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GASTROINTESTINAL HORMONES AND PATHOLOGY OF THE DIGESTIVE SYSTEM

Edited by Morton Grossman, V. Speranza, N. Basso, and E. Lezoche

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Preface

The discovery that the same or similar peptides are present in endocrine cells and in neurons is one of the most exciting and provocative recent developments in biology. Suddenly neurophysiologists and endocrinologists have found that they have a great deal to discuss with each other. Substances originally isolated as hypothalamic hormones turn out to be abundantly present in neurons of other parts of the brain and in endocrine cells and neurons of the gut and pancreas. Similarly, substances originally isolated as gut hormones are found not only in gut endocrine cells but also in gut neurons and in brain neurons. It turns out that the group of peptides that we are accustomed to call gastrointestinal hormones are not all confined to the gastrointestinal tract and are not all solely hormones. We are learning that the chemical transmitters of the neurocrine, endocrine, and paracrine systems form a single group of related substances. This volume contains the latest installments in this fascinating story. It tells how these peptides were isolated and their amino acid sequences determined, how the heterogeneity of most, perhaps all, of these peptides is being revealed as variant forms of them are discovered, how antibodies to these peptides are used as powerful tools to measure their concentrations in body fluids and to localize the cells in which they are synthesized and stored, and, finally, how the role of these substances in normal physiology and in pathological states is being unraveled. This book contains contributions from most of the leading authorities in this exciting field of study.

Morton I. Grossman

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THE GASTROINTESTINAL HORMONES : AN OVERVIEW

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In ancient times all roads led to Rome; and so it is on the present occasion when we are gathered here from many countries and continents to consider some of the hormonal activities in health and disease of what is now recognised to be the largest and most complex endocrine organ in the body - the digestive system. I am sure we are all conscious of the privilege of meeting in the capital city of that country in which originated the great revival of culture and learning in Europe after the long period of the 'Dark Ages'; and no doubt Dr. Grossman will remind us that this year is the 75th anniversary of the discovery by Bayliss and Starling of the "messenger function" of hormones as exemplified by secretin. This discovery brought to an end the Pavlovian era of the 19th century in which the gastrointestinal mechanisms were explained in terms of nervous reflexes; but although great advances soon followed in respect of other endocrine organs, 60 years were to elapse before the study of the gastrointestinal hormones could enter upon the astonishing expansion of knowledge of their nature and understanding of their functions which we are all now playing some part in furthering.

The truly remarkable developments of the past 15 years are clearly due to a combination of two circumstances. First of all there came the successful isolation of what are generally regarded as the major gastrointestinal hormones - and since then many peptides whose status has not yet been clarified - with the resultant provision of supplies of pure peptides, natural or synthetic, so that their physiological properties could be widely studied. Secondly, and deriving from those achievements, came the introduction of immunological methods of study which have made possible the measurement of the hormones in tissues and body fluids in health and

disease and the positive identification of their cells of origin in the gastrointestinal tract and elsewhere.

The old idea of 'one hormone, one function', which was widely assumed to follow from Bayliss and Starling's concept of the 'messenger' role of hormones, has long gone. The numerous actions on the glands and muscle of the digestive system which are exerted by most of the gastrointestinal hormones and the fact that these actions are often shared by more than one hormone means (1) that each hormone can be recognised by more than one target cell, and (2) that each target cell is capable of recognising more than one hormone. In the natural circumstances of the digestive response to a meal, all of the hormones are likely to be in circulation and exerting to some degree their characteristic actions on their target sites; and the problem is to decide, on the basis of experimental tests of hormonal actions (alone or combined), and the measurements of their circulating amounts by radioimmunoassay, what may be said to be the physiological role of each hormone, acting as it does not alone but in concert with the others.

This formidable problem is further complicated by the fact that probably all of the hormones are to some degree heterogeneous in their circulating forms. There was until fairly recently a general assumption, again based on Bayliss and Starling's original concept, that an endocrine cell released only a single version of its 'chemical messenger'. They at first believed that secretin was released by acid from an inactive 'prosecretin' in the mucosa; but they dropped the idea for lack of evidence, and there is nothing to show that they believed that 'prosecretin' might be secreted along with secretin. However, commencing with the classical discovery of proinsulin by Steiner and the later demonstration by radioimmunoassay of its release along with insulin (and the C-peptide) into the circulation, has come the recognition that probably all peptide hormones are heterogeneous in their tissues of origin and in the circulation because of the way in which they are synthesised in the cell, a large molecule being sequentially cleaved by specific enzymes to produce the final major active form. Besides the latter, the precursor forms and discarded fragments are likely to be released into the circulation, and their presence there may lead to errors in the measurement of the hormone by radioimmunoassay and in turn to errors in attempts to evaluate the physiological actions of a hormone by reproducing experimentally what is believed to be its normal postprandial level in the circulation. This situation, best exemplified so far by the case of gastrin which circulates in two major forms of very different activity, calls for increased sophistication of radioimmunoassays so that the different forms can be measured separately and their contributions to the final physiological response thus evaluated more precisely. There is already evidence, of which we shall hear in this Symposium, that CCK will pre-

