

## Identification and measurement of molecular variants of cholecystokinin in duodenal mucosa and plasma. Diminished concentrations in patients with celiac disease.

J Calam, ... , A Ellis, G J Dockray

*J Clin Invest.* 1982;69(1):218-225. <https://doi.org/10.1172/JCI110433>.

### Research Article

The amount and type of cholecystokinin (CCK) in duodenal extracts and plasma of celiac patients and normal subjects was studied by radioimmunoassay and gel filtration. In both groups there were similar patterns of molecular forms in extracts of duodenal biopsies, but concentrations in celiac disease were significantly depressed. In boiling water extracts of duodenal mucosa from both groups a factor with the properties of the COOH-terminal octapeptide of cholecystokinin predominated, but there were also significant amounts of a larger molecular weight form. In acid extracts of mucosa a factor with the properties of the 33 or 39 residue form was identified in amounts that were approximately 25% those of CCK8; there were also similar amounts of an acid-soluble form that had an apparent molecular weight higher than CCK39. Plasma immunoreactive cholecystokinin was studied after concentration by immunoaffinity adsorption and fractionation by gel filtration. In normal subjects fasting CCK-like immunoreactivity was less than 0.8 pmol/liter, and after a light breakfast increased to 2.0 +/- 0.7 (range 1.0 to 4.8) pmol/liter; CCK8-like activity accounted for all the increased immunoreactivity. In five of six celiac patients the concentrations of both fasting and postprandial CCK-like immunoreactivity in plasma were undetectable (less than 0.8 pmol/liter). We conclude that diminished production and release of CCK could account for the impaired pancreatic and gall bladder responses to [...]

**Find the latest version:**

<https://jci.me/110433/pdf>



# Identification and Measurement of Molecular Variants of Cholecystokinin in Duodenal Mucosa and Plasma

## DIMINISHED CONCENTRATIONS IN PATIENTS WITH CELIAC DISEASE

J. CALAM, A. ELLIS, and G. J. DOCKRAY, *Physiological Laboratory, University of Liverpool Gastroenterology Unit, Broadgreen Hospital, Liverpool, England*

**ABSTRACT** The amount and type of cholecystokinin (CCK) in duodenal extracts and plasma of celiac patients and normal subjects was studied by radioimmunoassay and gel filtration. In both groups there were similar patterns of molecular forms in extracts of duodenal biopsies, but concentrations in celiac disease were significantly depressed. In boiling water extracts of duodenal mucosa from both groups a factor with the properties of the COOH-terminal octapeptide of cholecystokinin predominated, but there were also significant amounts of a larger molecular weight form. In acid extracts of mucosa a factor with the properties of the 33 or 39 residue form was identified in amounts that were  $\sim 25\%$  those of CCK8; there were also similar amounts of an acid-soluble form that had an apparent molecular weight higher than CCK39. Plasma immunoreactive cholecystokinin was studied after concentration by immunoaffinity adsorption and fractionation by gel filtration. In normal subjects fasting CCK-like immunoreactivity was  $<0.8$  pmol/liter, and after a light breakfast increased to  $2.0 \pm 0.7$  (range 1.0 to 4.8) pmol/liter; CCK8-like activity accounted for all the increased immunoreactivity. In five of six celiac patients the concentrations of both fasting and postprandial CCK-like immunoreactivity in plasma were undetectable ( $<0.8$  pmol/liter). We conclude that diminished production and release of CCK could account for the impaired pancreatic and gall bladder responses to intraluminal stimuli in celiac disease.

## INTRODUCTION

Cholecystokinin (CCK)<sup>1</sup> has been isolated from hog intestine as a peptide of 33 amino acid residues

(CCK33), and as a variant of this molecule extended by a hexapeptide at its NH<sub>2</sub>-terminus (CCK39) (1). In addition, the COOH-terminal octapeptide (CCK8) of CCK33 has been isolated and characterized from sheep brain (2), and there is immunochemical evidence that this form also occurs in high concentrations in the intestine (3, 4). It is well established that the COOH-terminal heptapeptide amide of CCK contains all the information needed for biological activity (1). Moreover, CCK8 has been shown to be 2–10 times more potent on a molar basis than CCK33 or CCK39 in several systems including stimulation of gall bladder contraction in vivo and in vitro, and stimulation of pancreatic enzyme secretion in vivo (5–7). The possibility arises then, that an imbalance in the amount or type of CCK could contribute to pancreatic or gall bladder malfunction. However, this idea has not yet been intensively studied. In this context it is significant that in patients with celiac disease there are decreased pancreatic and gall bladder responses to luminal stimuli (8, 9), but responses to exogenous CCK are normal (8, 10), indicating that there may be decreased circulating CCK biological activity. The present series of experiments were undertaken to examine the possibility that in celiac disease there is an abnormality in the quantities or molecular forms of CCK produced and secreted by the small intestine. The first series of experiments were directed towards the identification and estimation of molecular forms of CCK in duodenal biopsy extracts. In further experiments plasma CCK responses to a standard meal were studied. Cholecystokinin was measured by radioimmunoassay (RIA) using an antibody that is COOH-terminal specific and therefore measures the biologically active part of the hormone. Because gastrin contains an identical COOH-terminal pentapeptide to CCK, and so reacts with COOH-terminal specific antisera, samples were fractionated by gel filtration to separate different molecular forms of the two hormones. In addition assays

*Received for publication 13 January 1981 and in revised form 8 September 1981.*

<sup>1</sup> Abbreviations used in this paper: CCK, cholecystokinin; RIA, radioimmunoassay.

were carried out using gastrin antisera that react weakly with CCK. The results reported here indicate that there are at least four major forms of CCK in human duodenum; in celiacs these forms occur in similar proportions to normal, but in decreased concentrations. After a standard meal, CCK8-like activity was consistently found in the plasma of normal subjects, but was virtually absent from patients with celiac disease.

