml. of water 30 minutes before giving the protein did not influence absorption, while a similar experiment (Fig. 6) carried out on a patient with adult coeliac disease who had a water-load of about 1 litre two hours before the protein was given showed little effect on the delay of absorption, maximum values for plasma amino-acid ^{15}N and urinary ammonia ^{15}N respectively occurring between two and two and a half hours after ingestion.

Two further mechanisms, hypomotility of the gut and excessive mucus production, could each contribute to the delay in absorption of both yeast protein and hydrolysate. Higgins *et al.* (1956) demonstrated that drug-induced hypomotility of the gut caused a delay of 8 to 10 minutes in the uptake of deuterium oxide from the intestine in normal subjects. Working with rats, Gupta *et al.* (1958) and Rosenthal and Nasset (1958) have shown that gastric emptying-time determines the overall absorption of test meals containing fat, carbohydrates, and protein, while May and MacCreary (1940) have pointed out that delayed gastric emptying and diminished peristalsis of the small intestine may be mainly responsible for the flat glucose-tolerance curves in children with coeliac disease. On the other hand, Cummins and Almy (1953), who review most of the literature in this field, and who examined the absorption of glucose and methionine from the intestine of normal persons and patients with sprue, concluded that hypomotility of the bowel is not the primary cause of malabsorption. Frazer *et al.* (1952) also reported that the absorption of both glucose and urea from the small intestine was delayed compared with normal subjects, even when solutions of these substances were injected into the small intestine of coeliac patients by intubation. The possibility that hypomotility of the gut is at least partly responsible for the delay of absorption of protein and amino-acids cannot be excluded and will be tested later.

Excessive production of mucus in the small intestine has been suggested in adult coeliac disease by Frazer *et al.* (1949), and this could interfere with the passage of the products of hydrolysis from the lumen of the gut into the intestinal mucosal cells. Heatley (1959) concluded from his *in vitro* studies of hog gastric mucin that films of this substance offered little barrier to the diffusion of either hydrochloric acid or pepsin, while Cooke *et al.* (1953b) have pointed out that even in the most severely ill patients the intestinal mucosal cells retain some selective ability with respect to the absorption of fat. Thus, while some of the possible explanations discussed above require further investigations, the most reasonable explanation of our findings appears to us to be as follows.

In a previous paper (Crane and Neuberger, 1960) we have considered the small difference in normal subjects between the rate of absorption of whole-yeast protein and that found with the hydrolysate. Borgström *et al.* (1957) have shown that high concentrations of both trypsin and chymotrypsin, 300–800 μg./ml. of fluid, are present in the small intestine soon after a test meal is given. We found that when unlabelled yeast protein was incubated at 37° C. in a phosphate buffer at *p*H 7.2 with concentrations of these enzymes equivalent in two experiments to 1,600 and 400 μg. of each enzyme/ml., about 60% and 40% of the insoluble protein respectively were hydrolysed within 5 minutes to trichloroacetic acid soluble material.

Newey and Smyth (1959) have shown that peptides are hydrolysed within mucosal cells, and it is probable that polypeptides are similarly degraded to amino-acids by peptidases which Wright *et al.* (1940) conclude are essentially intracellular. It is thus likely, then, that the breakdown of proteins into peptides is a very fast process and that these substances, as well as free amino-acids, are then taken up by the mucosal cells. As the amount of protein fed in our experiments was small, it is hardly surprising that little difference was observed between the rate of absorption of yeast protein and that of hydrolysate. It is thus assumed that in a normal subject hydrolysis of protein to peptides begins in the stomach by the action of pepsin, and is continued in the lumen of the gut by the action of pancreatic trypsin, chymotrypsin, and carboxypeptidase, while further

degradation of the peptides to amino-acids occurs mainly in the mucosal cells.

The observations of Paulley (1954) and of Shiner and Doniach (1959) have established that in adult coeliac disease the jejunal villi are either atrophied or clubbed and the cells of the columnar epithelium show evidence of disorganization. Rubin *et al.* (1960) made observations on jejunal biopsy material from patients with coeliac disease similar to those already mentioned, but emphasize that the most constant histological feature was the reduction of epithelial cells with resulting reduction of epithelial surface, and, in addition, there was disorganization of the small blood-vessels of the villi. Butterworth and Perez-Santiago (1958) have found similar changes in the small intestines of patients suffering from tropical sprue and have calculated that the absorptive surface of the jejunum in these patients may be reduced to one-quarter of normal. That this reduction cannot be the only factor in determining intestinal absorption follows from observations on patients who have undergone massive resection of the small intestine ; for Althausen *et al.* (1949) reported the case of a patient who later achieved nutritional equilibrium after the removal of all but 10% of his small intestine.

