II. DISTRIBUTION AND MOLECULAR HETEROGENEITY IN THE CENTRAL NERVOUS SYSTEM AND SMALL INTESTINE OF MAN AND HOG*

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Using sequence-specific radioimmunoassays, the distribution and heterogeneity of cholecystokinin (CCK) has been determined in extracts of tissues from the central nervous system and small intestine of adult man and hog. The CCK determinations were correlated with measurements with a radioimmunoassay specific for the structurally related peptide hormone gastrin. The amount and molecular forms of CCK extracted from brain as well as gut tissue were pH-dependent in that boiling at neutral pH released mainly small molecular forms, whereas acid released mainly large forms. Consequently, the tissue was first boiled at pH 6.6, after which acetic acid was added to pH 2.5. Large amounts of CCK were found in the central nervous system with a differential distribution: cerebral cortex, 200 to 2300 pmol/g; hypothalamus, 24 to 220 pmol/g; mesencephalon, 13 to 93 pmol/g; and brain stem, 6 to 43 pmol/g. In the pineal body, the predominating molecular forms corresponding to CCK\textsubscript{33} and CCK\textsubscript{39} (K<sub>v</sub> = 0.50 to 0.55, 8% of the immunoreactivity); 3) molecular forms presumably corresponding to the COOH-terminal dodecapeptide of CCK\textsubscript{33} (K<sub>v</sub> = 0.63 to 0.74, 6% of the immunoreactivity); 4) a large fraction corresponding to the COOH-terminal octapeptide of CCK\textsubscript{39} (K<sub>v</sub> = 1.10, 60% of the immunoreactivity); and finally 5) two peptides corresponding to each half of the COOH-terminal octapeptide (K<sub>v</sub> = 1.25 to 1.30, 16 to 20% of the apparent immunoreactivity). The existence and character of the different molecular forms of CCK were substantiated by rechromatography, chromatography with an 8 M urea gradient, affinity chromatography, and tryptic cleavage. The results suggest (a) that CCK and gastrin are distributed in both brain and gut in a unique, differential manner; (b) that the amount of CCK in the brain by far exceeds the amount of any other known hormonal brain peptide; (c) that CCK in both brain and gut is heterogeneous, the predominating molecular forms corresponding to the small COOH-terminal octa- and tetrapeptides of CCK\textsubscript{33}.

An important feature of peptide hormones is their molecular heterogeneity, i.e., the hormones are secreted as peptides of different size and biological activity within the same species. A fruitful way to study the heterogeneity of a known hormone has proved to be radioimmunochemical monitoring of tissue fractionation. Maximal information using such procedures is obtained by application of sequence-specific radioimmunoassays, which are assays that in a well defined manner recognize different regions of the hormonal sequence (1). In doing so it is possible to predict essential parts of the structure of the variant molecular forms of a hormone.

Cholecystokinin* is a hormone which regulates enzyme secretion from the pancreas and gallbladder emptying. It has been purified as a triacontatriapeptide and a triacontanonepeptide (2-4) from hog intestine. Recent measurements with cross-reacting gastrin assays have suggested that CCK\textsuperscript{1}-like peptides besides being located in the intestine are present also in brain tissue (5).

The preceding paper described the development and character of sensitive sequence-specific radioimmunoassays for CCK, cholecystokinin; CCK\textsubscript{33}, triacontanonepeptide cholecystokinin; CCK\textsubscript{39}, triacontatetrapeptide cholecystokinin; CCK<sub>α</sub>, COOH-terminal octapeptide of CCK\textsubscript{39}; gastrin<sub>α</sub>, heptadecapeptide gastrin; gastrin<sub>β</sub>, triacontatetrapeptide gastrin.

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jejuni were obtained at surgery at the University Hospitals in Copenhagen and Aarhus and immediately frozen on dry ice. Specimens of human brains, duodeni, and jejunum were morphologically normal from resections due to pancreatic cancers (Whipple's resection). The frozen tissues were small pieces (0.86, 2.40, and 3.42 g) of morphologically normal tissue in the periphery of resections of tumors in the frontal and parietal lobes. The specimens of human duodenum and jejunum were morphologically normal from resections due to pancreatic cancers (Whipple's resection). The frozen tissues were stored at -80°C. They were then weighed and extracted in the same way.