## METHODS

**Peptides and antisera.** Pure natural porcine CCK33 and CCK39 were generous gifts of Professor Viktor Mutt (Karolinska Institute Stockholm, Sweden). Synthetic sulphated CCK8 and unsulphated CCK8 were generous gifts of the Squibb Institute for Medical Research. Pure natural human unsulphated little gastrin (G17) and porcine sulphated big gastrin (G34) were generous gifts from Professor R. A. Gregory and Dr. Hilda J. Tracy (University of Liverpool). Antiserum 1296 was donated by Dr. J. H. Walsh (University of California at Los Angeles). Other antisera (L6 and L48) were raised in our own laboratory.

**Subjects.** The present studies were made on a total of 16 adults with celiac disease. All those involved in the study of tissue CCK (three male, seven female, mean age 32 yr) had total or subtotal villous atrophy at the time of study, and all those involved in the studies of circulating CCK (three male, three female, mean age 40 yr) had been shown to have total or subtotal villous atrophy 2–5 wk before the study. All patients subsequently showed clinical, hematological, or biochemical improvement on a gluten-free diet, with the exception of one patient in the study of circulating CCK who had both active dermatitis herpetiformis and total villous atrophy at the time of study. A total of 18 control subjects were studied. The controls for the tissue CCK study (five male, eight female, mean age 32 yr) were relatives of celiac patients who had no history of gastrointestinal disease, and had normal small intestinal histology. Controls (four male, one female, mean age 27 yr) for the study of circulating CCK were healthy laboratory workers. All patients and normal subjects gave informed consent, and the study was approved by the ethical committee of Broadgreen Hospital, Liverpool.

**Mucosal biopsy method.** About six biopsies (15 mg each) were taken from each of 10 celiac patients and 13 normal subjects, using a Quinton instrument maneuvered into the third part of the duodenum under fluoroscopic control. In both normals and celiacs the biopsies consisted almost entirely of mucosal tissue. Biopsies were frozen in a plastic tube using a 'Polar spray' (BDH Laboratories, Poole, Dorset, England) within 5 min of sampling, and were then stored at  $-20^{\circ}\text{C}$  until extraction, which was generally done within 4 d.

**Tissue extraction.** Previous studies have established that CCK8 is best extracted at neutral or alkaline pH, whereas CCK33 and CCK39 are best extracted in acid (3, 4, 11). Thus in the present studies the tissue biopsies were rapidly divided into two approximately equal parts, which were separately weighed and plunged, while still frozen, into boiling water (0.1 g/ml). One sample of tissue was boiled for 2 min and then homogenized (Ultra-Turrax) for 30 s. The extract was briefly reboiled, cooled at  $4^{\circ}\text{C}$ , and centrifuged (2,000 g, 10 min). The other sample was boiled and then glacial acetic acid added to a concentration of 0.5 M; the extract was ho-

mogenized, extracted at  $4^{\circ}\text{C}$  for 30 min, and centrifuged as above. Supernatant solutions were stored at  $-40^{\circ}\text{C}$  before analysis. In a few studies only neutral extracts were prepared, and in some others only acid extracts were obtained.

**Test meals.** The subjects fasted overnight and then ate a standard meal of two boiled eggs, two pieces of dry gluten-free toast, and a cup of beef extract (two cubes of Oxo in 150 ml hot water). Blood samples (80 ml) were taken immediately before the meal and at 40 and 80 min after feeding. The blood was collected in glass tubes at  $4^{\circ}\text{C}$  containing 1 mg/ml ethylene diamine tetraacetic acid (disodium salt). Plasma was obtained by centrifuging at 1,800 g for 10 min, and then again at 13,000 g for 20 min to remove protein precipitated at low temperatures. Any further cryoprecipitate was removed by filtration through glass fiber filter paper.

**Immunoabsorption.** Preliminary studies indicate that concentrations of CCK in ordinary plasma samples were at or below the limit of detection for our assay. Thus immunoreactive CCK in plasma samples was concentrated by immuno-affinity adsorption to a COOH-terminal specific antibody immobilized on Sepharose beads using methods that have previously been described in detail (12). Briefly, the IgG fraction of antibody L48 was immobilized on CNBr-activated Sepharose beads (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) using the methods recommended by the manufacturer. The beads were packed into a small column ( $3.0 \times 2.0$  cm—equivalent to 0.75 ml of original antiserum) and prewashed with 20 ml of 15% formic acid to elute endogenous antigen bound to antibody (12). The columns were then washed with 0.14 M sodium chloride and plasma samples passed twice through the column, followed by 0.14 M sodium chloride and 50 ml distilled water. Adsorbed CCK-related peptides were eluted with 15 ml of 15% formic acid, followed by 10 ml distilled water. The eluates were lyophilized, and the residue reconstituted in 1.5 ml of 0.05 M ammonium bicarbonate containing 1% bovine serum albumin. In some experiments the lyophilization vessels were then washed with 3% acetic acid to recover any acid soluble peptides. Overall recovery was monitored by addition of a small amount of  $^{125}\text{I}$ -labeled CCK8 (1,000 cpm) to the original plasma, and in 13 successive runs was  $63 \pm 7\%$ .

