MULTIPLE ENDOCRINE NEOPLASIA SYNDROMES

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I. INTRODUCTION

A. Classification

The appearance of tumors in various endocrine organs in the same individual is described in the literature as multiple endocrine adenomatosis, but, because hyperplasia or malignancy is involved it is more appropriate to use the term multiple endocrine neoplasia syndrome (MEN)*.[1]

Two main types can be distinguished: the first, (MEN-1), is characterized by the combined occurrence of tumors of the pituitary gland and pancreatic islets, and hyperparathyroidism; the second, (MEN-2), is the syndrome first described by Sipple (1961),[2] characterized by the combination of multiple pheochromocytomas and medullary thyroid carcinoma. Chong et al.[3] (1975) subdivided this syndrome into two phenotypes: MEN-2A, Sipple's syndrome combined with hyperparathyroidism, and MEN-2B, the marfanoid mucosaneural phenotype without concurrent involvement of the parathyroid glands.

Apart from the two distinct principal phenotypic MEN groups, there are several mixed forms which are mentioned in the literature with increasing frequency.

B. History of MEN-1

The syndrome of MEN-1 was first observed by pathologists about 80 years ago. Erdheim (1903)[4] was the first to document the occurrence of multiple endocrine tumors in a single patient. He described a patient with acromegaly who was found on post-mortem examination to have an eosinophilic adenoma of the pituitary gland and four enlarged parathyroid glands. Cushing and Davidoff (1927)[5] and Lloyd (1929)[6] were the first to record the association of

* Abbreviations used in text: ACTH = adrenocorticotropin hormone; APUD = amine precursor uptake decarboxylase; BCNU = 1.3 *bis*(β-Chloroethyl)-1-Nitrosurea; CCK = cholecystokinin; CEA = carcinoembryonic antigen; CRF = corticotropin releasing factor; CT scan = computed tomography scan; DNA = deoxyribonucleic acid; DOPA = dihydroxyphenylalanine; DTIC = dimethyltriazenoimidazole carboxyamide; ERCP = endoscopic retrograde cholangiopancreatography; 5-FU = 5-fluoro-uracil; HCT = human calcitonin; 5-HIAA = 5-hydroxyindoleacetic acid; HGH = human growth hormone; I^{131}MIBG = Iodine^{131}-metaiodobenzylguanadine; MEN = multiple endocrine neoplasia; MSH = melanocyte stimulating hormone; MTC = medullary thyroid carcinoma; MW = molecular weight; NGF = nerve growth factor; NSE = neuron-specific enolase; 17-OHCS = 17-hydroxycortico steroids; PNMT = phenylethanolamine-*N*-methyltransferase; PP = pancreatic polypeptide; PTH = parathyroid hormone; RNA = ribonucleic acid; STH = somatotropin hormone; STP = subtotal parathyreoidectomy; THPVS = transhepatic portal venous sampling; VIP = vasoactive intestinal polypeptide; VMA = vanillylmandelic acid; WDHA = watery diarrhea, hypokalemia, achlorhydria.

tumors involving three endocrine glands in one individual. The clinical observation of multiple endocrine tumors began in 1939 with Rossier and Dressler's[7] description of a family in which two sisters showed clinical and/or autopsy evidence of pluriglandular disease. Although they suspected the hereditary nature of the disease, they felt that the evidence then available was not sufficient to substantiate their hypothesis.

The first reported case of the combined occurrence of a pituitary tumor, hyperfunctioning parathyroid glands, and pancreatic islet-cell tumors, all recognized during life and treated, was that of Shelburne and McLaughlin[8] in 1945. This patient developed signs of hypoglycemia after an operation for an ureteral stone. After removal of three islet = cell adenomas the hyperinsulinism disappeared completely. Repeated elevated calcium values suggested that he also had hyperparathyroidism. At exploration, a single small parathyroid adenoma was found and after removal the value for calcium returned to normal. In addition, there was radiographic evidence of a pituitary tumor for which radiotherapy was given. By 1953, according to Underdahl et al.,[9] multiple endocrine adenomas of the pancreatic islets, the parathyroid glands, and the pituitary gland had been reported in various combinations in 14 or more patients. Of the eight new cases which they represented, four had a family history of involvement of one or another of the glands under consideration. In no instance, however, was there a proven family incidence of MEN-1.

In 1953, and 1954, Moldawer et al.[10,11] reported both a father and daughter with multiple parathyroid adenomas. The daughter had concurrent hypoglycemia, the father, his brother, and his mother had peptic ulcer disease. Wermer (1954)[12] then reported a family in which five members had evidence of involvement of one or more glands. He proposed a genetic basis for multiple endocrine neoplasia, suggesting that the disease was transmitted by a single dominant autosomal gene trait with a high degree of penetrance. Up to 1970, 31 families with MEN-1 comprising 107 affected members had been described.[13] Only a few reviews are available on the MEN-1 syndrome.[14-16]

The most extensive study made retrospectively from autopsy records and cases in the literature was published by Croisier et al.[15] and included 169 cases.

C. History of MEN-2

The concomitance of pheochromocytoma and thyroid carcinoma was first described by Eisenberg and Wallerstein (1932).[17] DeCourcy and DeCourcy[18] observed a high and a significant incidence of pheochromocytoma in patients with diffuse or nodular goitre, or with thyroid carcinoma. In The Netherlands, it was Smits (1959)[19] who in his thesis on a family with inherited pheochromocytoma described one patient with both pheochromocytoma and thyroid carcinoma, as well as a number of patients with pheochromocytoma and an unspecified type of goitre. In 1961, Sipple[2] reported the post-mortem findings in a 33-year-old man who had died of an intracerebral hemorrhage and made special mention of pheochromocytomas in both adrenals and multiple carcinomas in the thyroid gland; in addition, one of the parathyroid glands was enlarged. The thyroid carcinomas were described as follicular adenocarcinomas with a low degree of differentiation. In his review of the relevant literature, Sipple found, among 537 published cases of pheochromocytomas, reports on 27 patients (5%) with a malignant tumor of another organ. Six of these 27 tumors (22%) proved to be thyroid carcinomas. Compared with the expected incidence of thyroid carcinoma in a cross-section of the population, the incidence in patients with pheochromocytomas was 14 times higher. He concluded that the association of the two neoplasms could not be coincidental. It is of historical interest that in Sipple's original cases neither the familial association nor the precise identity of the thyroid neoplasms was recognized.

Medullary thyroid carcinoma as a distinct clinical and pathological entity was first recognized by Horn[20] in 1951 and further identified by Hazard et al.[21] in 1959. They described medullary thyroid carcinoma as a separate clinical and pathological entity with specific

histological and clinical features and with the presence of amyloid in the stroma as the principal characteristic. They called this unique tumor medullary or solid thyroid carcinoma (MTC).

The familial occurrence of MTC and pheochromocytoma was first reported by Cushman.[22] In the early 1960s an increasing number of cases of familial occurrence of MTC and pheochromocytoma was published.[23-26] In an analysis of 15 published cases of concomitant MTC and pheochromocytoma and two of his own, Williams[27] found that the adrenal tumors were bilateral in all of them, that the family history was often tainted (6 out of 17), and that the thyroid malignancy was of the medullary type in 11 of these 17 cases. He therefore concluded that the incidence of concomitant thyroid carcinomas and pheochromocytoma must be much higher than 14 times the expected incidence as given by Sipple.[2] Moreover, Williams suggested that the tumors in this syndrome, including the MTC, were of neuroectodermal origin.

In the same year, Schimke and Hartmann[26] described two families with MTC and pheochromocytoma in which autosomal dominant inheritance had been demonstrated, but with varying expression of the syndrome. They introduced the hypothesis of a single-gene inheritance for both MTC and pheochromocytoma. In 1968, Steiner et al.[1] published their detailed study of 186 patients with a MEN type 2A syndrome in seven generations of one family, thus confirming autosomal dominant inheritance with a high degree of penetrance and with varying expression of the syndrome. Apart from the family aspects, they added the presence of parathyroid adenopathy to the syndrome.

Cushing's syndrome caused by ectopic adrenocorticotropin hormone (ACTH) secretion was found in a patient with the MEN type 2A syndrome by Donahower et al.[29] in 1966 and Steiner et al.[1] in 1968. Steiner et al. introduced the designation MEN type 2 which was finally to become MEN-2A syndrome.

Williams and Pollock[30] described the combined occurrence of mucosal neuromas and marfanoid habitus in patients with MTC and pheochromocytoma, which Gorlin et al.[31] and Schimke et al.[32] subclassified as the MEN type 2B syndrome.

D. Etiology and Pathogenesis

1. Inheritance

A remarkable feature of all MEN syndromes is the inheritance as an autosomal dominant trait with a very high degree of penetrance. The predisposition to tumor formation in the pancreatic islets in the MEN-1 syndrome and in the thyroid and adrenal glands in the MEN-2 syndrome might be explained by the two-mutation hypothesis of genetic tumor formation developed by Knudson and Strong.[33,34] They postulated that a comparison of ages of onset of hereditary and sporadic types of certain tumors such as retinoblastomas, neuroblastomas, and pheochromocytomas, was compatible with a two-mutational-event model of the initiation of these neoplasms. In this theory, both events occur in somatic cells in the nonhereditary or sporadic cases, and the tumors are generally solitary and sporadic. In the hereditary neoplasms the first event is a germinal mutation making many cells susceptible and giving the genetic autosomal dominant inheritable defect that renders the involved cells susceptible to neoplastic changes. The second mutational event in the somatic cells in the post-zygotic phase is required to bring the latent genetic defect to expression and transform a mutant cell into a tumor cell, the tumors tending to be multiple and often bilateral. Jackson et al.[35] studied the age at onset in 20 cases of hereditary MTC and in 22 sporadic cases. Their findings were compatible with what might be expected according to the two-mutational-event theory of the initiation of cancer postulated by Knudson and Strong.[33,34]

Baylin et al.[36,37] found strong indications for a monoclonal origin of both MTC and pheochromocytoma. These authors described a patient who was mosaic for glucose-6-phosphate-dehydrogenase (G6PD) types A and B in normal tissues with several MTC nodules

each containing only G6PD type A or B. In tumors of the MEN type 2A syndrome, as for MTC and pheochromocytoma, each tumor focus was thought to arise from a single cell or a very small clone of cells.

The two-mutation theory of Knudson and Strong[33,34] and the final monoclonal mutation theory of Baylin et al.[36,37] offer a reasonable explanation for the multicentric origin of the tumors occurring in the MEN syndromes, however, the factors which may play a role in the genesis of tumor development are probably more complex.

Chromosomal instability may be a genetic factor in MEN patients. The term chromosomal breakage syndrome has been applied to a number of distinct genetic disorders such as Fanconi's anemia, Bloom's syndrome, ataxia telangiectasia, and xeroderma pigmentosum, which have in common an increased tendency to develop malignancies and a disposition to spontaneous chromosomal breakage in cultured cells, particularly blood lymphocytes. The chromosome analysis of cultured lymphocytes from patients with familial MEN-1 and MEN-2 showed an increased frequency of gaps and chromatid-type aberrations compared with controls and healthy relatives, perhaps due to a defective DNA repair mechanism. However, substantiation of these observations will require much additional data.

Recently, Van Dyke et al.[38] reported a minor deletion of chromosome 20p in two MEN-2 families. Prophase banding in patients by several genetic centers in the Netherlands failed to detect this deletion. Theoretically, if in some affected family the deletion involves a small segment, then even the resolution of prophase banding is not sufficient. Thus, negative data found by us and others in siblings with MEN-2 syndrome do not necessarily prove that the deletion found by Van Dyke et al. is absent.

Finally, the possibility exists that in a monoclonal cell-line such as that indicated by Baylin et al.[36,37] a latent cancer gene (oncogene) is activated (derepression) perhaps as a result of DNA rearrangements due to a random instability. This could be the second event mentioned by Knudson and Strong.[33,34]

2. Common Embryological Origin of MEN Tumors

Several hypotheses have been put forward to explain the concurrent appearance of tumors in different organs. Considering the MEN-1 syndrome, Wermer[39] proposed abnormal growth as a direct effect of an abnormal gene in the cells of different affected tissues (pleiotropism) and not as an effect mediated through hormonal factors. Vance et al.[40] proposed the expression of a genetic defect in the primordial cell of the islets of Langerhans resulting in multipotential hyperplasia or nesidioblastosis as the primary disorders. Changes in other endocrine glands would evolve as a consequence of islet cell hormone excess.

Because hyperparathyroidism is frequently the first abnormality to be observed in MEN-1, chronic hypercalcemia could also play an important role in further development of the other lesions. Calcium is a potent secretagogue for most of the hormones that are secreted in excess in MEN syndromes including calcitonin, gastrin, and insulin. This latter theory takes into consideration the important aspect of hormonal interrelationship. Overproduction of specific hormones promotes growth in several target organs. However, it is currently assumed that all neoplastic changes associated with the MEN syndromes arise from cells of what is called the diffuse endocrine system.

3. History of the Diffuse Endocrine System

The story of this cell system started as early as 1870, when Heidenhain[41] published the first description of argentaffin cells in the intestinal mucosa. These cells, which occur dispersed throughout the digestive tract, were later studied and described in detail by Kultschitzky (1897).[42] In 1906, Ciaccio[43] described them as enterochromaffin cells and Oberndorfer (1907)[44] recognized the potential malignancy of these cells and called the tumors originating from these cells carcinoid. In 1914, Masson[45] described the argentaffin reaction

of carcinoid as well as enterochromaffin cells. He regarded carcinoid as endocrine tumors arising from argentaffin cells and maintained that these cells are closely related to the autonomic nervous system (neuroectoderm). Masson elevated the argentaffin cell system to the status of *la glande endocrine de l'intestin,* which can be regarded as the oldest model of the diffuse endocrine system.