**Tissues**—Porcine brains and small intestines were obtained at a local abattoir from freshly killed pigs. The interval between killing of the hogs and freezing of tissue on dry ice was 15 to 20 min. In addition, brains were resected from three anesthetized pigs (Department of Experimental Surgery, Rigshospitalet, Copenhagen). These brains were immediately dissected on an ice-bath, and each region was homogenized in 5 ml of 0.05 M sodium phosphate, pH 7.5, or by acid extraction with 3% 0.5 M acetic acid, pH 2.5, which was added to the homogenate. The homogenates were centrifuged for 20 min at 15,000 x g (Servall). The supernatant fractions were decanted and frozen at -20°C until they were assayed in duplicate by sequence-specific radioimmunoassays in the dilution range from 1/10 to 1/1,000,000. Thus, the measurements were calculated as the mean of those dilutions resulting in concentrations measured within the working range of the assays, which generally required two or three degree of dilution.

**Validation of Extraction Procedures**—Control extractions with and without trypsin treatment of porcine jejunal tissue were performed using the synthetic COOH-terminal octapeptide CCK2, respectively, were performed with both boiling water extractions at neutral pH (6.6) and acid extractions (pH 2.5) of jejunal and cortical tissue. The extracts were assayed in duplicate in dilutions from 1/10 to 1/10,000,000 in order to measure recovery. 1.5-ml extracts taken for gel chromatography were described below in order to determine the molecular forms of CCK in the brain and jejunum.

**Fractionation of Tissue Extracts**—2- to 6-ml extracts were applied to Sephadex G-50 (superfine) columns (25 x 2000 mm) eluted with 0.25 M ammonium bicarbonate, pH 8.2, at 4°C at a flow rate of 20 ml/h. Fractions of 3.0 ml were collected. All columns were calibrated with 1-albumin and NaCl (Amersham) for indication of void volume and total volume. Besides, the columns were calibrated with 99% pure porcine CCK2 and CCK9 (generous gift from V. Mutt, Karolinska Institute, Stockholm), synthetic COOH-terminal octapeptide (SQ 19,844, batch NNO11NB), and synthetic COOH-terminal tetrapeptide (ICI, Alderley Park, Cheshire, England). The existence and nature of the different molecular forms in an extract of porcine jejunal and brain were substantiated by the following procedures: 1) refiltration on the Sephadex G-50 (superfine) columns of the individual components after pooling of fractions, lyophilization, and reconstitution in 2.0 ml of ammonium bicarbonate, pH 8.2; 2) dilution of the individual component and comparison of the dilution curves with CCK2 and synthetic fragments of CCK9; 3) gel filtration in an 8 M urea (Aristar) gradient as previously described (7); 4) gel filtration of the larger molecular forms of CCK after incubation with trypsin previously treated with L-1-tosylamide-2-phenylthethyl chloromethyl ketone, 10 μg of trypsin (Lot 37D778, Worthington) per ml for 20 min at 20°C; 5) gel filtration of the extracts after incubation with an immunosorbsorbent prepared as previously described (8) using antisera 2609 raised against synthetic heptadecapeptide gastrin and reacting against the common COOH-terminal pentapeptide sequence of gastrin and CCK (type A assay). Antiserum 4698 was used in the assay specific for region 25 to 30 of CCK2. This assay measures neither the COOH-terminal octapeptide nor tetrapeptide of gastrin nor any form of porcine CCK (type A assay). Antiserum 4698 was used in the assay specific for region 29 to 33 of CCK2. This antiserum, originally raised against heptadecapeptide gastrin (9), binds all cholecystokinin and gastrins containing the biologically active COOH-terminal tetrapeptide common to gastrin and CCK (type C assay). In all three CCK assays 2-1-hydroxyphenylpropionic acid-succinimide ester coupled to CCK2 was used as tracer. 99% pure porcine CCK2 was used as standard in type A assays and synthetic porcine CCK2 as standard in type B and C assays.