**Gel filtration.** A series of pilot experiments was directed towards obtaining optimal separation and recovery of immunoreactive CCK in human extracts and plasma. The procedures finally adopted were as follows: samples at neutral or slightly alkaline pH were fractionated on Bio-gel P-10, 200–400 mesh ( $1 \times 100$  cm) (Bio-Rad Laboratories, Richmond, Calif.), eluted by gravity at  $4^{\circ}\text{C}$  with 0.05 M ammonium bicarbonate containing 0.05% sodium azide. Samples dissolved in acetic acid were fractionated on similar columns equilibrated and eluted with 0.5 M acetic acid containing 0.1% BSA. Recovery of immunoreactive CCK in 20 neutral extracts was  $80 \pm 6\%$ , and in 13 acid extracts was  $79 \pm 8\%$ . There were no differences in the recovery of immunoreactivity between samples from celiac and normal subjects. Samples for chromatography (400–800  $\mu\text{l}$ ) were fortified with bovine serum albumin to mark the void volume, detected by absorbance at 280 nm, and with  $\text{Na}^{125}\text{I}$  to indicate the total volume. Column eluates were stored at  $-20^{\circ}\text{C}$  before assay. In some experiments, column eluates corresponding to the main peaks of immunoreactivity were pooled and lyophilized. The residues were then either dissolved in the appropriate column buffer and refractionated by gel filtration, or were used for construction of dilution curves in radioimmunoassays. The Bio-gel P-10 columns were calibrated with natural porcine CCK33 and CCK39,

natural porcine G34 and human G17, and synthetic sulphated CCK8 and COOH-terminal tetrapeptide of gastrin and CCK (G4).

**RIA.** RIA methods have been reported in detail (12-14). Routinely immunoreactive CCK was estimated by antibody L48 using desulphated CCK8 labeled with  $^{125}\text{I}$ . This antiserum was raised to sulphated CCK8 coupled to bovine serum albumin, and has been shown (12) to be specific for the COOH-terminus of CCK8 and to react equally with CCK8, CCK33, and G17. Immunoreactive gastrin in the samples was separately estimated using antiserum 1296 and  $^{125}\text{I}$ -labeled G17; this antiserum reacts weakly with CCK8 (0.05) compared with G17 (13). In addition, immunoreactive G17 was estimated using antiserum L6 and G17 label; the latter antiserum is highly specific for G17, and shows no significant cross-reactivity with other forms of gastrin, or with CCK (14). Tissue extracts were generally assayed at several dilutions and results expressed relative to CCK8 (L48) or G17 (1296, L6), as appropriate. The eluates of columns run in 0.5 M acetic acid frequently caused nonspecific inhibition in the assay. To overcome this, aliquots of acid eluates were lyophilized in the assay tubes, and reconstituted in assay buffer. In most cases column eluates could be satisfactorily assayed at high dilutions (1:200-1:1,000 in assay tube). However, in some cases, particularly after fractionation of immuno-affinity eluates of plasma samples, it was necessary to assay column eluates at low dilutions, e.g., 1:4; in these experiments nonspecific effects of column buffer in the assay were eliminated by constructing standard curves containing appropriate quantities of blank column buffer. The detection limit of assays with L48 was 0.5 fmol/ml; for 1296 detection limit was 0.3 fmol/ml, and for L6 was 0.7 fmol/ml. The detection limit of CCK in plasma was calculated to be 0.8 pmol/liter, allowing for recovery after affinity concentration and gel filtration, and assuming a starting plasma sample of 30 ml.

## RESULTS

**Neutral tissue extracts.** The concentration of total immunoreactive CCK in neutral extracts of celiac mucosa ( $88.4 \pm 21.0$  pmol/g, mean SEM,  $n = 7$ ) was significantly lower ( $P < 0.05$ ) than in normal subjects ( $214.4 \pm 36.0$  pmol/g,  $n = 13$ ). Gastrin 17, estimated by antibody L6 was undetectable ( $<0.2$  pmol/g) in all celiac extracts and in 6 of 13 normal subjects, in the remaining normals it was 3 pmol/g. In extracts of both celiac and normal duodenum antibody L48 revealed three major peaks of immunoreactivity after gel filtration on Bio-gel P-10 in ammonium bicarbonate (identified as IN, IIN, IIIN, in order of appearance; N = Neutral) (Fig. 1). Peak IN accounted for  $\sim 10\%$  of total immunoreactivity in both groups, and emerged in the position of G34. This material cross-reacted similarly with both L48 and 1296 (Table I, Fig. 1), and so had both chromatographic and immunochemical properties of G34. Peaks IIN and IIIN diluted in parallel with L48 (Fig. 2), and since they were read by antibody L48 (CCK8 standard) in  $\sim 20$  times higher concentrations compared with antibody 1296 (G17 standard), they were CCK-like rather than gastrin-like (Table I). Peak IIN emerged in a similar position to G17, CCK33, and CCK39. Refractionation of this ma-

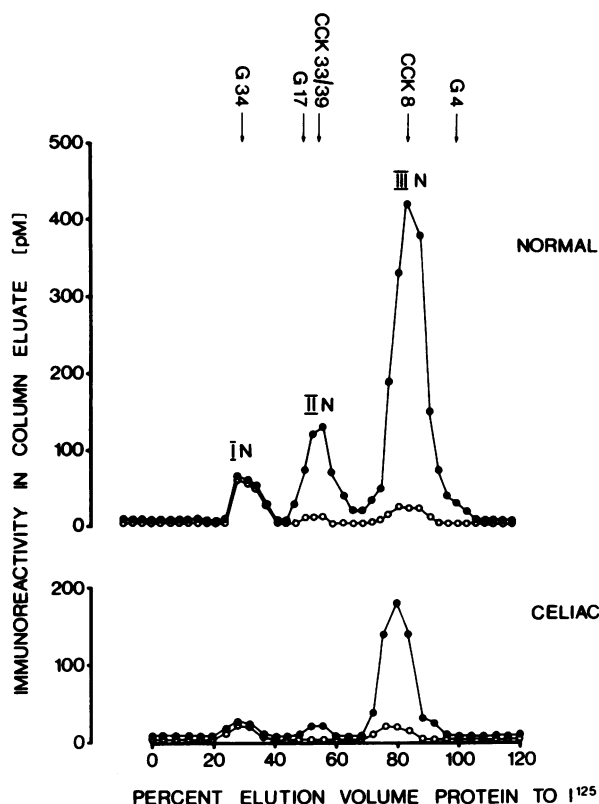


FIGURE 1 Separation on Biogel P-10 ( $1 \times 100$  cm,  $4^\circ\text{C}$ , 0.05 M ammonium bicarbonate) of boiling water extracts of duodenal biopsies from a normal subject (upper panel) and a celiac patient (lower panel). Each column run was assayed with both antibody L48 ( $\bullet$ ) and antibody 1296 ( $\circ$ ). Elution volumes of calibration standards (G34, CCK33, CCK39, G17, CCK8, and G4) are shown by arrows at the top. See text for details of elution and recovery.