In 1938, Feyrter's[46] first publication on this argentaffin cell system described a peripheral endocrine system of *helle Zellen* (clear cells) distributed diffusely in the mucosa of the digestive tract and the intestinal glands. After 1953, Feyrter[47] referred to "paracrine cells", thus indicating the probable function of these cells, i.e., to exert an influence on the cells in the immediate vicinity. He assumed that these paracrine cells were of endodermal origin and produced by *endophytie,* a process comparable to the *bourgonnement* or budding which Masson had described earlier. Altman (1940)[48] suggested that a number of these clear cells might be chemoreceptor cells originating directly from the neuroectoderm. Sunder-Plassmann (1939)[49] postulated that the neural crest was the origin of the clear-cell system, and Pagès (1955)[50] elaborated on this hypothesis.

4. The APUD Concept

After the Second World War, Pearse assumed that cytochemical techniques would make it possible to classify certain pituitary cell types on the basis of their hormone production. This hypothesis proved to be correct. At the same time he was engaged in studies on calcitonin in an effort to prove that this compound originated from the parathyroids. He failed to do this, but he found a calcium sensitive cell in the thyroid gland, i.e., the parafollicular cell of Nonidez,[51] which by virtue of its cytochemical and ultrastructural features, could fit into the (then still small) series of peptide hormone-producing cells. With the demonstration of calcitonin in these parafollicular C-cells, 1966, the amine precursor uptake decarboxylase (APUD) concept was born.[52] Specifically, APUD is derived from the principal cytochemical properties of these cells: fluorogenic *A*mine content (e.g., catecholamines or 5-hydroxytryptamine), amine *P*recursor *U*ptake (e.g., dopamine or 5-hydroxytryptophan) and the presence of the enzyme amino acid *D*ecarboxylase. When the APUD concept was first formulated, it was thought that the APUD cells probably had a common origin.[53] The amine storage process and the presence of cholinesterase might indicate a common embryonic origin in the neural crest. This hypothesis was supported by the common ultrastructural and functional characteristics of these cells. A strong indication was obtained by the research results published by Johnston (1966)[54] who had transplanted isotope labeled cells of the neural crest of one chicken embryo to another and found that the labeled cells migrated in ventral direction. Le Douarin and Le Lièvre[55] transplanted the neural tube of a Japanese quail embryo (7-10-somite stage) to a chicken embryo and observed that the recognizable quail cells migrated to the ultimobranchial body, which was later incorporated into the thyroid. It was thus demonstrated that the C-cells are of neuroectodermal origin.

Today, 17 years after the formulation of the APUD concept, experiments in various laboratories have shown that only 7 of the 40 APUD cell types originate from the neural crest. The original concept of the neural origin of APUD cells was modified by Pearse and Takor[56] in 1976 to the statement that all peptide hormones secreting APUD cells originate from the neuroectoderm, i.e., from cells of the ectoblast or epiblast that program the neuroendocrine system. In a later embryological stage, but before the formation of the somites (i.e., in the third week of human embryonic development), the APUD cells with the common epiblastic origin can be divided into three main groups: cells which migrate from the epiblast to the neural crest, cells which migrate to the various placodes, and cells which invaginate from the epiblast. The APUD cells of the digestive tract and the lungs were thought to be derived from the third group.

The APUD cells are responsible for the production of 40 or more bioactive endocrine peptides and a smaller number of bioactive amines. In the past few years at least 20 of these

peptides have been found to be common to the cells and processes of the central and peripheral divisions of the nervous system on the one hand, and to the non-neural endocrine cells of the central and peripheral divisions of the APUD series on the other; in short, the two principal sources of the common peptides can be referred to as brain and gut. In the brain these peptides are believed to act as neurotransmitters and releasing factors. In view of these considerations the nervous system can be divided into a somatic, an autonomic, and a diffuse endocrine nervous system.[57]

Tumors arising from APUD cells are called apudomas (Szijj et al.[58] 1969). According to Pearse and Welbourn,[59] any apudoma can potentially produce any of the APUD peptides (single or in combination, as a prohormone, or as a hormone fragment), bioactive amines, and enzymes. Medullary thyroid carcinomas, for example, have proved capable of producing, e.g., ACTH/β-endorphin, somatostatin, neurotensin, cholecystokinin, carcinoembryonic antigen (CEA), serotonin, and prostaglandins as well as calcitonin.

From a pathogenic aspect such a common embryonic origin hypothesis for APUD cells is attractive, and, if true, would explain the relationship among the different neuroendocrine neoplasms of the MEN syndromes. Unfortunately, however, the common ectoblastic origin is difficult to prove, because the migration of APUD-cell ancestors occurs in such an early stage that it is not accessible to experiments. Although similar morphological and cytochemical characteristics of the APUD cells suggest a common embryonic origin, the characteristics alone are not sufficient to prove it. At present, there is much contradictory evidence[60-63] especially about the origin of the gastrointestinal and respiratory APUD cells.[64-68] There are many reports of APUD activity in endodermally and mesodermally derived tumors. Perhaps a process of dedifferentiation explains the observed data more satisfactorily; the common APUD features may be acquired by external influences and are not necessarily indicative of a common site of embryonic origin. Based upon cytogenetic and biochemical studies,[36] it is now generally accepted that most, if not all, neoplasms develop as a clone of abnormal cells arising from a single cell. This clonal theory of evolution also suggests that there is a process of natural selection among neoplastic cells. During growth, abnormal subpopulations of cells develop increasingly, the most malignant of them eventually dominating. Concurrent with this evolutionary process there may be divergence of neoplastic cells as a result of the effects of local conditions (microenvironment) on the development of different subpopulations. Heterogeneity of cells in a neoplasm has been demonstrated with respect to growth rate and the ability to metastasize, the metabolic characteristics, hormone receptors, antigen properties, hormone and pigment production, and sensitivity to radiation and cytotoxic drugs. Genetic identity does not preclude phenotypic diversity. The evolution of diversity among the progeny of tumor cells is clearly a major factor in cancer progression and, particularly with respect to metastatic spread, it is probably the fundamental reason for the failure of current cancer therapy. The formulation of the APUD concept in 1966 initiated promising developments in the fields of embryology, histology, cytochemistry, and molecular endocrinology. Despite the fact that the common origin of all APUD cells in the crista neuralis is (extremely) doubtful, Pearse's contribution to the definition of a group of cells and neoplasms with similar phenotypes cannot be overemphasized.

E. Molecular Biochemistry of Ectopic Hormone Production

The production of hormones and other peptides is an important feature of the tumors associated with the MEN syndromes. The production of these substances is often not recognized clinically. A number of reasons can be put forward to explain this tendency for the true incidence to become obscured. Sometimes the patient dies quite soon after the tumor is diagnosed and before the distinct syndrome has reached its full development. In other

cases the tumor secretes only biologically inactive prohormones or fragments. In MEN patients, secretion of a biologically active product often occurs, but without a distinct clinical effect due to the compensatory production by the body of a natural antagonist. For instance, calcitonin production by MTC is counteracted by increased production of PTH. The identification of the produced peptide as a tumor marker is of clinical importance not only because it leads to the detection of tumors and a better understanding of the clinical picture, but also because it permits better quantitative evaluation of the behavior of tumor growth. Since the course of the blood levels of the tumor products can be determined (monitored), the tumor marker is a sensitive indicator of tumor growth and changes in tumor character during therapy. This permits alert management.

The products of MEN tumors can be divided into substances normally produced and secreted by the tissue from which the tumor originated (eutopic secretion) and substances ordinarily considered inappropriate for the tissue in which the tumor arose (ectopic secretion). The term ectopic hormone production is presumptuous because it implies that all sites where a hormone normally is produced are known. Many hormones originally suspected to be ectopic are formed in minute amounts in the normal tissue of origin too.

Before the age of peptide measurement by immunoassays, unequivocal demonstration of ectopic hormone production was difficult and based on the finding of a clinical syndrome in the presence of a tumor and regression of the symptoms after successful treatment. Detection of hormone concentrations above the normal range, the presence of an arteriovenous gradient across the tumor, and a fall of elevated serum levels ending in the normal range after removal of the tumor gave strong indications for the production of an ectopic hormone by the tumor. The presence of hormones could then be demonstrated in the tumor tissue by extraction and radioimmunoassay and/or bioassay. Precise histologic classification of tumor tissue was possible, and cytoplasmic secretory granules could be demonstrated electron-microscopically. Immunocytochemical study of the preparations completed this investigation. Evidence of production was obtained in vitro by the investigation of tumor-cell cultures. This method showed the incorporation of radioactive labeled amino acids into hormones as well as the production of hormones by long-term cell lines.

Ectopic hormone production by inherited tumors may start with changes on the DNA level (for instance by derepression of information usually present only in latent form and the translocation or deletion of information), by changes in the transcription of DNA in RNA or a different processing (splicing) of RNA, and by changes in the translation to RNA in polypeptide and the enzymatic degradation of the propeptide to the final hormone which is released into the circulation.

At present, the how and why of ectopic hormone production by tumor tissue is studied on this molecular biochemical level by the isolation of RNA from tumor tissue which permits determination of the biosynthetic precursor of the hormone. The extracted RNA could be translated in ribosomes of an in vitro translation system (e.g., erythrocytes lysate or a wheat germ system) and the product identified. These convenient methods for isolation and cell-free translation of messenger RNAs have yielded much insight into the biosynthesis of peptide hormones. For a variety of hormones the initial products of translation have been identified and shown to differ significantly from their secreted forms. Jacobs et al.[69] (1979) extracted messenger RNA from calcitonin secreting ultimobranchial glands of the codfish together with medullary thyroid carcinoma from rats and translated it in wheat germ and reticulocyte heterologous cell-free systems. Analysis of translation products by electrophoresis on polyacrylamide sodium dodecyl sulfate gels revealed that codfish and rat RNAs directed the synthesis of a number of polypeptides in the cell-free systems, one of them apparently with a molecular weight of 15,000, could be specifically immunoprecipitated with antisera raised against synthetic calcitonin (3500 mol wt). However, RNA translation procedures have the disadvantage inherent in the heterogeneity of RNA: this gives hetero-

geneity of translation products which in turn causes cross-reaction of antibodies with several products. As a result, exact identification of translation products is not possible, and no information is obtained about the transcription of DNA in RNA and about the processing of RNA.

Unequivocal identification of the messenger RNA coding for calcitonin was performed by Amara et al.[70] who used molecular cloning technology. Poly(A)-enriched messenger RNA was prepared from a line of rat MTC producing a high level of calcitonin and used as a template for complementary DNA synthesis (cDNA) with the enzyme avian myeloblastosis virus reverse transcriptase. Double-stranded DNA was prepared from cDNA with polymerase I and inserted into the plasmid pBR322. These recombinant plasmids were introduced into *Escherichia coli* and the clones containing the specific cDNAs were selected by using highly specific hybridization selection procedures based on the property of the cDNA containing the calcitonin coding sequence to hybridize specifically with calcitonin-coding RNA. The sequence analysis according to Maxam and Gilbert[71] showed that the inserts from the selected clones contained a nucleotide sequence encoding the complete amino acid sequence of preprocalcitonin. It appeared that the calcitonin messenger RNA (mRNA) encoded multiple polypeptides in a single precursor. During cellular biosynthesis, calcitonin arises from the large precursor protein by cleavages at both amino- and carboxyl-terminal residues of the hormone. Extending beyond the carboxyl-terminal of calcitonin is a 16-amino-acid segment, which is linked with a Gly-Lys-Lys-Arg tetrapeptide. Birnbaum et al.[72] (1981) presented a strategy for identification in vivo of this carboxyl adjacent peptide that was predicted by sequence analysis of the recombinant cDNA molecule. The predicted 16-amino-acids sequence was synthesized chemically using solid phase procedures. Antibodies raised against this synthetic peptide actually detected immunoreactive material in tissue extracts from both normal thyroid glands and calcitonin-producing MTC from the rat. In man, the preprocalcitonin cDNA encodes 21-amino acids in this carboxyl adjacent peptide region (Craig et al.[73] 1982) instead of the 16-amino acids in the rat. This synthetic COOH-terminal flanking peptide appeared highly biologically active in reducing the level of plasma calcium (Hillyard et al.[74] 1983). Human MTC cosecrete calcitonin and this carboxyl adjacent peptide, both of which are derived by proteolytic excision from the larger precursor, by processing mechanisms (Roos et al.[75] 1983).

The existence of simultaneously activated gene families and the presence of multiple hormones within a single primary translation product are two ways in which the assortment of peptide hormone produced in tumor tissue can become more diverse.

The discontinuity of genetic regions encoding mature RNA and the complexity of the pathways for RNA processing suggest that alternative splicing events could be an additional mechanism for increasing the flexibility of gene expression in normal and tumor tissues. Amara et al.[76] found that alternative processing of RNA transcripts from the calcitonin gene results in the production of distinct mRNAs encoding the hormone calcitonin or a predicted product referred to as "calcitonin gene related peptide" (GCRP). The calcitonin mRNA predominates in the thyroid, whereas CGRP-specific mRNA appears to predominate in the hypothalamus. These observations led to the proposal of a model in which developmental regulation of RNA processing is used to increase the diversity of neuroendocrine gene expression.

This potential versatility provided by alternative events in RNA processing has been used effectively too by several eukaryotic viruses to generate multiple protein products from a single transcription unit. Multiple mRNAs are generated from the hormone gene as a result of alternative RNA processing events.