In addition to the CCK radioimmunoassays, one gastrin radioimmunoassay was used for control and reference. Antiserum 2604 raised against human heptadecapeptide gastrin was used in this assay (9). It reacts with the COOH-terminal half of gastrin2. It is the largest molecular form of gastrin (10) with equimolar potency (11), whereas it binds <0.002 M CCK2 and <0.008 μM CCK2 as compared to gastrin2 on a molar base at inhibition dose 50. Monooiodinated gastrin2 was used as tracer (12), and synthetic human gastrin2 was used as standard in this gastrin assay (type D assay).

**RESULTS**

**Effect of Extraction Procedure on Cerebral CCK**—At neutral and as acid pH large amounts of immunoreactive CCK were extracted from porcine cerebrum (Table I). By boiling in neutral pH, however, 4 to 6 times more CCK was extracted than by acid extraction. Upon gel chromatography it appeared that at neutral pH 90% (mean, n = 6) of the cerebral CCK immunoreactivity was eluted in a peak at a position corresponding to that of CCK2. The remaining immunoreactivity was eluted later (Kn = 1.30) in a position similar to that for the COOH-terminal tetrapeptide of CCK2 (Fig. 2). At acid pH the smaller amount of CCK extracted was eluted mainly in three positions. The first peak constituting 90% of the immunoreactivity eluted in a position corresponding to that of CCK2. The second peak constituted approximately 10% of the immunoreactivity and eluted in a position corresponding to the COOH-terminal dodecapeptide of CCK9. Finally, the majority of the immunoreactivity (70%) eluted like CCK9 (Fig. 2).

Addition of exogenous CCK2 (Table I) or CCK9 (Table II) to boiling water or to acetic acid per se did not destroy the immunoreactivity nor degrade the added CCK to smaller molecular forms as revealed by gel chromatography. Of exogenous CCK2 added to frozen cerebral tissue pieces extracted...
Effect of Extraction Procedure on Jejunal CCK - At both neutral and acid pH large amounts of CCK were extracted from porcine jejunum. At neutral pH, however, twice as much CCK was extracted as at acid pH (Table I). Gel chromatography showed five different main components of CCK. The first fractions containing immunoreactive CCK were eluted in position corresponding to gastrin component I and proinsulin ($K_v = 0.50$) and CCK$_{38}$ ($K_v = 0.59$). The second region of CCK immunoreactivity was in a position corresponding to CCK$_{15}$ ($K_v = 1.10$). Finally, extracted at pH 6.6 an immunoreactive peak was eluted in a position corresponding to the COOH-terminal tetrapeptide of CCK$_{38}$ ($K_v = 1.30$). By extraction at acid pH the tetra- and octapeptide-like component constituted 92% of the total immunoreactivity (mean, $n = 6$), whereas the three components eluted earlier constituted from 1 to 8% of the immunoreactivity (Fig. 3). By extraction at acid pH the first peak comprised 20%, the third peak 11%, and finally the octapeptide-like peak the remaining 43% (Fig. 3). Based on these results it was decided for further extractions to boil intestinal tissue first in water at pH 6.6 and then to add acetic acid to pH 2.5 in order to obtain maximal extraction of both large and small molecular forms and CCK.

**Distribution of CCK in Central Nervous System** — The concentration of immunoreactive CCK in the different regions of the central nervous system varied from immeasurable levels to 2.3 nmol/g of tissue (Table III). The highest concentrations were found in the telencephalic cortex, the frontal, parietal, temporal, and occipital lobes. Also the rhinencephalic regions, especially the olfactory area, contained large amounts of immunoreactive CCK. The concentrations of CCK in the diencephalon were of an order of 10 times below the telencephalic concentrations. In the diencephalon, the hypothalamus contained the highest concentrations, 0.1 nmol/g of tissue. The more distal regions contained progressively less CCK. Some regions appeared devoid of CCK immunoreactivity. These regions were the epithalamus, including the pineal body, the mesencephalon including the cerebellum and pons, the spinal cord, and the pituitary. Also corpus callosum and the optic tract contained very little CCK.