sent a problem similar to that of gastrin in respect of heterogeneity and radioimmunoassay of circulating forms; and I have little doubt that a similar problem will to some degree unfold in turn for the other peptides with which we have to deal.

The highly active area of immunocytology and ultrastructural studies of the endocrine cells themselves, a field in which Professor Solcia and his colleagues have played such an eminent role, provides the indispensible basis for our physiological ideas. On the basis of their APUD and ultrastructural characteristics, it has defined a large number of endocrine cell-types and in most cases has successfully assigned to them peptides of established or putative hormonal role already identified by chemical isolation. It has shown us the precise location and general distribution of these cell-types in the digestive system and has offered us new and challenging ideas as to their functional roles. The suggestion that an endocrine cell may influence its near neighbours by local humoral transmission rather than by the circuitous route of the general circulation - 'paracrine' influence - awaits the decisive experimental evidence which will establish the existence of such a mechanism in the gut.

A particularly exciting area of study which leans heavily upon immunocytology is that of the brain-gut relationship. It was discovered many years ago that the characteristic pharmacological activity on smooth muscle attributed to an unidentified principle ('substance P') in crude extracts of intestine (particularly muscle) could also be found in brain; but the possible general significance of this observation never dawned on us until after the observation that the hypothalamic peptide somatostatin could also be found in the gastrointestinal tract and pancreas. Now there are appearing in rapid succession examples of peptides already known in the one tissue being present also in the other, e.g., VIP, neurotensin, enkephalin and what is probably CCK-octapeptide. What these peptides - and no doubt others yet to be discovered - are doing in these two situations is a fascinating problem. Obviously, activity of 'neuroendocrine' character is at least in part involved, for several of them are present in nerve-cells of the gut; but what their role may be in terms of neurotransmission or possibly influence of trophic character, we can hardly guess until further evidence appears, probably from the neurophysiologist and perhaps also the embryologist. Endocrine secretion by neuronal-type cells is a very primitive activity being found in the simplest forms of multicellular organisms and becoming diversified, though never lost, as the development of the "typical" activity of the nervous system proceeds in higher forms; and insofar as ontogeny recapitulates phylogeny, the study of the embryological development of hormones in brain and gut may throw some light on their role in these tissues in postnatal life.

Finally, the field of comparative studies of the endocrine cells and their peptides undoubtedly has further rich rewards to offer. The brilliant success of Professor Erspamer and his school in the exploration of the peptides present in amphibian skin has shown us the great predictive value of comparative studies of this kind, for each of the families of peptides they have discovered has proved to have its counterpart in mammalian forms; and it is safe to anticipate that further exciting discoveries will come from him along this line.

In the foregoing brief survey I have touched upon only a few of the many topics we shall discuss in the symposium; the physiological activities of the established and putative hormones, the measurement of them in health and disease and the clinical significance of the results in relation to diagnosis and treatment, the problem of heterogeneity, the brain-gut relationships and the prospects of further knowledge and understanding still to come. It never ceases to surprise me that each Symposium held in this field can offer a wealth of new work and ideas, always interesting and often challenging, and the present occasion will prove no exception, judging by the program.

A SHORT HISTORY OF DIGESTIVE ENDOCRINOLOGY

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In digestive endocrinology, as in other branches of science, most of the contributions have stemmed from a few epoch-making discoveries:

1. *PHYSIOLOGICAL ORIGINS.* On January 16, 1902, Bayliss and Starling[1] performed what they quite appropriately perceived to be "the crucial experiment" showing that putting acid into the jejunum still stimulated pancreatic secretion after all nervous connections between the two organs had been cut. Correctly deducing what the nature of the non-nervous mechanism must be, they made an extract of jejunal mucosa and showed that it stimulated pancreatic secretion when given intravenously whereas an extract of ileal mucosa did not. Bayliss and Starling recognized that they had not only discovered a new substance, secretin, but had also introduced a new concept, the regulation of bodily activities by blood borne chemical messengers or hormones. And thus was born the science of endocrinology in general as well as digestive endocrinology in particular. In due course, almost every digestive function was studied to determine whether it might have an endocrine component in its regulation. In addition to secretin, other studies with crude extracts which eventually led to isolation of chemically characterized peptides are: Edkins[2] 1905, gastrin; Ivy[3] 1927, cholecystokinin; Euler and Gaddum[4] 1931, substance P; Harper[5] 1941, pancreozymin (later shown to be identical to CCK); Brown[6] 1967, motilin; once again Brown[7] 1970, gastric inhibitory peptide (GIP); and Said and Mutt[8] 1970, vasoactive intestinal peptide (VIP). Many additional mucosal extracts have been made and given names but we don't know whether they contain new peptides since most of their biological actions are shared with known peptides.