II. MEN SYNDROME TYPE I

A. Terminology

This disease, characterized primarily by functioning or nonfunctioning tumors or hyperplasia of the pituitary gland, parathyroid glands, and pancreatic islet cells, is known by a variety of names. Underdahl et al.[9] (1953) called it multiple endocrine adenoma or adenomatosis. Because it was Wermer[12] who in 1954 first underscored the familial character of the disorder, the syndrome was named Wermer's syndrome. Berdjis[77] (1962) preferred the term pluriglandular syndrome because of the presence of hyperplasia rather than adenomas. According to Wermer[78] (1974), the previously accepted name for the type I syndrome, i.e., multiple endocrine adenomatosis, should be discarded because of the frequent concurrence of carcinomas and carcinoids. He proposed the term multiple endocrine neoplasia, which is the most popular name at present.

Recently, Friesen[79] suggested multiple endocrine adenopathy as the most appropriate name on the grounds that the syndrome can include the entire spectrum of hyperplasia, microadenomas, adenomas, carcinomas, and carcinoids.

B. Incidence

The incidence of the syndrome under consideration is difficult to assess. Among 450 randomly chosen autopsy cases, only one case of pluriglandular syndrome was found, an incidence of 0.22%. In another series comprising 1600 autopsies, 4 cases were recorded (0.25%).[77] In 1964, Ballard et al.[14] collected 74 cases from the literature. Croisier et al.,[15] 5 years later, were able to collect 169 cases of MEN-1 for review. In 1970, the number of known affected families in the literature was 31 (Karbach and Galindo),[13] and since then several more families have been discovered. Snyder et al.[80] detected five new families with MEN-1 during systematic screening of all patients with hyperparathyroidism, pituitary tumors, or islet cell tumors seen in a university hospital within a 10-year period. He suggested that the disorder is not as rare as was once thought. This was confirmed by a study done by Boey et al.[81] who, in 1975, screened 119 unselected patients with hyperparathyroidism and discovered 21 patients with proven or suspected MEN-1. The incidence of MEN-1 among patients with gastrinomas is even higher, i.e., up to 54% (Lamers et al.[82]). Stefanini et al.[83] reported that of 951 cases of insulinoma, 4% were associated with MEN-1. It is not known how frequently a pituitary tumor is a component of the MEN-1 syndrome. Since endocrine disorders in MEN-1 are often asymptomatic, the reported incidence is dependent on the thoroughness of the investigations. The Netherlands, with a population of 14,000,000, has about 20 known families with the MEN-1 syndrome.

C. Age/Sex

The disease has been described in all age groups. The average age of the patients is 41.9 years (42.7 for males and 41.3 for females).[14] The youngest patient described so far was a 5-year-old boy with biochemical evidence of hyperparathyroidism[14] and the oldest an 81-year-old woman with hyperinsulinism and hyperparathyroidism (Gelston et al.,[84] 1982). If extensive screening programs are instituted, the syndrome will be diagnosed at younger ages. Although female cases slightly predominate (52.7% vs. 47.3% for males)[15] no definite correlation was found between incidence and sex. The majority of the women developed clinically manifest disease during the third decade of life, whereas the peak for male patients occurs during the fourth decade.[14] The disease has been reported in most ethnic groups.

D. Symptomatology

The patients who are affected by the multiple adenomas tend to present a variable clinical picture. The first manifestation of the disease is usually produced by hyperfunctioning of

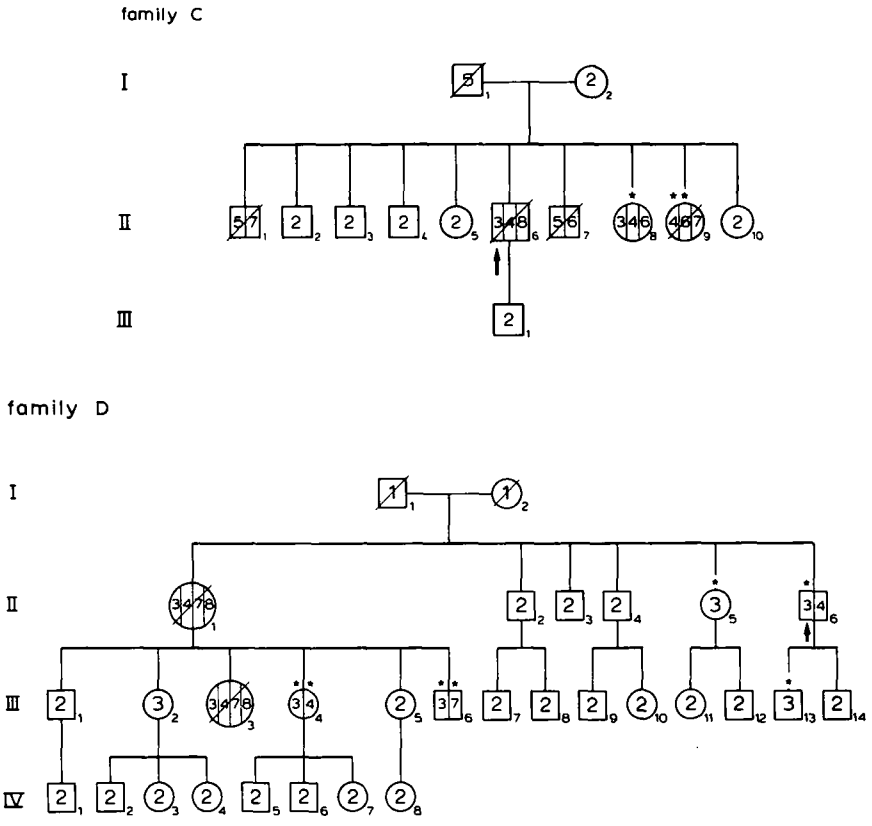

FIGURE 1. Two pedigrees showing interfamilial variation within the MEN-1 syndrome. In family C 3 members had insulinomas, whereas no insulinomas were found in family D. (1) insufficient data, (2) not affected, (3) hyperparathyroidism, (4) proven Zollinger-Ellison syndrome, (5) probable Zollinger-Ellison syndrome, (6) insulinoma, (7) pituitary tumor, and (8) adrenocortical hyperplasia; male □ female ○ deceased ⊘.

only one endocrine gland. With further progress, evidence of hyperfunctioning of the other gland(s) becomes manifest, and finally a complete clinical picture emerges. The most common clinical manifestations are, consecutively, peptic ulceration (42%), hypoglycemia (20%), symptoms of hyperparathyroidism (18%), and of pituitary tumors (15%).[15] Of 169 patients with the syndrome reported before 1969, 87.5% had evidence of parathyroid lesions, 84% had pancreatic islet-cell tumors, 50.8% had pituitary lesions, and 41.4% had adrenal lesions.[15]

The complete evolution of the syndrome may take years. A search of the literature in the English language over the period 1953 to 1978 done by Majewski and Wilson[85] for reports of complete autopsies of MEN-1-affected individuals indicated pathology in all three glands in 29 out of 32 cases. The clinical picture sometimes reflects the interaction of various hormones produced by the tumors. Thus, a patient with insulinoma and coexisting acromegaly may remain free of attacks of hypoglycemia.[78] In any given family the syndrome often manifests itself in a characteristic fashion (Figure 1).

E. Pathology

The key feature of MEN-1 is multiplicity. This applies not only to the coincidence of pituitary, parathyroid, and pancreatic adenomas, but also to the multiplicity of adenomas in the parathyroid glands and the pancreas. Although multiplicity of pituitary tumors has not been reported previously, Figure 2 shows multiple pituitary tumors in an MEN-1 patient with increased plasma prolactin and growth hormone concentrations. As already mentioned,

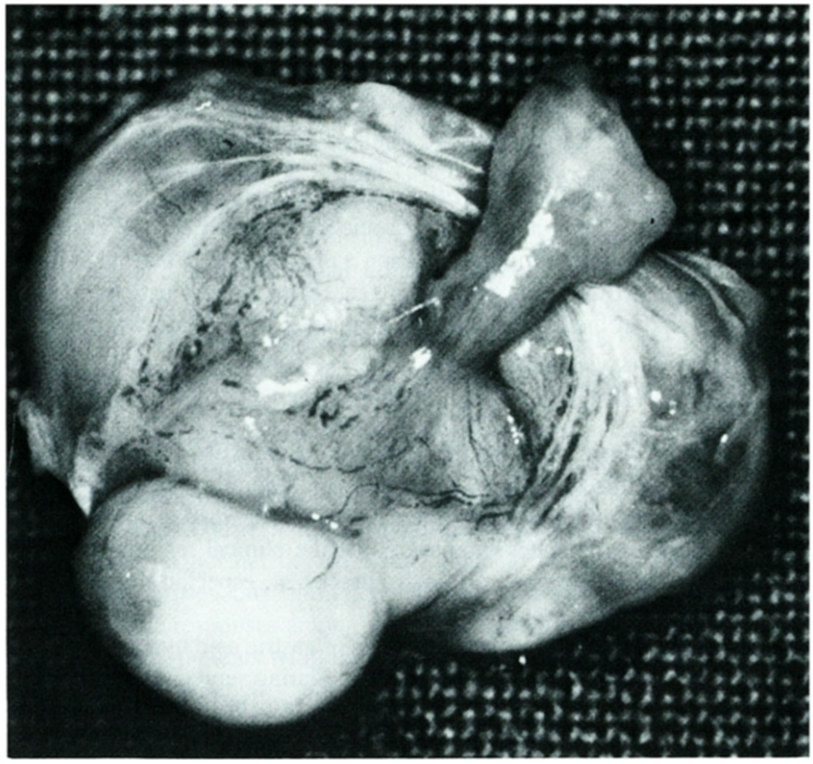

FIGURE 2. Multiple tumors of the pituitary gland in a patient with gastrinoma, hyperparathyroidism, and nodular adrenocortical hyperplasia as components of MEN-1. The patient had elevated plasma concentrations both of prolactin and growth hormone.

a complete spectrum of hyperplasia, microadenomas, adenomas, carcinomas, and carcinoid changes can be found on pathological examination.

F. Gastrinoma

Wermer[12] noted that peptic ulcers of the stomach and duodenum were frequently found in patients suffering from MEN. He suggested that in MEN-1 tumor formation and peptic ulceration were separate manifestations of a pleiotropic gene defect. Others believed ulcers to be a complication of hypercalcemia and hyperparathyroidism (Hellström).[86] This view was supported by the earlier finding of a high incidence of peptic ulcers in patients with hyperparathyroidism (Rogers et al.,[87] 1947). In addition, it was noted that cure of the hyperparathyroidism usually led to amelioration or cure of the ulcer symptoms. Subsequent studies showed, however, that the incidence of peptic ulceration in hyperparathyroidism was only slightly higher than that in the general population (Ostrow et al.[88]) and the majority of experimental results were inconclusive or in conflict with this hypothesis (Moldawer, 1962).[89] It is presently thought that the slightly higher incidence of peptic ulcers in hyperparathyroidism is attributable to inclusion of patients with MEN-1.

In 1955, Zollinger and Ellison[90] described two patients with recurrent peptic ulceration, marked gastric acid secretion, and islet-cell tumors of the pancreas. They suggested that the link between the pancreatic tumor and the ulcer disease was an ulcerogenic factor secreted by the tumor. Some years later, gastrin was extracted from such tumors (Gregory et al.)[91,92] and patients with recurrent peptic ulcers and non-β-islet-cell tumors were shown to have elevated gastrin concentrations in the circulation.[93] The gastrin-producing tumors have been called gastrinomas and the resulting syndrome the Zollinger-Ellison syndrome. It was shown that hypercalcemia induced by infusion of calcium produced a rise in serum gastrin levels

and gastric acid secretion in patients with gastrinomas.[94,95] Thus, in patients with the Zollinger-Ellison syndrome who also have hyperparathyroidism, the associated hypercalcemia may augment acid secretion and subsequently aggravate gastric ulcer disease. This might explain the significant reduction of acid secretion and clinical symptoms after parathyroidectomy in these patients.[96]

Two common variants of the Zollinger-Ellison syndrome have been described: the sporadic variety and the genetic variety occurring in association with MEN-1. Wermer[78] suggested that the former variety be called Zollinger-Ellison's disease, the latter the Zollinger-Ellison syndrome. Evidence of MEN-1 has been found in 15 to 54% of patients with Zollinger-Ellison syndrome.[97-101] On the other hand, the frequency of gastrinomas in MEN-1 ranges from 11 to 55%.[102]

1. Symptomatology

Peptic ulceration and diarrhea are the initial symptoms of MEN-1 in 45%.[15] Because early case reports emphasized the severe and often dramatic complications of atypically located peptic ulcers, the Zollinger-Ellison syndrome came to be regarded as a fulminating disease. Recent clinical experience indicates, however, that the clinical features and course of the syndrome is often less dramatic than originally described, especially in patients diagnosed during family screening.[101,103]

Most gastrinoma patients present with symptoms of gastric acid hypersecretion that cannot be differentiated from common peptic ulcer disease. During screening of relatives of known cases, even asymptomatic patients may be detected. In 16%, the ulcer is found at an unusual site (in the distal part of the duodenum, jejunum, and esophagus),[104] and in 14 to 25% there are multiple ulcers.[105] The atypical ulcers often occur after inadequate surgical treatment (in other words, less than total gastrectomy). When present, the atypically located ulcers are indicative for the diagnosis MEN syndrome. Diarrhea has been reported in between one third and three fourths of patients with Zollinger-Ellison's syndrome by Isenberg et al.[106] It must be distinguished from the massive watery diarrhea without acid hypersecretion seen in "pancreatic cholera" or the Verner Morrison syndrome, which is caused by vasoactive intestinal polypeptide (VIP) producing islet-cell tumors. About 7% of the sporadic form of Zollinger-Ellison syndrome have diarrhea without active ulcers.[107] The stools are either watery or fatty. The diarrhea observed in the Zollinger-Ellison syndrome is probably caused by multiple factors including delivery of excessive amounts of gastric acid to the duodenum, damage to the small-bowel mucosa by gastric juice, and increased motility. Steatorrhea has also been reported in many patients with the Zollinger-Ellison syndrome, i.e., as the result of inactivation of lipase and mucosal damage produced by low pH. In addition, bile salts are precipitated and inactivated at acid pH and are therefore unavailable for micel formation. Since total gastrectomy or antisecretory drugs abolish the diarrhea, it is evident that the diarrhea results from gastric acid hypersecretion.