It is suggested that in adult coeliac disease, therefore, there is both a reduction of total absorbing surface and changes in the mucosal cells which reduce the amount of peptidases (erepsin) available.

The reduction in total absorptive surface is sufficient to account for the delay, which was found to be between 30 and 75 minutes for the absorption of protein hydrolysates. It is of some interest in this connexion that Erf and Rhoads (1940) and Butterworth *et al.* (1958) reported delays in the absorption of glycine between 60 and 90 minutes for patients with sprue compared with normals.

There now remains to explain why the defect in absorption was more marked after ingestion of whole protein than after feeding the hydrolysate. It has already been pointed out that the maximum ^{15}N labelling of the urinary ammonia is of the same order in both normals and patients after feeding the protein when account is taken of the higher effective ratio of ^{15}N per body weight in normal subjects, and similar values apart from one patient were found for the urinary urea. It would appear, therefore, that the events subsequent to protein feeding are merely displaced in time from those occurring when the hydrolysate is fed. It is suggested that since normal concentrations of pancreatic enzymes (trypsin, chymotrypsin, and carboxypeptidase) are probably present in the intestine, reduction of the number of mucosal cells or changes in the enzymic composition consisting of a decrease in the amounts of peptidases elaborated leads to a prolongation of the time required for the overall hydrolysis of the protein to amino-acids, so increasing the delay already imposed by the reduced absorbing surface. In this connexion it is of considerable interest that Dawson and Isselbacher (1960) found that mucosa from the jejunum of patients with idiopathic steatorrhoea had a greatly diminished capacity to esterify labelled palmitate.

The response to the gluten-free regime may be rapid. French *et al.* (1957) found that normal faecal excretion of fat was achieved in three weeks in some of their patients, while Schwartz *et al.* (1957) have reported an almost immediate improvement in the faecal excretion of fat and nitrogen in one of their patients on introducing a gluten-free diet. The effect of treatment on the absorption of labelled protein will form a separate study, but it is of some interest that one patient, B. K., was found to have a normal uptake of the protein (Fig. 9) after only 14 days' treatment, at the end of which time she had made a remarkable clinical improvement. Shiner and Doniach (1959) concluded that the histological picture of the jejunal mucosa was unchanged in a patient with adult coeliac disease after periods of gluten-free diets plus folic acid of 67 and 158 days, and it is possible that the benefits of a gluten-free diet on the absorption of protein may be caused in some way by the elaboration of greater concentrations of intracellular peptidases without necessarily being associated with return to normal of the histology of the tissue.

It must be stressed that these results on protein absorption cannot be applied to everyday conditions of alimentation when larger amounts of protein, together with fat and carbohydrate, are ingested and when gastric emptying would be expected to exercise a very much greater influence on the rate of absorption. In one experiment to investigate this point we mixed labelled yeast protein (0.4 mg. ^{15}N/kg.) with 90 g. of skimmed-milk powder equivalent to 0.4 g. of protein/kg. body weight and fed the mixture to a normal subject. We found that absorption was much delayed, amounting to about 90 minutes, while the peaks of ^{15}N content occurred in the urinary ammonia and urea at two and a half and four hours respectively. Under normal circumstances, therefore, we may possibly find a much greater delay in absorption of protein in adult coeliac patients than has been found in the present investigation, and further work will be undertaken to investigate this.

SUMMARY

The intestinal absorption of proteins and amino-acids in patients with adult coeliac disease has been examined by administration of yeast protein and yeast protein hydrolysate labelled with the stable isotope ^{15}N. Urine was collected at half-hourly intervals after administration of the labelled material and the isotope content of the ammonia and urea in the urine, and in a few experiments that of the urea and amino-acids of the venous plasma, was measured. The appearance of the label in the various fractions mentioned was considerably delayed as compared with the behaviour shown by normal subjects. In the case of whole protein the maximum isotope content occurred two to two and a half hours after the ingestion of labelled material in contrast to normal subjects, in whom the maximum was observed about 50 minutes after ingestion. With protein hydrolysate, deviations from normal behaviour were still found, but were more variable than in the case of whole protein and on the whole less marked.