terial on Bio-gel P-10 in ammonium bicarbonate gave a single well resolved peak of activity in its original position (Fig. 3). In contrast, refractionation on Bio-

TABLE I  
Relative Immunoreactivities of Mucosal CCK  
Components Using Antibodies L48 and 1296\*

Peak	L48	1296
IN	1.0	1.15
IIN	1.0	0.059
IIIN	1.0	0.062
IA	1.0	0.045
IIA	1.0	0.05

\* Pools of immunoreactive factors were assayed with both L48 (CCK8 standard) and 1296 (G17 standard). The concentrations of immunoreactivity determined with 1296 are expressed relative to a value of 1.00 with L48. With antibody 1296 the immunochemical potency of synthetic CCK8 relative to G17 was 0.05.

gel P-10 in acetic acid (Fig. 3) gave a poorly resolved peak emerging between 65 and 105%, and on this basis the material was clearly distinguishable from CCK33 and CCK39, (see below). The major peak (IIIN) eluted in the characteristic position of CCK8, and had both chromatographic and immunochemical properties compatible with this peptide. The concentrations of all three peaks of immunoreactivity in neutral extracts were lower in celiac mucosa compared with normals, but the differences were only significant for peaks IIN and IIIN (Table II).

**Acid tissue extracts.** Concentrations of immunoreactive CCK in acid extracts of celiac mucosa ( $78.3 \pm 9.6 \text{ pmol/g}$ ,  $n = 7$ ) were significantly lower ( $P < 0.01$ ) than in normals ( $203.2 \pm 26.2 \text{ pmol/g}$ ,  $n = 6$ ). In both celiac and normal extracts there were two well-resolved peaks of immunoreactivity (designated IA and IIA, A = acid) after fractionation on Biogel P-10 in acetic acid (Fig. 4). There were also five smaller peaks; one of these (IIIA) emerged immediately after IIA at 30–42% of elution volume, there were then a group of three (IVA–VIA) incompletely resolved peaks emerging between 50 and 86% elution volume and a final peak (VIIA) at 92–115%. These peaks are probably attributable to minor CCK immunoreactive forms, and to incompletely extracted G34, IIN, and IIIN. Because peaks IA and IIA occurred in relatively high concentrations it was possible to study them in more detail; both diluted in parallel with CCK8 with antibody L48 (Fig. 2), and were read in  $\sim 20$  times higher concentrations with L48 than with antibody 1296 (Table I). Peak IA emerged between the void volume and the elution position for CCK33 and CCK39; peak IIA coeluted with porcine CCK33 and CCK39 and so probably corresponds to the human forms of one or both of these peptides. Both peaks emerged in their

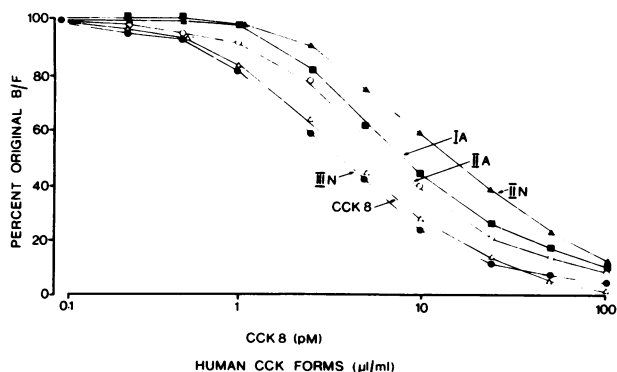


FIGURE 2 Dilution curves of synthetic CCK8 ( $\Delta$ ) and pools of peaks of human CCK forms with antibody L48. Pools were prepared from appropriate tubes of column eluates of normal and celiac subjects. IA ( $\blacksquare$ ); IIA ( $\circ$ ); IIN ( $\blacktriangle$ ); IIIN ( $\bullet$ ).

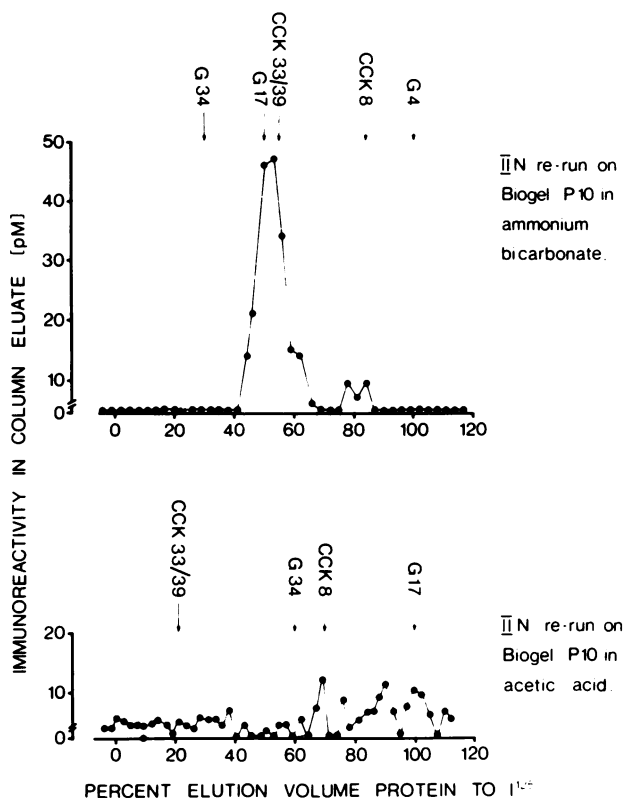


FIGURE 3 Refractionation of peak IIN on Biogel P-10 in ammonium bicarbonate (upper panel) and in acetic acid (lower panel). Note that the material emerges in its characteristic position when eluted with ammonium bicarbonate, and that poor recovery and resolution are obtained when eluted with acetic acid.