2. Diagnosis

Because the treatment of the Zollinger-Ellison syndrome differs from that for ordinary peptic ulcers (chronic cimetidine therapy or, in some patients, total gastrectomy), correct diagnosis is important. In patients with gastrinoma with pronounced gastric hypersecretion and hypergastrinemia (>1000 pg/mℓ) presenting with prominent and dramatic clinical features, diagnosis is relatively easy. Before the development of serum gastrin determination, the measurement of gastric acid was an accepted diagnostic method for Zollinger-Ellison syndrome. At that time, the criteria most generally used for the diagnosis were a basal output higher than 15 meq/hr (5 meq/hr in patients with previous gastric surgery) or a ratio of basal acid output to maximal acid output higher than 0.6.[108,109] However, these criteria gave both false-positive and false-negative results.[108,110] Like the measurement of acid secretion the

serum gastrin determination does not provide complete discrimination between patients with and without the Zollinger-Ellison syndrome. Hypergastrinemia may be found in other clinical conditions, for example achlorhydria, severe hypochlorhydria antral G-cell hyperfunction, pyloric obstruction, renal failure, and retained gastric antrum after Billroth-II gastrectomy.[105]

Recently, a new syndrome called antral G-cell hyperfunction has been described which is characterized by basal acid hypersecretion, elevated fasting serum gastrin levels, an enhanced postprandial gastrin response to a meal, and in some patients, association with hyperpepsinogenemia I and autosomal dominant inheritance.[82,111] Antrectomy results in normalization of serum gastrin and gastric acid in such patients.

In some patients with proven gastrinoma the serum gastrin level may be only slightly elevated. Approximately 40% of patients with Zollinger-Ellison syndrome have fasting serum concentrations ranging between 100 and 500 pg/mℓ.[112] These observations indicate the need for additional diagnostic tests. Soon after the development of the gastrin radioimmunoassay, reports of serum gastrin response to various stimuli in patients with gastrinoma appeared. In 1969, the observation was made that a slow infusion of calcium intravenously (IV) over 3 hr in a patient with the Zollinger-Ellison syndrome increased serum gastrin levels in excess of the increase seen in normal controls and duodenal ulcer patients.[94] Since then many investigations have verified this finding.[113-115] However, recent studies indicate the occurrence of false-negative responses among gastrinoma patients and false-positive responses in some hypergastrinemic patients without the Zollinger-Ellison syndrome.[116,117] Furthermore, the Ca-stimulation test is lengthy and may be associated with adverse reactions especially in patients with hypercalcemia due to hyperparathyroidism.[116-118] For these reasons the test should probably not be used routinely. In 1972, Isenberg et al.[119] reported that in patients with Zollinger-Ellison syndrome IV secretin infusion produced paradoxical increase of the serum gastrin concentration. Of eight controls, none responded to secretin. This observation led to the conclusion that serum gastrin response to secretin infusion may be of diagnostic value in patients suspected of the syndrome. With the exception of two studies done by one group,[103,120] all investigations published since then[112,116,117] support this conclusion. No adverse reactions to secretin were observed. There was pronounced divergence in the method of administration (bolus vs. infusion), the dose of secretin (1 vs. 2 U/kg body weight), the type of secretin, the timing of sampling, and criteria for positive response (50% increase;[116] 110 pg/mℓ over basal;[117] 200 pg/mℓ over basal;[112] or peak response of more than 500 pg/mℓ[118]). It is evident that a criterion combining the absolute and the relative increase in serum gastrin after secretin is preferable. A recent study has shown that an increase in serum gastrin of more than 50% with a minimum rise of 100 pg/mℓ is indicative for gastrinoma.[121] When the diagnosis criterion of an absolute increase of 200 pg/mℓ gastrin in response to secretin injection was used, no false-positive and few false-negative responses were recorded, provided that two units of secretin per kilogram body weight were administered and blood samples were drawn at 5-min intervals for 30 min.[112] However, it has been shown that larger doses of secretin than 1 cU/kg do not result in better separation between patients with and without gastrinoma.[122] It should be kept in mind that the value of the test is greatest when the basal gastrin level is less than five times the upper limit of normal. Because, as already mentioned, a false-negative response after secretin injection does occur in some patients with the Zollinger-Ellison syndrome, a negative response should not be considered diagnostically conclusive.

The calcium test is not useful as a second-line test in patients with suspected Zollinger-Ellison syndrome and negative secretin test results since Zollinger-Ellison syndrome patients with negative secretin provocation tests do not respond to calcium infusion.[116] The gastrin response to a standard meal is helpful in differentiating Zollinger-Ellison syndrome from antral G-cell hyperfunction and hyperplasia.[82,111] The glucagon test seems to have no clinical value.[103]

Procedures that have been used to detect a primary gastrinoma or metastatic hepatic tumors include abdominal ultrasound, CT scanning, and visceral angiography. Lesions were only detected by these procedures in 20 to 30% of the patients with Zollinger-Ellison syndrome.[110,123-126] Although the combined detection rate with angiography, CT scanning, and ultrasound has not been investigated, this probably will be low because of multiplicity and small size of the tumors. Upper gastrointestinal endoscopy may lead to the finding of a duodenal tumor. Radioimmunoassay of gastrin in blood samples from pancreatic veins obtained during transhepatic portal catheterization is now used in the localization of an islet-cell tumor.[127,128] Sporadic tumors may be localized by this procedure, making surgical cure by excision of the tumor possible. However, in patients with the Zollinger-Ellison syndrome as part of MEN-1 this method is of limited value due to the multiplicity of tumors. As could be expected, Glowniak[129] found diffuse hypersecretion of gastrin from the pancreas.

3. Pathology

Gastrinomas derive from gastrin-producing cells, the so-called G-cell. Although G-cells have been found in the fetal rat pancreas, most cytologists have been unable to identify them in the normal adult pancreas. Histologically, the majority of the tumors are characterized by a ribbon pattern. The cells are arranged in cords which are separated by delicate strands of connective tissue. The tumors show considerable variation in size, ranging from microscopically small to 10 cm or more in diameter. The malignancy of a pancreatic islet-cell tumor is generally reflected not by its histology but by the presence of metastases in lymph nodes and the liver. Malignant disease at the time of diagnosis has been reported in 61% of patients with Zollinger-Ellison snydrome in general (both the sporadic and genetic variety taken together),[97] and in 35.7% of patients as part of MEN-1,[15] probably due to periodic screening of families at risk. The localization of the tumor is not limited to the pancreas, reported sites include the wall of the duodenum (13%),[130] the stomach, and the hilus of the spleen. Multiple lesions in the pancreas are found in half the cases of Zollinger-Ellison syndrome in general[97] and are probably present in all patients with Zollinger-Ellison syndrome as part of MEN-1.[15] There is no evidence that hypergastrinemia can result from hyperplasia of G-cells in the absence of tumors.

4. Treatment

The standard treatment of Zollinger-Ellison syndrome in general has traditionally been surgical, in the form of total gastrectomy with excision of all accessible tumor tissue. In recent years this approach has been modified by the recognition of the fact that cimetidine can induce ulcer healing in the majority of patients. Total gastrectomy is now reserved for patients who fail to respond completely to cimetidine or ranitidine and for those patients who do not wish to take cimetidine for the rest of their lives. In the past the major cause of death was related to the underyling peptic ulcer complications. With the accumulation of more longitudinal information, it is evident that an increasing number of patients are now dying because of malignant behavior of these progressive slow-growing tumors.[106] Regardless of the ability of cimetidine to control the patients' symptoms, most of the authors agree that surgical exploration should be done in all patients with Zollinger-Ellison syndrome to determine the location and extension of the tumor growth with the hope of total removal.[79,128,131,132] It seems reasonable to give the patients with a potentially curable gastrinoma the opportunity to be permanently free of the threat of death from progression of the neoplasm, but because of the presence of multiple lesions in about 50% and of malignancy in about 60% of the cases, true cure resulting from excision of tumors appears to be an exceptional outcome.[133] Hofmann et al.[130] and Bonfils et al.[132] reported successful excision in 2.5 and 5.4% of all patients with Zollinger-Ellison syndrome (and 20 and 80% of patients with tumor located in the duodenum wall). Because of the relatively good prognosis of the

disease even when metastatic disease is present, McCarthy[134] considers chemotherapy to be the treatment of choice for elderly patients, especially those suffering from concurrent illnesses.

In the literature no special approach is mentioned for the treatment of patients with Zollinger-Ellison syndrome as part of MEN-1. Whereas in Zollinger-Ellison syndrome in general, as already mentioned, multifocal lesions are found in about 50% of the cases, the lesions are multiple in more than 70% of patients with Zollinger-Ellison syndrome as part of MEN-1.[15] The only curative treatment for these patients would be total pancreaticoduodenectomy, but because of the high mortality and morbidity associated with the procedure, this approach is not justified. Whether noncurative excision or debulking of the active endocrine tumor would improve the management of these patients and prolong life is an appropriate question for further studies. Because of the very slow rate of progression of many of these tumors, only studies with a very long follow-up period can provide evidence of therapeutic benefit. Until more data are available on this point we recommend conservative medical treatment in patients with Zollinger-Ellison syndrome as part of MEN-1. With or without tumor excision, therapy with cimetidine must be continued in most of the patients because hypergastrinemia persists in almost all cases. When cimetidine is properly used, virtually all patients reach a clinical remission with respect to acid hypersecretion that is often sustained for several years. McCarthy[135] reported that cimetidine therapy controlled acid peptic disease in a large proportion of 61 cases. Although two other authors also reported success in the treatment of Zollinger-Ellison syndrome with cimetidine,[105,136] others thought that the results of cimetidine therapy were less impressive.[133,137-139] In these latter series, however, all patients who failed to respond to medical treatment had received less than 2.5 per day cimetidine, which suggests that the poor results may have been due to inadequate doses of the drug.

In a study from the National Institutes of Health of 27 patients with Zollinger-Ellison syndrome, 8 received a mean dose of cimetidine of 2.6 g per day and 16 a mean dose of 5.1 g per day cimetidine in combination with an anticholinergic drug.[105] Some patients even needed 10 g per day cimetidine. However, in a study reported by McCarthy,[135] the disease was controlled in two thirds of the patients by 1.2 g per day and in one third by 2.4 g per day. Resistance to cimetidine may develop gradually, and appeared to be correlated with tumor growth and rising gastrin levels in some but not all studies.[140,141] Anticholinergics (especially the more selective drug pirenzepine) may be useful as adjuvant drug. Malagelada[142] prefers to add anticholinergics rather than increase cimetidine dosage above 2.4 g per day. Side effects of cimetidine treatment, which include transient elevation of serum transaminases, gynecomastia, and breast tenderness, are rare.[143] Some of the adverse effects are probably due to the antiandrogenic action of cimetidine. Ranitidine, a newer, more potent, and longer acting H_2-receptor antagonist, may prove to be a valid alternative drug in some cases because it lacks this antiandrogenic effect.

Highly selective vagotomy might facilitate the response to H_2-blockers,[144] but more reports of long-term results are needed to confirm the efficacy of this approach. Prolonged cimetidine administration requires continuous compliance with treatment. Stopping of the treatment will result in rapid recurrence of the symptoms. Two methods to monitor therapy have been proposed. According to Wyke et al.,[145] the optimum dose is best titrated on an out-patient basis when the patient is exposed to the stresses of everyday life. Raufman et al.[146] recommends gastric analysis every 6 to 12 months and proposes an adjustment in the daily dose of H_2-blockers to keep acid output below 10 meq/hr (2 hr before the next dose), because at this low rate of acid output endoscopically demonstrated lesions are rare. Because the majority of the patients mentioned above have multiple malignant lesions, complete resection of all tumor tissue is usually impossible, and death will occur due to cachexia and tumor extension in due course. Since most of the tumors grow slowly and invasive disease may

take years to develop, chemotherapy should only be considered for patients with metastatic disease who have symptoms related to tumor growth or whose clinical course indicates the presence of a more progressive malignant disease. In 1968, Murray-Lyon et al.[147] reported the first successful response to streptozotocin by an islet-cell tumor producing multiple hormones (gastrin, glucagon, and insulin). However, later reports indicated that when streptozotocin was used to treat islet-cell tumors producing only gastrin, the drug was without effect.[148,149] Streptozotocin, an antibiotic derived from *Streptomyces achromogenes*, produces a specific islet-cell toxicity in animal models.[150] Adverse effects of the drug included nausea/vomiting in 83%, renal toxicity in 39%, leukopenia in 5%, and thrombocytopenia in 5% of 84 cases.[151] Streptozotocin has been administered in a wide variety of dosages and via both the intravenous and the hepatic arterial routes. At the dosage level used by Moertel et al.[151] (500 mg/m^2 of body surface area given daily for 5 consecutive days every 6 weeks), renal toxicity seemed to be controllable. Experience in the treatment of a small number of patients with Zollinger-Ellison syndrome suggests that intraarterial administration of the drug may be more effective.[152-154] Ruffner[155] reported a patient with metastatic Zollinger-Ellison syndrome who responded to treatment with streptozotocin in combination with 5-FU. The effect of streptozotocin alone or together with other drugs has been reported for 27 patients with gastrinomas, among whom 8 out of 18 responded to streptozotocin and 3 out of 4 to streptozotocin combined with 5-FU.[105]

Regarding the facts that streptozotocin does not cure the patient, induces only incomplete remissions in only few patients, is accompanied by severe side effects, and has not been shown to improve the prognosis, one should be reluctant to administer this drug to patients with the Zollinger-Ellison syndrome.