**Molecular Forms of CCK in Central Nervous System** — As shown in Table III, the concentrations of CCK in the central nervous systems of hogs varied considerably depending on the assay used. The highest concentrations were, except for the pituitary gland, always measured with type B and type C assays and always in a similar order of magnitude. Since type A and type D assays measured much lower concentrations, the findings suggest that the predominating molecular form of CCK is the COOH-terminal octapeptide of CCK$_{38}$. This suggestion was confirmed by gel chromatography of extracts from both telencephalon (gray cortex and olfactory area), diencephalon (hypothalamus), mesencephalon (nigral substance), and myelencephalon (vagal nucleus). Fig. 4 shows the elution pattern of an extract from the porcine parietal lobe of the cortex. The biopsies from human cerebral cortex contained immunoreactive CCK in concentrations of a magnitude similar to those found in porcine cerebral cortex (Table IV); and the predominating molecular form in human cerebral also resembled the COOH-terminal octapeptide of CCK$_{38}$ (Fig. 5). Both porcine and human brains contained, in addition to the octapeptide-like CCK, a larger molecular form of CCK with chromatographic and radioimmunochemical characteristics like CCK$_{38}$. This form constituted 2 to 5% of the immunoreactivity. Finally, the brain tissue contained little immunoreactivity apparently corresponding to the COOH-terminal tetra-
peptide of CCK and gastrin (Figs. 4 and 5).

**Gastrin in Central Nervous System** — While the gastrin assay (type D assay) measured nothing or very low concentrations of immunoreactivity in most brain regions (Table III), and while these small amounts of immunoreactivity reflected only the weak cross-reactivity of the octapeptide-like CCK in the gastrin assay (Figs. 4 and 5), the pattern differed in the posterior lobe of the pituitary and the pituitary stalk (Table III). In the pituitary neither of the specific CCK assays (type A and B assays), devoid of cross-reactivity with gastrin, measured any immunoreactivity, while the cross-reacting CCK assay (type C) and the more specific gastrin assay measured substantial amounts of true gastrin immunoreactivity.

**Distribution of CCK in Small Intestine** — As shown in Table V the concentration of CCK varied considerably in the upper intestinal tract of hogs when measured with the four different types of assays. A similar distribution and variation were found in the intestinal tract of man (Table VI). In the antral mucosa the specific CCK assays, type A and B, did not measure significant amounts of immunoreactivity, whereas the cross-reacting type C assay and the more specific gastrin type D assay measured high concentrations. The midportion of the duodenum contained comparatively low concentrations of gastrin, whereas high concentrations of immunoreactive CCK were found. The jejunum contained similar amounts of CCK as found in duodenum, whereas the content of gastrin as measured with type D assay was minute.

**Molecular Forms of CCK in Proximal Small Intestine** — Since type B and C assays measured the highest concentrations and concentrations in a similar range (1 to 2 nmol/g of tissue) in duodenum and jejunum, whereas type A assays measured concentrations between 0.2 and 0.9 nmol/g of tissue, the findings indicate that the predominating molecular form of CCK corresponds to the COOH-terminal octapeptide of CCK\(_{26}\). By gel chromatography the interpretation of measurements on extracts was confirmed. Almost identical patterns of immunoreactive CCK components were found in duodenum and jejunum, without significant species differences between man and hog (Figs. 6 and 7). Immunoreactive CCK was eluted mainly in five positions as described above in the comments on the extraction procedure. The gastrin assay (type D) showed that small amounts of gastrin immunoreactivity also eluted in four positions: component I, component II (gastrin)\(_{17}\) with a \(K_v\) of 0.42, component III (gastrin)\(_{13}\) with a \(K_v\) of 0.76, and finally a peak corresponding to the COOH-terminal octapeptide of CCK\(_{26}\), which cross-reacts weakly with antisemum 2604.