original positions when refractionated on Biogel P-10 in acetic acid. The two peaks occurred in significantly lower concentration in celiac compared with normal subjects, as did peaks IIIA and VA (Table II).

**Plasma CCK.** When normal postprandial plasma was concentrated by immunoaffinity adsorption and fractionated on Biogel P-10, antibody L48 revealed three peaks of immunoreactivity (Fig. 5). The first two peaks were identified as G34 and G17, respectively, since they eluted in the characteristic positions of these peptides and reacted similarly with L48 and 1296. The third peak generally accounted for  $<20\%$  total immunoreactivity and was identified as CCK8, since it eluted in the position of synthetic CCK8 and reacted weakly with 1296 compared with L48. There were no significant quantities of CCK8-like material in basal plasma of normal subjects ( $<0.8 \text{ pmol/liter}$ ), but measurable amounts were found in three of five normal subjects at 40 min after feeding and in all five subjects at 80 min. In sharp contrast, in five celiac patients there was no significant peak of CCK-like activity in basal plasma or at 40 or 80 min after feeding (Fig. 6).

TABLE II  
Concentrations of Immunoreactive CCK in Extracts of Normal  
and Celiac Duodenal Mucosa

		Concentrations		
		Normal	Celiac	
		pmol/g*		
Neutral extracts: total		214.4 ± 36.0 (n = 13)	88.4 ± 21.0 (n = 7)	P < 0.05
Biogel P 10 peak	IN	9.1 ± 3.3	4.7 ± 2.3	NSD
	IIN	37.1 ± 8.4	9.1 ± 1.6	P < 0.01
	IIIN	105.7 ± 20.3	43.5 ± 7.5	P < 0.01
Recovery†		70.6 ± 4.5	76.1 ± 9.4	
Acid extracts: total		203.2 ± 26.1 (n = 6)	78.3 ± 9.6 (n = 7)	P < 0.05
Biogel P 10 peak	IA	28.3 ± 4.8	11.1 ± 1.1	P < 0.05
	IIA	32.2 ± 7.5	6.0 ± 1.1	P < 0.05
	IIIA	7.3 ± 1.3	2.4 ± 0.6	P < 0.01
	IVA	14.6 ± 2.9	4.3 ± 1.3	NSD
	VA	20.7 ± 4.8	8.9 ± 2.0	P < 0.01
	VIA	14.1 ± 5.1	6.9 ± 1.7	NSD
	VIIA	11.6 ± 2.1	13.2 ± 2.6	NSD
Recovery†		74.9 ± 8.5	77.7 ± 11.8	

\* Concentrations expressed as means ± SE (Values for n, given in parentheses). Peak IN was defined as material eluting at 22–38%, peak IIN at 42–65% and peak IIIN at 70–106% of Biogel P-10 columns eluted with ammonium bicarbonate. Peak IA was defined as material eluting at 2–15%, peak IIA at 16–29%, peak IIIA at 30–42%, peak IVA at 50–66%, peak VA at 67–75%, peak VIA at 76–86%, and peak VIIA at 92–115% of Biogel P-10 columns eluted with acetic acid.

† Recovery is based on material eluting in the peaks defined above. Actual, or total, recovery was 80–100% for each group since a small and variable proportion of total immunoreactivity emerged in regions other than those included in the main peaks.

In a single case there were trace amounts of CCK8-like immunoreactivity in basal plasma and after 80 min. There were, however, significant quantities of G17- and G34-like immunoreactivity in the celiac samples (Fig. 5). Interestingly there was generally a higher proportion of G17- relative to G34-like immunoreactivity in postprandial plasma of celiacs compared with normals; the significance of the latter observation will be considered in more detail in a future report. When plasma samples of either celiac or normal subjects were fractionated on Biogel P-10 in acetic acid there were no measurable amounts of material eluting in the positions of IA or IIA found in tissue. Measurable quantities of activity were also absent from lyophilized immuno-affinity elutes that were redissolved in acetic acid, rather than ammonium bicarbonate.

## DISCUSSION

In the present study we have sought to characterize and estimate the major molecular forms of CCK in the plasma and intestine of normal, healthy subjects and patients with celiac disease. The study has revealed at least four major forms of immunoreactive CCK in nor-

mal human duodenal mucosa. The predominating form (IIN) had the properties of CCK8, while another form (IIA) had properties similar to CCK33 or CCK39 isolated from pig intestine (or both, since they are not separated in the systems we used). One of the other forms (IA) eluted earlier than CCK33/39 on gel filtration and so is probably a larger peptide, while the remaining form (IIN) could well be intermediate in size between CCK33 and CCK8. All four forms occurred in lower concentration in celiac mucosa compared with normals. In plasma of normal subjects after feeding we were able to demonstrate an increase of CCK8-like immunoreactivity, but significant amounts of CCK-like peptides were not consistently found in the circulation of celiacs after feeding. The results raise the possibility that the decreased postprandial circulating CCK in celiacs is a direct consequence of diminished tissue stores of hormone. These results provide a basis for interpretation of earlier studies in which it was suggested that circulating CCK might be decreased in celiac disease, because target organ responses (gall bladder and exocrine pancreas) to intraluminal stimuli were impaired, whereas responses to exogenous CCK were normal (8–10).