G. Insulinoma

Insulinomas are tumors of pancreatic origin that are characterized by an inappropriate secretion of insulin and present clinically with hypoglycemia. In 1927, Wilder et al.[156] were the first to note the association between hypoglycemic symptoms and β-islet-cell tumors of the pancreas. Eight years later, Whipple and Frantz[157] established their famous triad: hypoglycemic attacks precipitated by fasting or exertion, abnormally low blood glucose concentration, and symptoms relieved by oral administration of glucose. Since then the diagnosis has been made more frequently and there have been many reviews, the most voluminous by Stefanini et al.[83] in 1974, covering 1067 cases of proven functioning insulinomas. In 4% of the cases, insulinoma represents the pancreatic element of the MEN syndrome. On the other hand, hyperinsulinism has been reported in 26% of 169 cases of MEN-1.[15]

1. Symptomatology

Insulin-producing islet-cell tumors of the pancreas cause recurrent symptoms of hypoglycemia which mainly occur late in the afternoon after work or early in the morning before breakfast. The clinical manifestations related to the sympathetic overactivity are palpitations, tachycardia, sweating, anxiety, and nervousness. The second group of symptoms is due to the deleterious effect of hypoglycemia on brain functions. These symptoms vary from mild fatigue, mental dullness, and headache to severe symptoms with abnormal behavior, seizures, and episodic unconsciousness. Because of these manifestations, many patients were initially admitted to neurological or psychiatric wards. A carefully taken history will often distinguish hypoglycemic attacks from epilepsy, postural hypotension, Adams-Stokes attacks, or transient ischemic attacks. Less frequently, patients with insulinoma present with gastrointestinal symptoms include hunger, vomiting, and occasionally epigastric pain. Their increased appetite and hyperinsulinism often lead to obesity. The only difference in the clinical picture between sporadic and familial insulinoma (as a part of MEN-1) is that obesity is less frequent in the latter.[15]

2. Diagnosis

The most important clue provided by history in patients with insulinoma is the relation of the symptoms to meals and exercise. In spite of characteristic symptoms and the many procedures available at present for verification, diagnosis is often delayed. In the 1067 cases reviewed by Stefanini et al.,[83] the diagnosis insulinoma was made in 34% within 1 year, in 47% within 1 to 5 years, and in 20% after 5 years. If blood can be sampled during an attack, a high insulin concentration coinciding with hypoglycemia may be sufficient to confirm the diagnosis. The insulin/glucose ratio provides the reliable parameter to confirm or establish the diagnosis insulinoma in patients with mild abnormalities. Turner et al.[158] suggested that the amended I/G ratio (plasma insulin (CμU/mℓ) $\times$ 100 divided by plasma glucose (mg/dℓ) $-$ 30) would give better discrimination between patients with hypoglycemia due to insulinomas or other causes. This ratio will be above 50 in patients with insulinoma. However, in the study done by Seyer-Hansen and Lundbek,[159] 3 out of 15 patients with proven insulinoma had an amended I/G ratio that was below this value. Most patients with insulinoma have hypoglycemia after an overnight fast, and the basal insulin levels, which are inappropriately high in relation to low glucose, are diagnostic; in these cases no further investigation is needed. If hypoglycemia is not induced, fasting should be continued up to 70 hr and the patient should be vigorously exercised at the end of the test. When fasting hypoglycemia is mild or absent, additional tests are necessary to confirm the diagnosis. Stimulatory tests with tolbutamide, leucine, or glucagon are rarely employed today, because they produce both false-positive and false-negative results.[160] However, stimulation of insulin secretion by calcium infusion has recently been suggested as another useful provocation test. The cumulative data of Roy et al.[161] and Kaplan et al.[162] include only one false-negative study in 14 insulinoma patients and no false-positive studies.

A number of suppression tests have been reported. Hypoglycemia induced with fish insulin suppresses endogenous immunoreactive insulin in normal subjects, but this suppression is impaired in insulinoma patients.[163] However, fish insulin is difficult to obtain. A similar test consists of the injection of commercial insulin and the measurement of C-peptide instead of insulin.[164] Because C-peptide release from an insulinoma may be very low, this test is not completely reliable and normal or near normal suppression of C-peptide has been reported in patients with proven islet-cell adenoma.[165,166] Mandelkow et al.[167] have found that the C-peptide suppression test yields a pathological result in 85% of the patients with insulin-producing tumors. The estimation of fasting levels of proinsulin may be helpful in the diagnosis of insulinoma because these tumors often release excess amounts of proinsulin.[168] Other causes of fasting hypoglycemia include extra-pancreatic tumors, hypopituitarism, Addison's disease, severe liver insufficiency, alcohol ingestion, and factitious administration of insulin or sulfonylurea. Hypopituitarism, Addison's disease, liver insufficiency, and alcohol abuse are easily recognized clinically. Factitious induced hypoglycemia can be detected by measuring immunoreactive insulin and C-peptide or proinsulin, since exogenous insulin will suppress endogenous insulin release and inappropriate low C-peptide and proinsulin will be apparent. Self-medication with sulfonylurea can be recognized by analysis of a blood sample.[169] The extra-pancreatic tumors which give rise to hypoglycemia are rare and usually sufficiently large to be detected by ordinary clinical examination.

Once the presence of an insulinoma has been confirmed biochemically, the next step is to attempt to locate the tumor. The surgeon's difficulty in finding an insulinoma is well known. Because surgical morbidity and mortality are greatly increased when major blind pancreatic resections are performed, preoperation localization procedures are very important. Usually insulinomas are relatively small and are thus beyond the resolution of pancreatic ultrasound and computed tomography (CT) scanning. This accounts for the disappointing results reported in the literature on these techniques.[170] Similarly, endoscopic retrograde cholangiopancreatography (ERCP) is not helpful because the tumors are not ductal in origin.

The advent of selective angiography was a major advance in the localization of islet-cell tumors. The success of this procedure varies widely (38 to 91%),[171,172] but in experienced hands, arteriography — improved by new techniques (highly selective injections, magnification, and subtraction) — is able to localize most insulinomas prior to surgery. The deficiencies of the arteriographic method of localizing insulinomas led to the development of a new technique: pre- or peroperative percutaneous transhepatic portal-venous sampling (THPVS). With this technique the sampling of pancreatic venous blood and its assay for insulin has permitted the localization of small insulinomas previously undetectable by arteriography or by palpation of the pancreas.[173,174] Although Daggett et al.[175] questioned the value of this procedure, most authors[176-180] consider THPVS to be a safe, useful, and reliable method for the localization of insulinomas. Recently, Cho et al.[179] reported the successful use of this technique in 12 patients with organic hyperinsulinism.

3. Pathology

Among patients with an insulinoma, a number of pathologic entities can be recognized including adenoma, microadenoma, hyperplasia, and carcinoma. Most nonfamilial insulinomas are single adenomas (83 to 92%.)[83,162] When multiple insulinomas are found the MEN-1 syndrome should be suspected because in insulinoma as part of this syndrome, multiple lesions have been reported in 93% of cases.[15] Whereas islet-cell carcinoma is found in 8% of the patients with sporadic insulinoma, malignant lesions have been reported in 21% of the cases of insulinoma as part of MEN-1.[15] About 1% of the insulinomas arise in ectopic sites outside the pancreas.[162]

4. Treatment

The main objective of the medical management of patients with insulinoma has been the prevention of prolonged and repeated hypoglycemic attacks which can cause permanent cerebral damage. This may be accomplished by frequent high-carbohydrate feedings. For some, the inclusion of a bedtime feeding is sufficient, for others a midmorning or midafternoon snack will be necessary. The prognosis for patients with insulinoma was greatly improved by the introduction of diazoxide.[181] This drug, a benzothiadiazine, directly inhibits the release of insulin from the β-cells. Patients with benign insulinoma have been successfully managed with diazoxide for many years. The starting dose is 50 mg every 8 hr and this may be increased to more than 1 g daily. The adverse effects of diazoxide include edema, nausea, hirsutism, and hypotension. The addition of a diuretic may not only correct edema but also synergize the hyperglycemic effect of diazoxide. The treatment of choice in patients with sporadic insulinoma is surgical removal. In these patients most single lesions in the body or tail of the pancreas can be dealt with by a distal pancreatectomy, although enucleation can be performed for small single lesions on the surface of the organ. Lesions of the head are usually enucleated. If the tumors cannot be located by imaging, sampling, or palpation at operation, the treatment likely to give the best result is progressive blind pancreatic resection from left to right, with examination of the excised specimen, and frequent blood glucose measurements.[83,162,182,183] However, accurate preoperative or peroperative sampling for insulin measurements will usually obviate the need for blind resection. Some authors[184,185] avoid major surgical intervention in these patients if the hypoglycemia can be controlled by diazoxide.

In patients with insulinoma as part of MEN-1, theoretically, the only curative treatment is complete resection of the pancreas because multiple lesions are found in 92% of the cases. Surprisingly, after partial resection of the pancreas containing multiple insulinomas, hypoglycemic attacks often do not recur. Furthermore, since medical treatment with diazoxide in patients with insulinoma is not always successful, a more aggressive approach is justified. In patients with symptoms due to metastases of insulinoma, chemotherapy is required. The

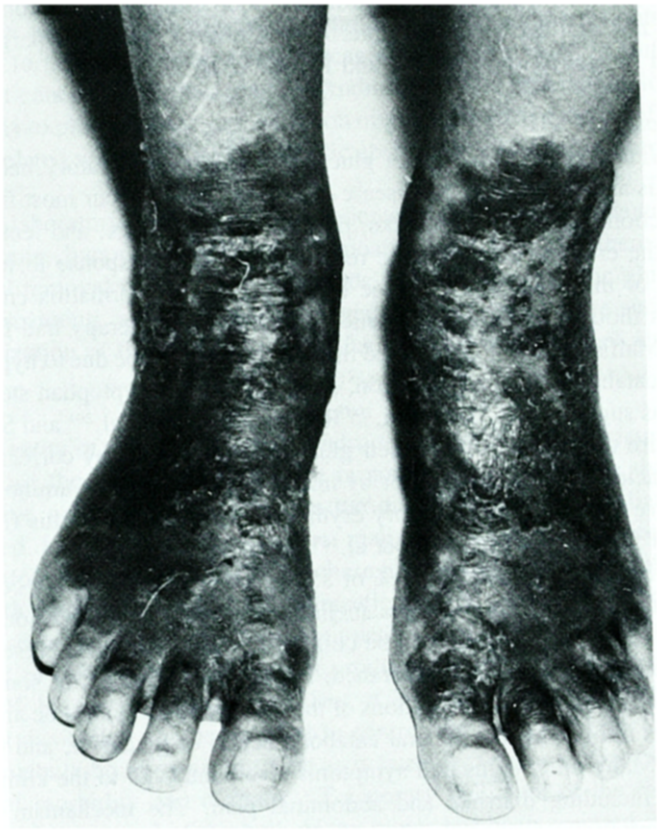

FIGURE 3. Skin lesions in a patient with glucagonoma syndrome.

most active agent for achieving a permanent remission of insulinoma is streptozotocin. Of 30 patients with functioning malignant insulinomata treated with this drug, 50% showed an objective reduction of the tumor mass.[186] Moertel et al.[151] reported seven patients with insulinoma of whom six responded to treatment with streptozotocin in combination with 5-FU.

H. Glucagonoma

The third major clinical syndrome associated with an islet-cell tumor results from the synthesis of glucagon. In 1942, Becker et al.[187] first described the association of a distinctive cutaneous eruption with an islet-cell carcinoma of the pancreas. In 1966, McGravan et al.[188] reported the case of a 42-year-old diabetic woman with an unusual bullous and eczematoid dermatitis of the hands, feet, and legs who was found to have an α-cell tumor of the pancreas. He was the first to show glucagon secretion by these tumors by radioimmunoassay. In 1974, Mallinson et al.[189] published a review of nine cases and described the major features of the syndrome: diabetes mellitus, characteristic skin lesions, stomatitis, anemia, and weight loss in association with elevated plasma glucagon levels. These authors suggested that the term glucagonoma syndrome was appropriate for their patients. Wilkinson[190] described the skin lesions in great detail and used the term necrolytic migratory erythema to describe the rash clinically (Figure 3). Up to 1981, about 84 cases had been reported.[191] Glucagonomas may be a very rare component of the MEN-1 syndrome. As far as we know, glucagonomas seem to have been part of the MEN-1 syndrome in only five cases reported in the literature.[192-196] On the other hand, the glucagonoma syndrome was not mentioned in an extensive

review of 169 cases of the MEN-1 syndrome,[15] published in 1971, probably because the syndrome was not well known at that time. A syndrome of familial hyperglucagonemia has been described by Boden and Owen[197] and Palmer et al.[198]

1. Symptomatology

The most widely reported aspect of glucagonoma is the migratory necrolytic erythema and the glossitis associated with the disease. The skin lesions occur most frequently in areas exposed to friction, such as the buttocks, perineum, groins, legs, and feet. Becker et al.[187] believed that the eruption might be the result of the host's response to tumor cells in the skin. Because of the resemblance of the dermatosis to acrodermatitis enteropathica, zinc deficiency was thought to be a pathogenic factor, but zinc therapy had failed to improve the lesions.[199] Mallison et al.[189] speculated that the lesion could be due to hypoaminoacidemia caused by the catabolic effect of glucagon. Depletion of the tryptophan store with resultant skin lesions was suggested by Galdabini.[200] Recently, Norton et al.[201] and Stacpoole et al.[196] were able to cure the skin lesions of their glucagonoma patients by correcting the levels of plasma amino acids by total nutrition or by infusion of a mixture of amino acids. Although the precise cause of necrolytic migratory erythema remains unclear, this finding makes the explanation put forward by Mallinson et al.[189] the most likely.