**Validation of Existence of Different Molecular Forms of CCK** — By refiltration of the various molecular forms of CCK in extracts of porcine jejunum, they eluted in positions corresponding to those found by gel filtration of the crude extract (Fig. 8). In an 8 M urea gradient the position of the individual peaks remained unchanged (Fig. 9). Immunosorption using a gastrin antisemur with COOH-terminal reactivity removed all CCK components measured with an assay specific for sequence 29 to 33 of CCK\(_{26}\) (type C assay), which suggests that none of the peaks measured with this assay were due to unspecified interference. Also, the peaks measured by type A assay, presumably specific for sequence 20 to 25 of CCK\(_{26}\), were removed. However, type B assay, specific for sequence 25 to 30 of CCK\(_{26}\), still revealed one peak after immunosorption with the gastrin antisemur. This peak was eluted after CCK\(_{26}\) with an elution constant of 1.30 (Fig. 9). Incubation with trypsin displaced immunoreactive CCK to the position corresponding to CCK\(_{26}\) (Fig. 10). The CCK\(_{26}\)-like peptide in porcine jejunal extracts showed a dilution curve parallel to that of pure porcine CCK\(_{26}\) (Fig. 11A). The CCK\(_{26}\)-like materials in porcine jejunal and cerebral extracts diluted in parallel with synthetic porcine CCK\(_{26}\) (Fig. 11B), and material from the peak corresponding to the COOH-terminal tetrapeptide in extracts from porcine jejunum and cerebral diluted in parallel with synthetic COOH-terminal tetrapeptide (Fig. 11C).

**DISCUSSION**

The results presented in this paper argue that CCK, although first isolated from the small intestine of hogs, is also widely distributed throughout the central nervous system in a unique differential manner and in amounts beyond those known for other hormonal peptides in the brain. Second, the results indicate that CCK in tissues is more heterogeneous than previously known, without significant species differences between man and hog. The predominating molecular forms in brain and gut from both species are peptides resembling the COOH-terminal octa- and tetrapeptides of CCK\(_{26}\). Finally, the results demonstrate that the central nervous system, in addition to its content of different molecular forms of CCK, also contains the related gastrointestinal hormone gastrin differentially located in the pituitary.

**Extraction of CCK from Tissue** — The study emphasizes the importance of evaluation of the extraction procedure for hormones present in different molecular forms. While the different gastrin components are all extracted in maximal amounts using boiling water at neutral pH (13-15), the structurally related CCK components differ. The larger molecular forms of CCK in intestines and nervous tissue are, thus, only partly extracted at neutral pH, whereas the octa- and tetrapeptide-like CCK are extracted in maximal amounts. Conversely, the large molecular forms of CCK are extracted more efficiently at acid pH, at which the small forms are only partly released (Figs. 1 and 2). The rational procedure is, consequently, first to boil the tissue at neutral pH to release the octa- and tetrapeptide-like CCK and then to add acid to release the larger molecular forms. Since these, like CCK\(_{26}\) and CCK\(_{26}\) (I), are readily dissolved in water or buffers at neutral pH (Tables I and II), the necessity for acid probably reflects ionic binding in the tissue of the large molecular forms of CCK. The pH-dependent extraction explains in part why the small molecular forms of CCK, in particular the highly bioactive COOH-terminal dodecapeptide- and octapeptide-like CCK (16), were not previously discovered in the intestines since acid extraction was used to isolate CCK (2-4, 17). Moreover, failure of algicin acid to absorb the octa- and tetrapeptides, late elution of these peptides on Sephadex G-50 and G-25 columns, and finally disposal of water used for initial boiling of the intestine (2-4, 41) may further explain the discrepancy between the present and earlier extraction results with regard to the molecular nature of CCK in the intestinal tissue.