2. *THE BIOCHEMICAL ERA.* Completely new avenues were opened when gastrointestinal hormones became available as pure substances of known chemical structure. In 1964, Gregory and coworkers[9] announced the amino acid sequence of gastrin and since that time the sequences of secretin (Mutt[10] 1970), cholecystokinin (Mutt[11] 1971), substance P (Chang[12] 1973), GIP (Brown[13] 1971), motilin (Brown[14] 1973), and VIP (Mutt[15] 1974) have been reported. Proof of structure by synthesis of fully biologically active peptides has been accomplished for gastrin,[16] secretin,[17] motilin,[18] and VIP.[19] Homologies of amino acid sequences place gastrin and CCK in one chemical family and secretin, GIP, VIP, and pancreatic glucagon in another. The shared carboxyl-terminal fragment of gastrin and CCK has all the biological actions of both hormones. Gastrin and CCK display molecular heterogeneity in the form of molecules with different chain lengths; 3 forms of gastrin and 2 of CCK have been sequenced but all have the same carboxylterminal pentapeptide sequence. Once the hormones were available in pure state the remarkable range of their actions and interactions was revealed, including their trophic actions.[20] The availability of pure hormones makes it possible to make antibodies with which to measure their concentrations in blood and to identify the cells of origin. The pure hormones are now being applied to studies on the nature of the receptors on cell surfaces and the second messengers released by activation of these receptors.

3. *RADIOIMMUNOASSAY.* While studying the metabolism of insulin in 1956, Berson and Yalow[21] serendipitously discovered that insulin-treated patients had antibodies to insulin in their blood. They quickly recognized that such antibodies could be used to measure the amount of insulin in body fluids and thus was born the science of radioimmunoassay which has now been applied to every hormone, including those from the gut, as well as to many other substances. Reliable radioimmunoassay of gastrin is well established and assays for all of the other gut peptides are being perfected.

4. *CELLULAR LOCALIZATION.* Antibodies are also the tools used to determine which cells make and store the various peptides. Using well established principles of immunocytochemistry, McGuigan[22] identified the gastrin containing or G-cells in antral mucosa in 1968 and since that time Pearse, Polak, Solcia and others have demonstrated cells that react with antibodies to each of the peptides that have been extracted from the gut (secretin,[23] CCK,[24] GIP,[25] motilin,[26] VIP,[27] substance P[28]). There appear to be separate cells of origin for each peptide and the cells storing each peptide have a distinctive distribution along the gut, some being confined to small areas, others being found throughout the tract. Once an antibody to a peptide is available, the entire body can be surveyed to determine which organs contain cells that react with it. Such immunofluorescent surveys have given some surprising results.

Organs outside the gut have been shown to have cells reactive to antibodies to gut peptides and conversely the gut has cells that react with antibodies to peptides isolated from other organs. Thus cells reactive with antibodies to glucagon[29] from the pancreas, bombesin[30] from frog skin, and somatostatin[31] and neurotensin[32] from the hypothalamus have been found in intestinal mucosa. Similarly, substances reactive with antibodies to gastrin[33] and to VIP[34] have been found in the brain.

5. *THE BRAIN-GUT AXIS.* When Euler and Gaddum[4] attempted in 1931 to study the distribution of acetylcholine in tissue extracts, they discovered an interfering substance in extracts of brain and intestine that was later to be identified as the peptide substance P. This was the first instance in which a peptide was shown to be present in both brain and gut. Now there are five additional examples of peptides found in both brain and gut: somatostatin,[31] gastrin,[33] VIP,[34] neurotensin,[32] and enkephalin.[35] The list is certain to grow. These findings that certain peptides occur in both brain and gut and in some instances in both endocrine and neural cells of the gut indicate that the chemical messenger cells of the body comprise a single system in which the same or similar peptides or amines may be utilized for neurocrine, paracrine, or endocrine transmission. Thus all of the major mechanisms involved in coordinating bodily activity can be viewed as belonging to a unified system.

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