Glucose intolerance was found in 90% of 85 proven or probable cases collected from the literature by Stacpoole.[191] The diabetes mellitus is usually mild and not associated with ketoacidosis and most of the cases can be controlled with a diet. The presence of glucagon excess might explain the glucose intolerance, but this is the subject of some debate.[202,203]

Other frequently reported manifestations of the glucagonoma syndrome are loss of weight, also possibly resulting from the general catabolic action of glucagon, and thromboembolic complications. Many other signs and symptoms also contribute to the clinical spectrum of the syndrome, including diarrhea and abdominal pain. The mechanism of the diarrhea occurring in about 50% of cases is unknown.

2. Pathology

In all cases of glucagonomas in which histological characteristics of the tumor were determined, the lesion was composed of the α type of islet cells. Classification of the type of islet cell was made on the basis of special staining procedures and the ultrastructural examination revealing characteristic secreting granules. At the time of diagnosis, 50% of the glucagonomas had metastasized.[191] Multiple tumors have been reported in only a minority of glucagonoma patients.[191] The tail of the pancreas has been the most frequent site of origin.[191] Two cases of extra-pancreatic glucagonoma have been described, one in the proximal duodenum[196] and the other in the right kidney.[205] The kidney tumor is the only known glucagonoma producing enteroglucagon. The clinical symptoms of the patient were constipation and the small intestine showed villous hypertrophy.

3. Diagnosis

The glucagonoma syndrome should be considered in all patients with the characteristic skin lesions. Once suspected, the diagnosis can be established by determination of glucagon in plasma. If the concentration is above 1000 pg/mℓ the diagnosis is certain. Other disorders involving hyperglucagonemia include diabetes mellitus, burn injury, acute trauma, bacteremia, and liver cirrhosis.[191] The basal hormone concentration reported in patients with the glucagonoma syndrome varies between 320 and 96,000 pg/mℓ.[191] Additional tests are rarely required to differentiate between patients with and without glucagonoma. The most widely used are the arginine test (abnormal rise of glucagon) and the glucose tolerance test (no fall of glucagon).[203] Recently, Stacpoole et al.[196] reported that secretin induced a rapid and marked increase of glucagon in a patient with the glucagonoma syndrome, whereas no

stimulatory effect was detected in normal subjects. These authors suggested that responsiveness to secretin may be of diagnostic importance in glucagonoma patients.

Four molecular forms of immunoreactive glucagon have been found in variable amounts in the serum of patients with the glucagonoma syndrome.[203] The different biological potency of each species may explain why the concentration of glucagon in the plasma of these patients is not always correlated with clinical or metabolic abnormalities resulting from the glucagon excess.[206]

Other common laboratory findings in these patients include hypersedimentation, anemia, hypercholesterolemia, hypoproteinemia, and hypoaminoacidemia. All these abnormalities are correctable by removal of the tumor. Methods to localize pancreatic glucagonoma are echography, CT scanning, and angiography. Ingemansson et al.[207] have successfully used selective catheterization of the pancreatic veins for local determination of glucagon.

4. Treatment

Because of the rarity of the glucagonoma syndrome, experience with treatment is limited. Surgical removal of the tumor offers the best prognosis, but since more than 50% of the glucagonomas have metastasized by the time the diagnosis is made, palliative therapy is frequently required. Like other islet-cell tumors, malignant glucagonoma appears to be slow-growing and control of the hormonal effect of these tumors can therefore result in substantial palliation. As with other endocrine active pancreatic tumors, administration of somatostatin results in decrease of hormone levels. A long-acting analogue of somatostatin has been successfully used in two patients in whom it produced a reduction of the glucagon levels for 12 to 24 hr.[208] However, data on the effect of long-term treatment with subcutaneous injections are not available. Another method to control the hormonal effect of the tumor is laparotomy with debulking of as much tissue as is feasible, whenever this can be done with a minimal risk of morbidity and mortality. Temporary palliation of the malignant glucagonoma syndrome by removal of the bulk of the tumor has been reported.[206,209]

Chemotherapy is required when the symptoms recur after debulking or initially in patients with an inaccessible tumor. Even when metastasis has already occurred, excellent palliation provided by chemotherapeutic drugs can result in prolonged survival. Streptozotocin is usually effective in inducing tumor reduction and decrease in glucagon levels in plasma. An excellent and prolonged response to dimethyltriazenoimidazole carboxamide (DTIC) has been described in five patients.[206,210-212] However, in a recent report concerning another series, the drug had to be discontinued because of severe thrombocytopenia.[196]

I. Vipoma (Verner-Morrison Syndrome)

Priest and Alexander[213] in 1957 and Verner and Morrison[214] in 1958 described a syndrome of refractory diarrhea and hypokalemia associated with islet-cell tumors of the pancreas. Based on the clinical findings, two other names have been applied. Matsumoto and Peter[215] suggested the term pancreatic cholera because of the clinical similarity between this syndrome and that produced by a *Vibrio cholerae* infection, whereas Marks et al.[216] proposed the acronym WDHA syndrome (watery diarrhea, hypokalemia, achlorhydria). It should, however, be noted that most patients with a vipoma are not achlorhydric, although gastric acid secretion is often low. In 1973, Bloom et al.[217] showed that plasma and/or tumor extracts from six patients suffering from the Verner-Morrison syndrome contained large amounts of VIP. They suggested that tumors containing VIP should be called VIPomas, and the syndrome itself the VIPoma syndrome. Animal studies have shown that this peptide hormone strongly stimulates intestinal secretion of water and electrolytes,[218,219] inhibits acid secretion,[220] promotes hepatic glycogenolysis and hyperglycemia,[221] and dilates peripheral blood vessels.[222] Since these actions fit well with the frequent clinical findings of severe watery diarrhea, hypo- or achlorhydria, diabetes mellitus, and flushing, excessive production of VIP has been

thought to be responsible for the Verner-Morrison syndrome.[217,223] However, there are now several reports of tumor-bearing patients with the typical Verner-Morrison syndrome in whom a normal or slightly elevated plasma VIP concentration was found and in these cases other agents such as pancreatic polypeptide,[224] prostaglandin,[225] and calcitonin[226] were thought to be responsible. Although the frequent finding of increased plasma and tumor concentrations of VIP in patients with the Verner-Morrison syndrome favors the view that this hormone plays a role, these data suggest that it is not the sole etiologic agent. There are also reports of extra-pancreatic tumors underlying the Verner-Morrison syndrome, e.g., medullary carcinoma of the thyroid, carcinoma of the bronchus,[227] ganglioneuroblastoma,[228] a renal tumor,[229] and a pheochromocytoma.[230]

Cases of VIPoma as part of MEN-1 have rarely been reported. One of the cases in the original description[214] of the syndrome by Verner and Morrison concerned a patient with MEN-1. In a review of 65 cases collected from the literature by Burckhardt,[231] five had abnormalities in other endocrine organs. Of the 50 patients with VIP-producing tumors accompanied by diarrhea analyzed by Long et al.,[228] two had a family history of pancreatic endocrine tumors and there was one case of MEN-1. The mean age of the patients in these series was 49 years. There was a slight preponderance of women.

1. Symptomatology

The predominant symptom in this group of patients is profuse cholera-like diarrhea, which can amount to 10 to 15 ℓ/24 hr, but may also be mild and intermittent. The stools have the appearance of weak tea and are rich in electrolytes. Weight loss is a constant feature of the syndrome. Abdominal pain, nausea, and vomiting may occur during the more fulminant episodes of diarrhea. As the diarrhea progresses, symptoms due to hypokalemia and dehydration appear. The patient complains of general muscular weakness and even paralysis. Correction of the hypokalemia, often associated with acidosis, often requires large quantities of exogenous potassium. Other common manifestations of the Verner-Morrison syndrome are hypercalcemia, diabetes mellitus, and achlorhydria, which have been reported in 56, 54, and 33% of the cases, respectively.[231] Hypercalcemia in the Verner-Morrison syndrome might be due to simultaneous hyperparathyroidism (MEN-1) or secretion by the tumor of a substance with calcium-elevating properties.[226] In addition, cutaneous flushing and a dilated gallbladder are observed in some patients.

2. Diagnosis

The average time from onset of the symptoms to diagnosis of the Verner-Morrison syndrome has been reported to be 3 years.[232] When the Verner-Morrison syndrome is suspected in patients with severe watery diarrhea and hypokalemia, measurement of plasma VIP concentrations can establish the diagnosis.[217] The plasma VIP level in 62 patients with VIPomas (pancreatic tumors and ganglioneuroblastomas) ranged between 48 and 760 pmol/ℓ, with a mean value of 203 pmol/ℓ (normal 6.4 ± 0.4 pmol/ℓ).[228] However, since some patients with the full clinical Verner-Morrison syndrome have normal VIP levels, other hormones that could possibly be responsible should be measured. In most of the reported cases, VIP-producing islet-cell tumors have been localized by selective angiography. In doubtful cases portal venous catheterization and sampling for VIP determination may be helpful.[233]

3. Pathology

The pathologic lesion can be a benign or malignant tumor or islet-cell hyperplasia. Among 65 cases summarized by Burckhardt,[231] 42% had a malignant and 37% a benign tumor and 14% had islet-cell hyperplasia. Multiple lesions were found in only 5% of the cases. Most of the tumors were located in the tail of the pancreas. There is a considerable variation in

size, but most of the tumors were less than 8 cm in diameter. The tumor cells react positive to VIP antibodies. Ultrastructural studies may show numerous electron-dense secretory granules having a diameter of about 170 nm.[228]

4. Treatment

Treatment of the Verner-Morrison syndrome caused by pancreatic islet-cell disease is primarily surgical. For a solitary benign pancreatic tumor, excision or resection has been curative. In patients with an unresectable tumor, treatment with chemotherapy is indicated. Remissions lasting several years have been reported after streptozotocin.[234,235] Other drugs, such as indomethacin,[225] lithium carbonate,[236] trifluoperazin,[237] metodopramide,[238] corticosteroids,[230,233] and long-acting somatostatin[228] have provided relief of the syndrome in single cases.

Measurement of VIP may be useful for monitoring the effect of therapy and to predict regrowth of the tumor.[228]

J. Parathyroid Lesions

Hyperparathyroidism is the most common endocrine abnormality in MEN-1 patients. Of the 169 cases reviewed by Croisier et al.,[15] 148 (87%) had parathyroid involvement. On the other hand, in about 16% of the patients, primary hyperparathyroidism was a component of the MEN-1 syndrome.[81,239] Because the other manifestations of MEN-1 may be delayed for many years, a patient with this oligo symptomatic form of MEN-1 may be erroneously classified as a case of familial hyperparathyroidism.

1. Symptomatology

The patient may present with a long history of vague arthralgia or bone ache with or without gastrointestinal or urogenital disturbances, but the majority of the patients with hyperparathyroidism as a part of MEN-1 are asymptomatic. Croisier et al.[15] saw involvement of the renal tract in only 27% of their MEN-1 cases in contrast with the incidence of renal involvement reported for two large series of hyperparathyroidism in general, (i.e., 57 to 77%).[240,241] The relatively low incidence of kidney involvement in patients with hyperparathyroidism associated with MEN-1 might be explained by the tendency for the hyperparathyroidism to be diagnosed in an early asymptomatic stage in such patients due to screening studies initiated because of a familial history of endocrine neoplasia or because of the presence of pituitary or islet-cell tumors in the patients themselves.

In addition, since the introduction of routine calcium screening 3 decades ago, the clinical spectrum of nonfamilial hyperparathyroidism has shifted dramatically from the classic description of "bones, stones, and abdominal groans" to the asymptomatic patient. In Albright and Reifenstein's[242] original series 2% of 64 cases of primary hyperparathroidism were asymptomatic. In 1961, Keating[240] described a series of 380 patients, 5% of whom had no symptoms. "Biochemical" or asymptomatic patients accounted for 47% of 100 cases reported by Lafferty[243] and 46% of 318 cases reported by Purnell et al.[244] This may explain why, in two recent studies, Boey et al.[81] and Lamers and Froeling[245] found no significant difference in the incidence of symptoms and complications between patients with hyperparathroidism as a component of MEN-1 and patients with isolated hyperparathyroidism. Although the incidence of peptic ulcers in studies on hyperparathyroidism has ranged up to 24%, a figure of 9.1% proven incidence resulted from a critical analysis of 429 cases reported in six series by Ostrow et al.[88] These authors concluded that the incidence of peptic ulcers is not strikingly higher than the frequency of peptic ulcers among the population at large. More recent authors too, have pointed out that the increased susceptibility to peptic ulcers with hyperparathyroidism is rare.[239,243]

2. Pathology

Whereas 18% of the patients with hyperparathyroidism have multiple parathyroid tumors or chief-cell hyperplasia,[246] the majority of the patients with hyperparathyroidism associated with MEN-1 have multiple adenomas or hyperplasia. In Croisier's review[15] of the literature, 60% of the patients (77 out of 116 cases) had multiple adenomas or hyperplasia. In Lamers' series[245] 10 out of 13 patients with MEN-1 proved to have multiple enlarged parathyroid glands. Proper interpretation of the pathological changes in the parathyroid glands is difficult. Reports of parathyroid lesions in studies on the MEN syndrome differ between observers, which is probably a reflection of the controversy concerning the detection of adenomatous and hyperplastic changes in the parathyroid glands of these patients.