Several lines of evidence support the contention that some of the radioimmunoassayable molecular forms of CCK in brain and in gut are closely related and probably identical with the known triacontatriapeptide CCK and its COOH-terminal octa- and tetrapeptide. (a) Parts of the radioimmunoassayable CCK in brain and gut extracts elute by gel chromatography on Sephadex G-50 (and G-25 using different buffer systems) in positions identical with those of CCK\(_{26}\), CCK\(_{26}\), and the COOH-terminal tetrapeptide (Figs. 3 and 5).
(b) The substances in cerebral and intestinal extracts chromatographically similar to CCK₁₀, CCK₈, and the COOH-terminal tetrapeptide react with the four different sequence-specific antisera in proportions identical with the ¹²⁵I-CCK displacements of the pure peptides (Figs. 3 to 6, Fig. 11, and Table III in Ref. 6). (c) Trypsin known to cleave the peptide bond between the arginine and aspartic acid residues in positions 25 and 26 cleaves all the larger molecular forms to the octapeptide-like CCK (Fig. 10). The same lines of evidence as discussed above support the contention that porcine and human CCK are very closely related, at least in the COOH-terminal half of their sequence.

**Distribution and Heterogeneity of CCK in Central Nervous System**—In recent years an increasing number of hormonal peptides have been isolated or demonstrated by radioimmunoassays with a dual localization in both the central nervous system and the intestinal tract. Some of these peptides, substance P (18, 19), somatostatin (20, 21), neurotensin (22, 23), and enkephalin (24, 25) were first isolated from brain tissue. Of peptides primarily isolated from the gut, vasoactive intestinal polypeptide (VIP) was recently located in the central nervous system (26, 27). The present study extends the list of gut peptides located in the central nervous system with two classical hormones, CCK and gastrin. It has previously been suspected that a small peptide related to CCK was present in the brain, since gastrin radioimmunoassays that cross-reacted with CCK indicated that the gastrin-like peptide earlier reported (28) rather resembled the COOH-terminal region of CCK₁₀ (6). However, the present results based on specific CCK radioimmunoassays give direct evidence for the existence of peptides similar to native CCK octapeptide in the brain. Moreover, the results argue that molecules similar to the large CCK₁₃ and the small COOH-terminal tetrapeptide also are present in brain tissue. Finally, the simultaneous use of assays specific for both CCK and gastrin demonstrated that not only CCK but also proper gastrin are located in the central nervous system (Table III). Further studies have shown that pituitary gastrin in the pituitary almost exclusively is present as gastrin₁₇ (“big” gastrin) and gastrin₁₇ (29).

The distribution pattern for CCK in the central nervous system is unique and differs markedly from that of other hormonal brain peptides like thyrotropin-releasing hormone (TRH) (30, 31), somatostatin (32), substance P (33), vasoactive intestinal polypeptide (26, 27), neurotensin (21), and luteinizing hormone-releasing hormone (LHRH) (34, 35). While the latter peptides are all located mainly in the hypothalamus and present only sporadically and in low concentrations in the cerebral cortex, CCK is predominantly located to the cerebral cortex, particularly in the neocortical part of the telencephalon, the pallium (Table III), which dominates the central nervous system in man. CCK is, however, still present in substantial amounts in the diencephalon, mesencephalon, and the brain stem (Table III). In all regions CCK appears like the other peptides located in neurons (36, 37).

Another unique feature of cerebral CCK is the amount present in the brain. While the other hormonal peptides mentioned above mainly are found in the hypothalamus in concentrations of the order 10 to 100 pmol/g of tissue (21, 26, 30–35), hypothalamic CCK is present in similar concentrations, 25 to 100 pmol/g (Table III). However, in addition, CCK is present in concentrations 10 to 20 times higher in cortex. Since adult porcine and human hypothalamus constitute only 3 to 4 g, whereas the weight of cortex in man for instance is 400 g (38), the brain in pig and man thus contains on an estimate approximately 10⁶ times more CCK than any of the other peptides on a molar basis. Thus, human brains contain totally 1 to 2 mg of CCK₁₀, whereas other hormonal peptides are present in microgram amounts. The central nervous system also contains on a molar basis more CCK than is present in the small intestine in man.