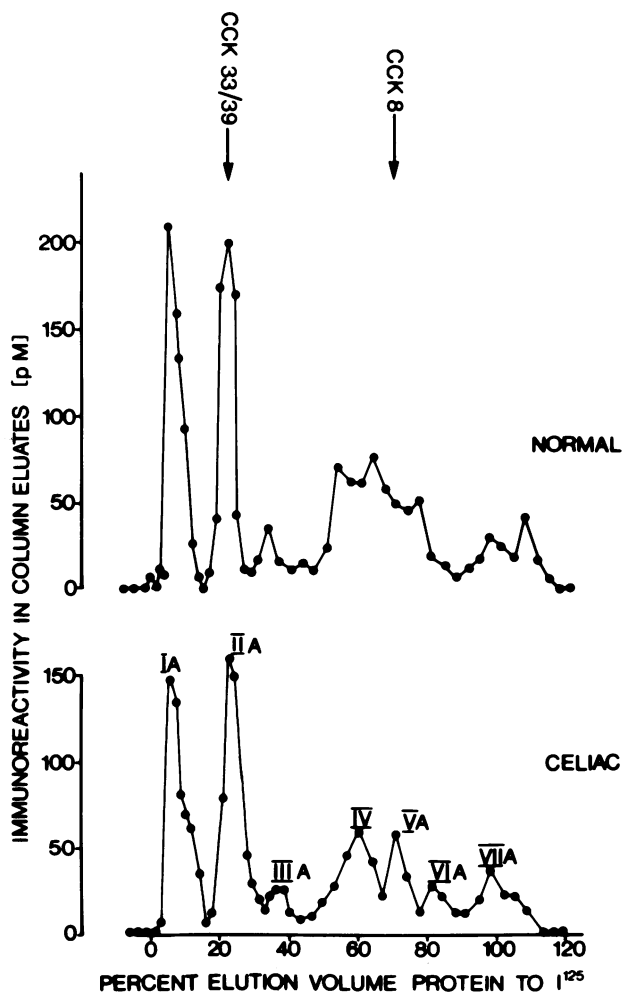


FIGURE 4 Separation on Biogel P-10 ( $1 \times 100$  cm,  $4^\circ\text{C}$ ,  $0.5$  M acetic acid containing  $0.1\%$  BSA) of acid extracts of duodenum from a normal subject (upper panel) and a celiac patient (lower panel). Results are shown of assays with antibody L48 (CCK8 standard). See Fig. 1 and text for further details.

There have been several recent studies on the molecular forms of immunoreactive CCK in the intestine of various animal species (3, 4, 11, 12, 15). Previously Rehfeld (4) has studied immunoreactive CCK in extracts of intestine taken from patients with pancreatic cancer. It is possible that pancreatic disease alters the forms of CCK in intestine. In normal subjects we found the ratio of CCK33-like activity to CCK8-like activity was about 1:4, whereas Rehfeld previously described relative proportions of about 1:8. Conceivably there might be a true difference between normal subjects and those with pancreatic cancer, or alternatively, the differences may be attributable to variation in technique. We took considerable care to obtain optimal extraction and fractionation conditions. Moreover, it may well be that other antisera, such as those specific

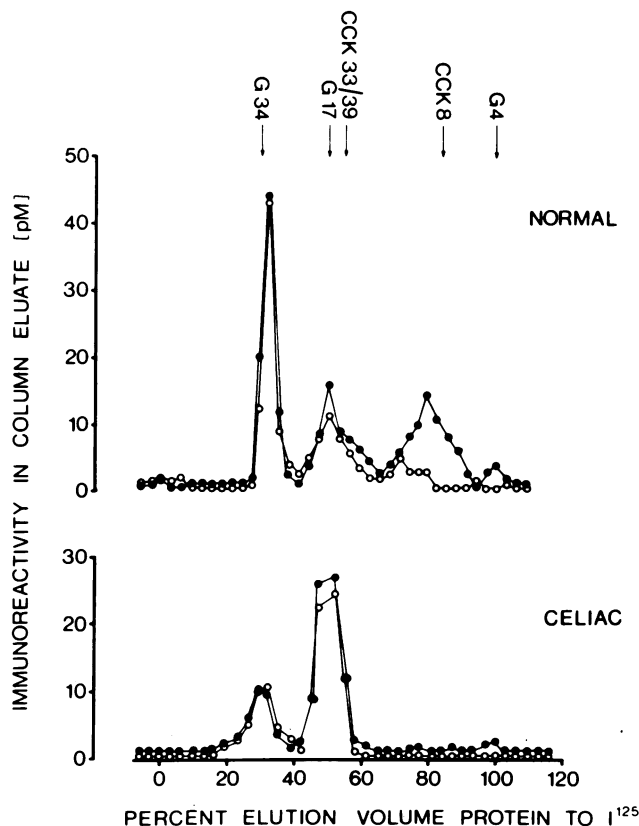


FIGURE 5 Separation on Biogel P-10 ( $1 \times 100$  cm,  $4^\circ\text{C}$ ,  $0.05$  M ammonium bicarbonate) of immuno-affinity concentrate of 80-min postprandial plasma from a celiac subject (lower panel), and normal subject (middle and upper panel). The middle and upper panels show elution patterns of the same column after assay with L48 (CCK8 standard) and 1296 (G17 standard), respectively. The lower panel shows assays with antibody L48. See Fig. 1 for details.

for the mid- or  $\text{NH}_2$ -terminal regions of CCK would reveal a different pattern. Such antisera are probably of limited use in human studies in any case, as there