Hyperplasia, adenomas, and carcinomas appear to form a continuous spectrum. Although every type of parathyroid cell can be involved in hyperplasia, the majority are chief cells. Malignant degeneration is rare.

3. Diagnosis

The existence of hyperparathyroidism can be established by the finding of elevated levels of serum calcium and serum alkaline phosphatase, combined with a depressed plasma level of inorganic phosphate. The immunoassay for parathyroid hormone is proving to be of increasing value as a specific diagnostic test for hyperparathyroidism, but there are still numerous problems associated with the interpretation of assay results.

Histological examination of bone specimens from patients with severe hyperparathyroidism reveals a number of changes that collectively define the presence of parathyroid overactivity. There is a reduction in the number of trabeculae and an increase in the number of giant multinucleated osteoclasts as well as substantial replacement of normal cellular and marrow elements by fibrous tissue. In milder forms of skeletal involvement, early changes can sometimes be detected by radiography of the hands and skull. The phalangeal tufts may be resorbed and an irregular outline replace the normally sharp cortical outline of the bone in the digits (subperiosteal resorption).

4. Treatment

The only effective treatment for hyperparathyroidism is surgical. Subtotal parathyroidectomy (STP) was first proposed by Cope et al.[247] in 1958 for the treatment of hyperparathyroidism due to chief-cell hyperplasia. Block et al.[248] reported a success rate of 86% for STP in multiple-gland disease. However, among their six patients with MEN-1 syndrome there was one case of persistent hyperparathyroidism and two patients developed recurrent hyperparathyroidism. In a recent review by Prinz et al.[249] of 12 patients with MEN-1 undergoing STP, two remained persistently hypercalcemic and two developed recurrent hypercalcemia. Thus, it seems that in patients with MEN-1 the results of STP are not as favorable as in patients without MEN-1 syndrome.

Wells et al.[250] recommended total parathyroidectomy and autotransplantation as suitable treatment for chief-cell hyperplasia, and Prinz et al.[249] proposed this approach as the treatment of choice for MEN-1 patients requiring surgical treatment for primary hyperparathyroidism. For patients undergoing reoperation for persistent or recurrent hyperparathyroidism due to multiple gland disease, Saxe and Brennan[251] recommended total parathyroidectomy, reserving delayed autografting with cryo-preserved tissue for patients with prolonged hypocalcemia. This approach may prevent confusion about the source of postoperative hypercalcemia and minimize the risk of graft-dependent hypercalcemia by restricting autotransplantation to those truly in need of additional tissue.

When a second operation is indicated, selective thyroid venous catheterization with sampling for PTH levels may be helpful for preoperative localization.[252] Accuracy of localization of both side and site of parathyroid tumors can be of the order of 80%.[253,254]

As mentioned above, the majority of the patients with hyperparathyroidism (HP) as part of MEN-1 and an increasing proportion of the patients with nonfamilial HP are asymptomatic and have a moderate degree of hypercalcemia. It is not clear whether all such patients require surgical treatment. There need be no hesitation in recommending parathyroid exploration when major manifestations of the disease are present, for example osteitis fibrosa, recurrent renal stone formation, decreased renal function, hypertension, psychological disturbances, and/or a repeated serum calcium level above 11 mg/dℓ. Scholz and Purnell[255] left 141 patients with mild biochemical hyperparathyroidism without surgery for 10 years. Of this group, 14 were lost to follow-up and 10 refused to cooperate in follow-up assessment. The authors found that only 24.6% of the remaining patients developed indications for surgery, including serum calcium above 11 mg/dℓ, radiological evidence of bone disease, impaired renal function, active or inactive renal stones, impracticality of prolonged observation, and/or gastro-intestinal complications. They were unable to define criteria that would predict which patients with asymptomatic hyperparathyroidism will ultimately require surgery.

Most authors,[81,255-261] however, advocate surgery in all patients with asymptomatic HP in the absence of definite contraindications. Some authors[262,263] think a conservative approach is justifiable in some patients provided they are kept under regular review. The rationale for surgical treatment has included the prevention of important complications, particularly renal damage. In addition, cervical exploration in skilled hands is safe and effective,[261] whereas intensive follow-up is expensive and time consuming with a high rate of patient drop-out.[264]

However, the surgical treatment of HP as part of MEN-1 is associated with a high percentage of recurrence after parathyroidectomy. In these patients careful follow-up should in any case be continued to insure early recognition of other endocrine abnormalities. The critical problem in these cases is the possibility of progressive impairment of renal functions if hyperfunctioning parathyroid tissue is not extirpated. Worsening hypercalcemia, osteitis fibrosa, and psychological disturbances are reversible complications. Even nephrolithiasis need not be very worrisome, because it rarely causes renal damage and disappears after parathyroidectomy.[259,265] But renal damage caused by hypercalcemia fails to heal and can progress to renal failure.[266,267]

Significant deterioration of renal functions was demonstrated in only 6 of the 142 patients with HP left untreated for 10 years.[255] Since renal function decreases for many reasons in the middle-aged, who also have the highest incidence of HP,[268] it is not certain that the declining renal function in the patients of Scholz and Purnell[255] was due to HP.

When all considerations are taken into account, it could well be argued that, particularly in the elderly, surgical treatment for HP as part of MEN-1 can be delayed provided follow-up is strict. It must not be forgotten, however, that we lack a prospective trial performed to determine the incidence of declining renal function in treated and untreated patients. When parathyroidectomy is indicated, the operation should be done by an experienced surgeon.

It would be of great value to have an alternative to surgical treatment. An agent that inhibited the secretion of parathyroid hormone (PTH) would be potentially useful. Caro et al.[269] reported significant reduction of the PTH and serum Ca concentrations in eight patients with primary HP given propranolol. Other investigators[270-272] have shown that propranolol does not reduce PTH or serum Ca levels in patients with primary HP. Sherwood et al.[273] reported that the H_2-receptor antagonist cimetidine reduced the calcium concentration and suppressed PTH levels in 12 patients with primary HP.

Others found that cimetidine has little effect in reducing calcium concentrations.[274,275] The diphosphonate clonodrate disodium is a powerful inhibitor of bone resorption and has been used successfully for the reduction of increased bone resorption and hypercalcemia in patients with primary HP.[276,277]

It is apparent from this review that more data are needed before unequivocal conclusions can be drawn with respect to the efficacy of propranolol, cimetidine, and diphosphonate in the treatment of primary HP.

K. Pituitary Lesions

Pituitary lesions were detected in 65%[14] and 50.8%[15] of cases of MEN-1. Although some of these tumors produce STH (HGH) or ACTH and result in acromegaly and Cushing's disease, the majority were thought to be nonsecreting chromophobe adenomas. However, recent studies have shown that in sporadic cases as many as 70%[278] of nonsecreting pituitary tumors in fact secrete prolactin. Furthermore, an increasing number of reports on prolactin-producing pituitary adenoma occurring in MEN-1 have appeared.[279-282] Stabile et al.[283] found that 54% of their patients with Zollinger-Ellison syndrome as part of MEN-1 have elevated prolactin levels. Because of this high prevalence, determination of prolactin levels has been recommended in these patients.[281,283] The exact incidence of MEN-1 in association with prolactinoma is unknown.

1. Symptomatology

The pituitary tumors present classically in two ways. First, they can cause compression of adjacent structures, e.g., the optic chiasm and the normal pituitary tissue, which results in bitemporal hemianopsia and hypopituitarism. Second, tumors can secrete an excess of pituitary hormones, including prolactin, STH, and ACTH. Impotence, amenorrhea, and infertility are recognized to be important manifestations of prolactin hypersecretion. Clinical features of STH excess are acral enlargements, hyperhidrosis, headaches, paresthesia, hypertension, and diabetes mellitus. Hypersecretion of ACTH by the pituitary lesion leads to bilateral adrenocortical hyperplasia with corticosteroid excess resulting in Cushing's disease. Pituitary tumors may be the first manifestation of endocrine disease in MEN-1. Of 169 cases collected from the literature by Croisier et al.[15] 15% presented in this manner.

2. Diagnosis

The diagnosis of functional pituitary tumors depends on the demonstration of elevation of basal hormone levels. Sometimes additional tests are required for confirmation of the diagnosis. Polytomography of the sella turcica and high-resolution computed tomography with use of the thin-slice technique can reveal most pituitary tumors.

3. Treatment

Treatment of pituitary tumors as part of MEN-1 is not essentially different from that in patients with isolated pituitary tumors.

There are several modes of treatment for pituitary lesions. Selective transsphenoidal microsurgery has supplanted intracranial exploration and conventional supervoltage irradiation for pituitary adenoma, except when tumors have invaded surrounding structures. Irradiation should be considered after subtotal removal of tumors.[284]

However, some workers[285] have advocated heavy-particle irradiation as initial therapy. Recently, several types of drugs have become available to reduce hormone secretion in tumors producing prolactin, STH, or ACTH. The dopamine agonist bromocriptine, as primary therapy, has been used successfully to lower serum prolactin levels and reduce the size of the adenoma in the great majority of patients.[286-288] Bromocriptine treatment of acromegaly has been controversial.[289-291] Recent reports suggest that bromocriptine may also be of benefit in the management of some patients with nonfunctioning tumors.[292,293]

Serotonin antagonists such as cyproheptadin have been reported to be effective in some patients with (mild forms of) Cushing's disease.[294]

L. Other Lesions

Adrenal cortical involvement is very common in patients with MEN-1. The adrenal glands were affected in 41.4% of 169 cases of MEN-1.[15] The pathologic picture may be that of cortical adenoma, hyperplasia, nodular hyperplasia, or even carcinoma. In only a few cases

does the clinical history indicate that the tumors have functioned. Several patients with Cushing syndrome secondary to an ACTH-secreting pituitary tumor have been described and one patient with hyperaldosteronism has been reported.[14] Thyroid disease was present in 26.6% of the 169 MEN-1 patients collected from the literature by Croisier et al.[15] The pattern of involvement is inconsistent. Thyroid adenoma, colloid goitre, thyroid carcinoma, and thyrotoxicosis have been reported. Because of the high prevalence in the general population of abnormalities in the thyroid and adrenal glands found at autopsy, it is questionable whether these lesions should be considered an intrinsic part of the MEN-1 syndrome.

Less common findings reported in MEN-1 patients include lipoma, carcinoid tumors, thymic tumors, and pinealoma. Williams and Celèstin[295] were the first to note the frequent association between the bronchial adenoma of the carcinoid variety and other endocrine tumors. Carcinoid tumors were found in nine cases in Croisier's series.[15] These occur in the bronchi, the stomach, the duodenum, the jejunum, the ileum, and the thymus, and they do not differ from nongenetic carcinoid tumors. Recently, the vertical transmission of carcinoid tumors as part of the MEN-1 syndrome has been reported.[282]

M. Prognosis, Causes of Death, Screening

The prognosis of MEN-1 is dependent on several factors, i.e., the quality of the diagnostic and therapeutic procedures applied and the information made available to the patient and his family doctor. Instructions should be given with respect to symptoms, complications, treatment, prognosis, and inheritance of the syndrome. The patients should be told that the only form of prevention of the disease is to have no children.

Complications of peptic ulceration are the most common causes of death among members of families with MEN-1. In the group of Lamers,[100] 8 of the 12 patients whose death was related to MEN-1 died of complicated peptic ulcer disease. In Ballard et al.'s study,[14] complications of peptic ulcers were responsible for 48% of the deaths among MEN-1 patients. Other reported causes of death have been complications related to hypoglycemia, disseminated carcinomatosis, renal failure secondary to hyperparathyroidism, electrolyte imbalance secondary to intractable diarrhea, pituitary insufficiency, and complications of pancreatic and pituitary surgery.

The finding of any endocrine tumor in the pituitary or parathyroid glands or the pancreas in one individual calls for a thorough family history and an appropriate study to detect other endocrine lesions. It has been stressed by many authors.[13,16] that all relatives of patients with MEN-1 should be screened because the probability that they will be affected amounts to 50%. From an analysis of the literature, Betts et al.[296] concluded that in the MEN-1 syndrome HP invariably accompanies the Zollinger-Ellision syndrome and they suggested that screening of affected members of such families could therefore be restricted to the measurements of serum calcium levels. However, others[16] have proposed an extensive scheme of diagnostic tests. In our opinion, the basic screening program for relatives of MEN-1 patients should include an assessment of clinical symptoms due to pituitary lesions, HP, or pancreatic lesions, the determination of fasting glucose, calcium, phosphate, gastrin levels, and prolactin, and radiography of the skull. Further investigations should be restricted to those patients in whom these tests give positive results, (i.e., determination of levels of GH, ACTH, cortisol, oral glucose tolerance test (for acromegaly), daily excreted urinary 17-hydroxycorticosteroids, dexamethason suppression test, PTH, gastrin, secretin test, insulin, glucagon, VIP, PP, urinary 5-HIAA). These screening procedures should be repeated at regular intervals, because the occurrence of endocrine disorders increases with age. Since the incidence of endocrinopathy is very low in children, the screening program should be started with relatives of MEN-1 patients aged between 15 and 20.