CCK in the brain is heterogeneous. A minor fraction corresponds to CCK₁₃, but the predominating form appears similar to CCK₈. However, based on chromatographic behavior and immunoreactivity it can be assumed that the brain also contains a peptide closely related to the COOH-terminal fragment of CCK₁₃, Trp Met Asp Phe NH₂ (Figs. 1, 4, and 5). Since the potency of this tetrapeptide is much smaller than that of the octapeptide, used as standard, with respect to displacement of ¹²⁵I-CCK₁₃ from antiserum 2609 (Fig. 11 and Table III in Ref. 6), the true molar concentrations of the tetrapeptide-like substance are 30 times above those indicated in Figs. 4 and 5. Thus, the tetrapeptide-like fragment of CCK₁₃, which quantitatively appears to predominate in cortical tissue, may be the principal chemical messenger in the family of CCK- and gastrin-like peptides, and the larger molecular forms may merely be biosynthetic precursors. This hypothesis is partly supported by the observation that the tetrapeptide on an equimolar basis releases insulin more potently than the larger heptadecapeptide gastrin (39). It is not likely that the COOH-terminal octa- and tetrapeptide-like fragments of CCK₁₃ are artifacts due to degradation of CCK₁₃ during the extraction, since pure CCK₁₃ and CCK₈ added to the tissue were not degraded during extraction. Furthermore, CCK immunoreactivity in spinal fluid, which is not subjected to extraction appears like CCK₈ and the COOH-terminal tetrapeptide (40).

**Distribution and Heterogeneity of CCK in Small Intestine**—In the duodenum and the jejunum of both man and hog CCK is present in concentrations (Tables V and VI) of the same order of magnitude as measured for other gastrointestinal hormones (41). Also, in the gut CCK is highly heterogeneous (Figs. 6 and 7), and more different molecular forms of CCK are present than previously described (2–4). The predominating forms are, as in brain tissue, small peptides resembling the COOH-terminal octa- and tetrapeptide of CCK₁₃. In addition, type B assay (antiserum 4698) measured a small peptide eluted after CCK₁₃ (Figs. 6B and 7B). This peptide was not removed by immunosorption using the gastrin antiserum and is present than previously described (2–4). The predominating forms are, as in brain tissue, small peptides resembling the COOH-terminal octa- and tetrapeptide of CCK₁₃. In addition, type B assay (antiserum 4698) measured a small peptide eluted after CCK₁₃ (Figs. 6B and 7B). This peptide was not removed by immunosorption using the gastrin antiserum and is present than previously described (2–4). The predominating forms are, as in brain tissue, small peptides resembling the COOH-terminal octa- and tetrapeptide of CCK₁₃. In addition, type B assay (antiserum 4698) measured a small peptide eluted after CCK₁₃ (Figs. 6B and 7B). This peptide was not removed by immunosorption using the gastrin antiserum and is present than previously described (2–4). The predominating forms are, as in brain tissue, small peptides resembling the COOH-terminal octa- and tetrapeptide of CCK₁₃. In addition, type B assay (antiserum 4698) measured a small peptide eluted after CCK₁₃ (Figs. 6B and 7B). This peptide was not removed by immunosorption using the gastrin antiserum and is present than previously described (2–4). The predominating forms are, as in brain tissue, small peptides resembling the COOH-terminal octa- and tetrapeptide of CCK₁₃. In addition, type B assay (antiserum 4698) measured a small peptide eluted after CCK₁₃ (Figs. 6B and 7B). This peptide was not removed by immunosorption using the gastrin antiserum and is present than previously described (2–4).
Distribution and Heterogeneity of Cholecystokinin in Brain and Gut

needed to clarify their structure and role as putative biosynthetic precursors.

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Addendum — Since the present article was submitted, Muller, Straus, and Yalow ((1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3035-3037) have reported the presence of radioimmunoassayable CCK in pig cerebral cortex, and Dockray ((1977) Nature 270, 359-361) the presence of octapeptide-like CCK in porcine intestines.

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Imunochemical studies on cholecystokinin. II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog.

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