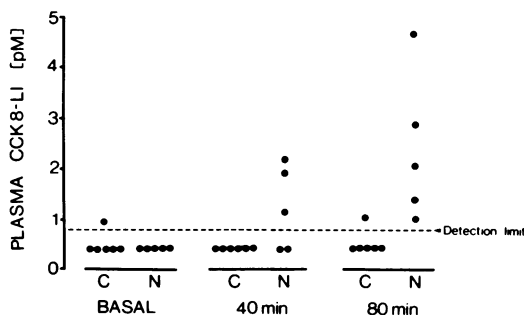


FIGURE 6 Plasma CCK8 concentrations estimated from integration of peaks on Biogel P-10 of fasting, and 40-min and 80-min postprandial plasma from normal subjects (N) and celiac patients (C). Detection limit was  $0.8$  pmol/liter, assuming a  $30$ -ml starting volume of plasma.

is evidence of species differences in the antigenic determinants of the mid- and  $\text{NH}_2$ -terminal regions CCK (15, 16), and all presently available antisera have been raised to porcine CCK or its fragments. Estimation of the true concentrations of the human peptides must obviously await their isolation and characterization. The approach we have taken for the estimation of immunoreactive CCK depends on the use of C-terminal specific antibodies that react equally with CCK8, CCK33, and the gastrins. These antibodies have the merit of reacting with biologically active forms of the hormone, but not inactive mid- or  $\text{NH}_2$ -terminal fragments. In the present study immunoreactive gastrins were identified in a second RIA using an antibody that reacts weakly with CCK. It is important to emphasize that the present estimates of tissue or plasma CCK are not based on subtraction of immunoreactive gastrin from total C-terminal immunoreactivity. In particular, we carefully selected chromatographic systems that separated the molecular variants of gastrin and CCK, so that it was possible to integrate peaks corresponding to different forms of CCK to obtain direct and definitive estimates of their concentrations.

Immunocytochemical studies indicate that CCK occurs in both endocrine cells and nerves in the gut (17). However, CCK-containing nerve fibers are relatively sparse in duodenal mucosa, whereas endocrine cells are abundant. Thus immunoreactive CCK in the mucosal biopsies we studied is likely to have originated mainly from endocrine cells. Previous immunocytochemical studies have indicated that celiac disease CCK endocrine cells are more numerous and more intensively stained than in normal subjects (18, 19). These reports are difficult to relate to the present findings. It is, however, worth emphasizing that immunocytochemistry is scarcely an ideal method for estimating concentrations of peptides in tissue.

Several earlier studies on concentrations of CCK-like immunoreactivity in human plasma after feeding have suggested concentrations considerably higher (up to 16 ng/ml, or  $\sim 4$  nmol/liter) than those reported here (20–22). In addition, it has been reported that basal CCK concentrations in celiacs are higher than normal, but postprandial increases are less than normal (23). It seems probable that in these studies plasma hormone concentrations were overestimated for several reasons, among which are poor quality tracer, poorly characterized antisera, difficulties with standards, and non-specific effects of plasma proteins (24). Recently, Lamers et al. (25) studied circulating CCK-like immunoreactivity in normal subjects after intraduodenal fat, using a similar approach to the one used here, i.e., immuno-affinity concentration of plasma CCK followed by gel filtration. These authors found increases of CCK8-like activity up to  $\sim 10$  fmol/ml in peripheral plasma, and like us, did not find evidence

of significant increases of other forms of CCK. There appears to have been no previous attempts to document molecular forms of immunoreactive CCK in plasma after a normal meal. The increases found in the present study (1–4.6 pmol/liter) are lower than those of Lamers et al. (25), but this is readily attributable to the fact that we used a light meal, while they gave a relatively stronger stimulus for CCK secretion (intraduodenal fat). Infusions of exogenous CCK8 in doses that produce peripheral plasma concentrations of  $\sim 10$  pmol/liter have been shown to stimulate about half-maximal pancreatic enzyme output in the presence of secretin (26). The concentrations of CCK8-like immunoreactivity found in the present study are therefore likely to be within the dynamic range for stimulation of the exocrine pancreas. The absence of CCK33-like peptides, or of other forms of CCK from plasma, need not be particularly surprising because concentrations of these forms in tissue are only  $\sim 20\%$  those of CCK8. If relative proportions of different CCK forms in plasma resemble those in tissues, the concentrations of large forms in plasma would be below our limit of detection.

It is of interest that at least two other upper intestinal hormones, secretin and glucose-dependent insulin-releasing peptide, have also been shown to be depressed after feeding in celiacs, whereas hormonelike peptides from the ileum, eg. neurotensin and the enteroglucagons, are elevated (27). It is possible that the small pancreatic responses to feeding that have been recorded in celiacs (10) are mediated by enteropancreatic nervous reflexes of the type postulated by Solomon and Grossman in the dog (28). Nevertheless, the data now available strongly support the view that there is impaired upper intestinal endocrine function in celiacs and that this accounts for the diminished gall bladder and pancreatic function of these patients.

#### ACKNOWLEDGMENTS

We are grateful to Dr. R. B. McConnell for allowing us to study his patients. Mrs. Eva McElroy kindly typed the manuscript.

We are also grateful to the Medical Research Council for supporting this work.