The various factors responsible for this delay in absorption are considered and the possibility that hypomotility of the small intestine may be partly responsible for the slower absorption has not been excluded. However, in view of the cytological changes observed by other workers, it is assumed that the rate of absorption of peptides and amino-acids is reduced in adult coeliac disease by a reduction in effective absorptive surfaces. In addition, the view is put forward that in the normal subject hydrolysis of proteins to products which are mainly peptides occurs in the first place in the lumen of the gut by the action of pancreatic enzymes such as trypsin and chymotrypsin. These primary products are

then further degraded to amino-acids by peptidases present in the cells of the mucosa.

In adult coeliac disease it is suggested that the activity of these cellular enzymes is reduced, and this, in addition to a reduction in absorptive area, produces a slowing down in the later stages of proteolysis. The possibility that the changes of absorption observed in the hydrolysis are associated with the rate of absorption of water has been excluded by appropriate control experiments. It was of interest that treatment for several weeks with a gluten-free diet in one of our cases produced a normal type of absorption, as shown by the results of isotope experiments.

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The inaugural meeting of the International Society of Tropical Dermatology was held earlier this year at the Rockefeller Institute in New York. An international congress was suggested for 1962 or 1963.

MIGRAINE AS A DEADLY DISEASE

BY

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The vast majority of patients in need of medical attention because of headaches are sufferers from migraine. The occurrence of migraine in the population has been estimated as from 3 to 30%. If only females are considered, and if temporary periods of migraine, say, for one or a few years, are included, 30% is hardly an overstatement, high as it may seem (Ask-Upmark, 1953). If both sexes are considered, and only severe cases embittering life for a number of years are included, 3% may be a more likely approximation (Essen–Möller, 1956).

Possible Causes of Death from Migraine

Although migraine more often than not reduces or abolishes the working capacity of the patient, it is not usually regarded as a fatal disease. Increasing evidence, however, suggests that it is a real danger to life, not least by means of iatrogenic activities. As a matter of fact, death may ensue from migraine under the following conditions.

1. Ophthalmoplegic migraine is more often than not due to an aneurysm of an artery belonging to the circle of Willis. The topography of this aneurysm may involve the oculomotor nerve, so that a distension of the aneurysm will not only produce pain but also an oculomotor palsy with ptosis, etc. The rupture of such an aneurysm may be fatal.

2. Paroxysmal tachycardia of auricular origin is to be looked upon as an equivalent to migraine. Not only is it apt to occur in families with migraine, but there is also the possibility that in one and the same individual some attacks are typical migraine, whereas others are paroxysmal tachycardia. The peculiar phenomenon of urina spastica is characteristic for both types of attacks. It is well known that most attacks of paroxysmal tachycardia are innocent enough, at least if handled properly. There is always, however, the possibility that the heart rate attained may be so rapid as to prevent the auricular contraction from ejecting the blood into the ventricles because of the almost simultaneous contraction of the ventricles. This so-called critical level of the heart rate is about 180. If no adequate assistance is given in such a case the circulation may be blocked just as efficiently as, for instance, by a prolonged Stokes–Adams attack by a solid pulmonary embolism, or by the obstruction of the mitral passage by a pendulating auricular myxoma.

3. There are (rare) instances of migraine which are connected with an outstanding bradycardia during the attacks, say, a heart rate of 20–40. If in such a case ergotamine tartrate is injected intravenously a cardiac standstill may easily be induced. This happened to me once, although by good luck and adrenaline I managed to get the heart going again. As a means of stopping an attack of migraine ergotamine tartrate has decided merits, and it is most efficient if given intravenously. However, if bradycardia is present a word of warning must be issued, at least so far as intravenous administration is concerned. That such an injection in susceptible persons may bring about an attack of status asthmaticus with all its implications is another feature to be remembered.

4. Whereas the fatalities mentioned are rare, there is another complication which, for unknown reasons, has become increasingly common and which obviously is a very real danger. This complication is renal; its anatomical character is interstitial nephritis, and it is by no means uncommon as a cause of death from uraemia. It is apt to