#### REFERENCES

1. Mutt, V. 1980. Cholecystokinin: isolation, structure and functions. In *Gastrointestinal Hormones*. G. B. J. Glass, editor. Raven Press, New York. pp 169–221.
2. Dockray, G. J., R. A. Gregory, J. B. Hutchison, J. I. Harris, and M. J. Runswick. 1978. Isolation, structure and biological activity of two cholecystokinin octapeptides from sheep brain. *Nature (Lond.)* 274: 711–713.
3. Dockray, G. J. 1977. Immunoreactive component resembling cholecystokinin octapeptide in intestine. *Nature (Lond.)* 270: 359–361.
4. Rehfeld, J. A. 1978. Immunochemical studies on cho-



- lecystokinin. II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog. *J. Biol. Chem.* **235**: 4022-4030.
5. Rubin, B., S. L. Engel, A. M. Drugis, M. Dzelalus, E. O. Grigas, M. H. Waugh, and E. Yiacas. 1969. Cholecystokinin-like activities in guinea pigs and dogs of the C-terminal octapeptide (SQ 99, 844) of cholecystokinin. *J. Pharm. Sci.* **58**: 955-959.
  6. Ondetti, M. A., B. Rubin, S. L. Engel, J. Pluscec, and J. T. Sheehan. 1970. Cholecystokinin-pancreozymin. Recent development. *Am. J. Dig. Dis.* **15**: 149-156.
  7. Debas, H. T., and M. I. Grossman. 1973. Pure cholecystokinin: pancreatic protein and bicarbonate response. *Digestion.* **9**: 469-481.
  8. Di Magno, E. P., V. L. W. Go, and W. H. J. Summerskill. 1969. Pancreozymin secretion is impaired in sprue. *Gastroenterology.* **56**: 1149.
  9. Low-Beer, T. S., K. W. Heaton, S. T. Heaton, and A. E. Read. 1971. Gall bladder inertia and sluggish enterohepatic circulation of bile salts in coeliac disease. *Lancet.* **I**: 991-994.
  10. Di Magno, E. P., V. L. W. Go, and W. H. J. Summerskill. 1972. Impaired cholecystokinin-pancreozymin secretion, intraluminal dilution and maldigestion of fat in sprue. *Gastroenterology.* **63**: 25-32.
  11. Muller, J. E., E. Straus, and R. S. Yalow. 1977. Cholecystokinin and its COOH-terminal octapeptide in the pig brain. *Proc. Natl. Acad. Sci. U. S. A.* **74**: 3035-3037.
  12. Dockray, G. J. 1980. Cholecystokinins in rat cerebral cortex: identification, purification and characterization by immunochemical methods. *Brain Res.* **188**: 155-165.
  13. Dockray, G. J., and J. H. Walsh. 1975. Amino terminal gastrin fragment in serum of Zollinger-Ellison syndrome patients. *Gastroenterology.* **68**: 222-230.
  14. Dockray, G. J., and I. L. Taylor. 1976. Heptadecapeptide gastrin: measurement in blood by specific radioimmunoassay. *Gastroenterology.* **71**: 971-976.
  15. Straus, E., and R. S. Yalow. 1978. Species specificity of cholecystokinin in gut and brain of several mammalian species. *Proc. Natl. Acad. Sci. U. S. A.* **75**: 486-489.
  16. Go, V. W. L., R. J. Ryan, and W. H. J. Summerskill. 1971. Radioimmunoassay of porcine cholecystokinin-pancreozymin. *J. Lab. Clin. Med.* **77**: 684-689.
  17. Larsson, L-I., and J. F. Rehfeld. 1979. Localization and molecular heterogeneity of cholecystokinin in the central and peripheral nervous system. *Brain Res.* **165**: 201-218.
  18. Polak, J. M., S. R. Bloom, M. V. McCrossman, C. M. Timson, D. R. Johnston, D. Hudson, M. Szelke, and A. G. E. Pearse. 1978. Cholecystokinin abnormalities in coeliac disease. *Gastroenterology.* **74**: 1097.
  19. Sjölund, K., J. Alumets, N. O. Berg, R. Håkanson, and F. Sundler. 1979. Duodenal endocrine cells in adult coeliac disease. *Gut.* **20**: 547-552.
  20. Reeder, D. D., H. D. Becker, N. J. Smith, P. L. Rayford, and J. C. Thompson. 1973. Measurement of endogenous release of cholecystokinin by radioimmunoassay. *Ann. Surg.* **178**: 304-310.
  21. Go, V. W. L., S. Cataland, and W. M. Reilly. 1974. Radioimmunoassay (RIA) of cholecystokinin-pancreozymin (CCK-PZ) in human serum. *Gastroenterology.* **66**: 700.
  22. Harvey, R. F., L. Dowsett, M. Hartog, and A. E. Reed. 1973. A radioimmunoassay of cholecystokinin-pancreozymin. *Lancet.* **II**: 826-828.
  23. Low-Beer, T. S., R. F. Harvey, E. R. Davies, and A. E. Read. 1975. Abnormalities of serum cholecystokinin and gall bladder emptying in coeliac disease. *N. Engl. J. Med.* **292**: 961-963.
  24. Dockray, G. J. 1979. Gastro-intestinal hormones II. Gastrin, cholecystokinin and secretin. In *Hormones in Blood*. C. H. Gray and V. H. T. James, editors. pp. 355-399.
  25. Lamers, C. B., J. E. Valenzuela, and J. H. Walsh. 1979. Demonstration of cholecystokinin (CCK-8) like immunoreactivity in the circulation of man after introduction of fat. *Gut.* **20**: A925. (Abstr.)
  26. Valenzuela, J. E., C. B. Lamers, G. Buga, I. M. Modlin, and J. H. Walsh. 1979. Comparative study of circulating cholecystokinin (CCK) and CCK octapeptide (CCK8) as stimulants of pancreatic secretion in man *Gut.* **20**: A925. (Abstr.)
  27. Besterman, H. S., S. R. Bloom, D. L. Sarson, A. M. Blackburn, D. I. Johnson, H. R. Patel, J. S. Stewart, R. Modigliani, S. Guerin, and C. N. Mallison. 1978. Gut hormone profile in coeliac disease. *Lancet.* **I**: 785-788.
  28. Soloman, T. E., and M. I. Grossman. 1979. Effect of atropine and vagotomy on response of transplanted pancreas. *Am. J. Physiol.* **236**: E186-190.