Recently, pancreatic polypeptide (PP) has been suggested as a reliable marker for endocrine pancreatic tumors in patients with MEN-1.[297] This 36-amino acid polypeptide was isolated

from impurities in insulin preparations[298,299] and was traced to the secretory granules of a distinct endocrine cell type[300] (F or D_1 cell) in the pancreas. Despite extensive studies[301] on the physiological actions of pancreatic polypeptide, the clinical expression of its biological effect is obscure. Only a small number of cases of pure PP cell tumors have been reported.[297,302,303]

It is not known which symptoms should alert the physician to a possible PPoma. High PP concentrations are found in 10 to 77% of the patients with endocrine pancreatic tumors[304-308] (insulinomas, gastrinomas, vipomas, and glucagonomas). Elevated PP levels are frequently reported, especially in patients with pancreatic tumors as part of MEN-1.[297,301] In all, high PP levels have been reported in three patients with pancreatic tumors as part of MEN-1 studied by Friesen[297] and in four out of five MEN-1 patients with pancreatic tumors described by Floyd et al.[301] However, in a recent study by Lamers and Diemel[309] only three of the eight MEN-1 patients with pancreatic neoplasms showed moderately elevated serum PP levels.

In sum, it may be said that the determination of PP as a screening marker seems to be of limited value. Since normal PP secretion is regulated mainly by vagal activity, an atropin suppression test has been advocated[310] to differentiate between elevated serum PP concentrations of tumor and nontumor origin. In Lamers and Diemel's study,[309] however, all three patients with endocrine pancreatic tumors and elevated serum PP levels showed atropin-induced decreases of the serum PP concentration.

More recently, Prinz and Marangos[311] suggested that neuron-specific enolase (NSE) may be a useful marker for diagnosis and the monitoring of the response to therapy in patients with neuroendocrine tumors. NSE is an isomer of the glycolytic enzyme enolase and is found exclusively in neuroendocrine cells.[312] Prinz and Marangos[311] found elevated levels of NSE in 9 out of 21 patients with a variety of neuroendocrine tumors. However, since NSE is an enzyme, it is not released into the plasma under normal conditions. It presence in plasma may reflect the presence of a very large tumor with overflow or necrosis. Thus, NSE is probably not a useful marker for the early diagnosis of endocrine tumors in the MEN-1 syndrome, but is indicative of tumor burden.[313]

Periodic screening of first-degree relatives of MEN-1 patients may improve the diagnosis and increase life expectancy, because potentially life-threatening tumors can be detected and treated in an early stage. However, it is not certain whether screening on such a large scale can satisfy the criteria to be maintained in epidemiologic screening programs. For example, the natural history of the disease should be known, i.e., what would happen in the absence of treatment? Adequate knowledge about the natural course would permit effective and uniform strategies for diagnosis and treatment. The only way to assess a positive effect of screening and treatment would be to perform a randomized controlled trial, but such an investigation would seem to be ruled out on ethical grounds. Another approach would be a strict follow-up of treated patients combined with an inventory of the causes of death in earlier generations of a large group of patients. The results of this kind of retrospective study could provide a basis for decision making about whether to screen families with this syndrome.

III. MULTIPLE ENDOCRINE NEOPLASIA TYPE 2

A. Pheochromocytoma

A pheochromocytoma[314] is a tumor that arises from chromaffin cells called pheochromocytes[315] and induces an overproduction of catecholamines, epinephrine, and/or norepinephrine thus leading to a syndrome with hypertension as one of the features. The hypertension can be permanent (65% of cases) or paroxysmal (30%) and in a small percentage of cases of pheochromocytom it is entirely absent.[316-319] The clinical expression of pheochromocytoma, which is often dramatic and explosive, is so variable that it has rightly

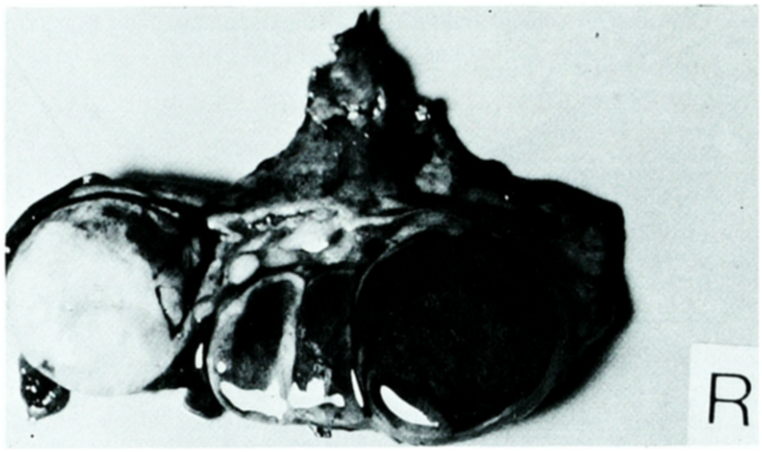

FIGURE 4. Gross specimen of the enlarged right adrenal gland showing multinodular pheochromocytomas. Cross section of this gland demonstrates a small rim of cortex above the central tumor nodules. The left nodule shows fibrosis and the right one is hemorrhagic and partly necrotic.

earned the title of the great mimic.[18,330] Especially during the last decade, familial investigation and improvements in biochemical documentation, radiological localization, and anesthetic management have led to safer management of pheochromocytomas in patients with the MEN-2 syndromes.

1. Incidence

Pheochromocytomas occur relatively infrequently but are by no means rare. Van Way et al.[321] estimated the number of patients with pheochromocytomas in the U.S. to be 75,000, i.e., 0.037% of the total population, and Melicow[319] and Manger and Gifford[322] put the incidence in the U.S. at 0.018%. Each year 400 new cases of pheochromocytoma are diagnosed in the U.S.[323]

Pheochromocytomas occur as solitary or sporadic tumors or as a hereditary familial disease (in the latter case often showing a bilateral and multiple localization). The familial form is less common than the sporadic; according to Steiner et al.[1] at least 6% of all pheochromocytoma are familial, which means that there are probably 5000 MEN-2 patients in the U.S. Whenever bilateral pheochromocytomas are found a familial occurrence should be suspected.[324,321] Calkins and Howard[325] gave the first description of familial occurrence. Smits and Huizinga[326] reached the conclusion that familial pheochromocytoma constitutes a hereditary disease with an autosomal dominant mode of transmission.

Comparison of the biological behavior of the two phenotypes of pheochromocytoma reveals a difference in age of onset (3 to 78 years for the sporadic form; 20 to 60 years for the MEN-2 syndrome). The sex ratio for both is 1:1.

2. Pathology

Since pheochromocytomas arise from chromaffin cells, they can occur not only in the adrenal gland (90 to 95%) but also at the other sites where sympathetic nerve tissue is present. They can arise from paraganglionic cells of the entire sympathetic side-chain system — thoracic as well as abdominal — and in the solar plexus, the organ of Zuckerkandl, the wall of the bladder, the region of the internal and external genitals, and also in chromaffin cells left behind during embryological migration.

It has been established that in the MEN-2 syndrome the lesions of the adrenal medulla are generally bilateral (70 to 80% of cases) and multicentric (Figure 4) and that as a rule

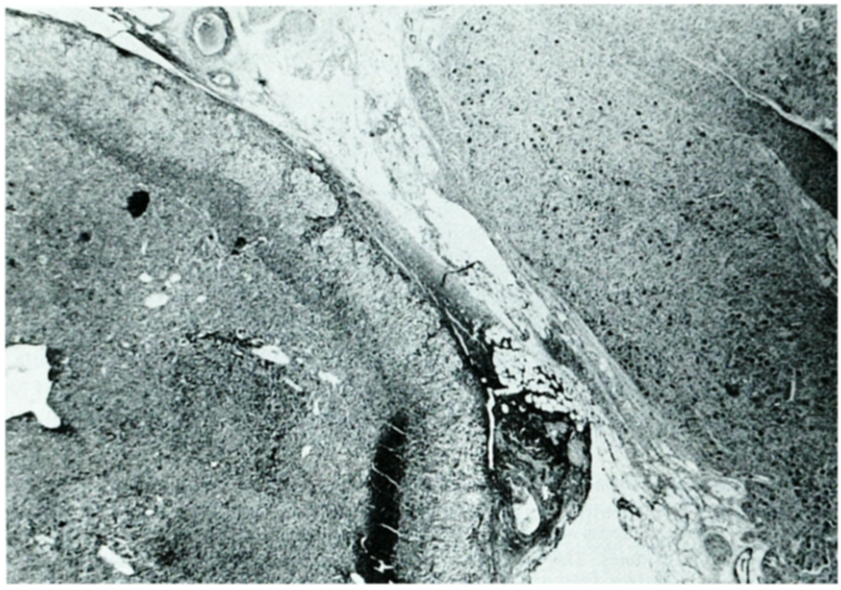

FIGURE 5. Complete accessory adrenal gland adjacent to a celiac ganglion containing a small central pheochromocytoma histologically characterized by the presence of very large vacuolated pheochromocytes, pronounced anisokaryosis, atypical nuclei, and intracytoplasmic spherical eosinophilic inclusions (hyaline globules).

pheochromocytomas develop.[327] Beside distinct tumor formation, diffuse and/or nodular hyperplasia of the adrenal medulla can occur.[328,329]

Extra-adrenal pheochromocytomas have been described frequently in the MEN-2 syndrome,[1,330] especially in complete accessory glands. Ljungberg[327] found pheochromocytoma tissue in an accessory adrenal at autopsy in one patient. Sjoerdsma et al.[331] and Scully et al.[332] too described the presence of extra-adrenal pheochromocytomas in complete accessory adrenal glands in patients with the MEN-2A syndrome . The incidence of complete accessory adrenal glands was exceptionally high in our series of patients.[333] Solitary complete accessory glands containing small central pheochromocytomas were found in about 50% of our MEN-2 patients operated on for pheochromocytomas, and multiple complete accessory adrenals were found in one patient (Figure 5).

Less than 1% of all pheochromocytomas develop outside the abdominal region, mostly in the thoracic sympathic trunk.[318,334-336] Intrathoracic pheochromocytomas have never been described in the MEN-2 syndrome.

Microscopically, the tumor consists of bands or nests of polyhedral cells separated by thin strands of connective tissue, rich in blood vessels. The cytoplasm is abundant, finely granular, and basophylic. The nuclei vary widely in size. Upon immersion of the tumor in a dichromate solution, the color becomes dark brown.

Malignant degeneration occurs in 2.6 to 11% of the sporadic tumor (in 24% for tumors situated in ectopic sites)[337] and is rare in the familial form. The usual hallmarks of malignancy do not hold for this type of tumor. According to Davis et al.,[338] who defined the criteria for the diagnosis of malignant pheochromocytoma, malignancy is only certain if secreting metastases are present in nonendocrine tissue (liver, bone). The histological differences between benign and malignant pheochromocytomas are difficult to define.

In five of Carney's cases[339] the tumor penetrated through the tumor capsule and invaded the periadrenal fat. Tumor growth through vessels has been identified in three cases and metastatic spread occurred in four cases. Metastases have been found in the lung, liver, and

bone. Westfried et al.[340] described a case of Sipple's syndrome with malignant pheochromocytoma that metastasized to the pericardium and both lungs.

3. Symptomatology

A complaint pattern is subjective and therefore difficult to characterize as absolutely negative or positive. The pleomorphism of the clinical picture of pheochromocytomas is so great that a wide range of complaints is possible. The symptoms caused by excessive production of catecholamines are dependent on the character of the catecholamines that reach the circulation, i.e., the ratio between epinephrine and norepinephrine, as well as the amount in which they are secreted in a given time. On the basis of this ratio, adrenal or extra-adrenal localization could often be predicted.[341,342]

A given endocrine pattern can enhance certain features of the clinical picture. There is, for instance, a form characterized by paroxysms and one with sustained hypertension that can have a benign or a malignant course. Sometimes the course is relatively asymptomatic[1,343,344] and sometimes metabolic anomalies develop such as hyperglycemia/glucosuria and a resistance to insulin. In cases characterized by paroxysms, the intensity, frequency, and duration of the attacks can vary widely. As a result of the sudden release of predominantly epinephrine, the patient develops feelings of apprehension and fear; tachycardia follows as well as a drop in the peripheral resistance, as a result of which the blood pressure may drop. Subjectively, the patient experiences palpitation, headache, dizziness, a dead feeling in the limbs or over the entire body, pallor, and breaking out in cold sweat, sometimes with shaking of the whole body. Immediately after the attack, polyuria develops together with polymiction and glucosuria; the temperature and basal metabolism rise and the blood glucose level is often elevated.

When norepinephrine dominates, bradycardia is more likely, with a sharp rise of the blood pressure to as much as 300 mmHg. At certain ratios of epinephrine to norepinephrine both pictures can occur in various combinations. Sometimes the patient knows what induces the attacks, and sometimes he can even precipitate one himself.

There are also patients who never have distinct paroxysms but may predominantly show more general symptoms, such as increased appetite despite leanness, shortness of breath, anginal or asthmatic complaints, persisting headaches, dizziness upon standing up, and abdominal complaints such as an excess of gastric acid and stubborn obstipation. There may also be severe abdominal pain (occasionally due to hepatic congestion or spasm of the pyloric sphincter). The picture of an acute abdomen may even develop (total bowel obstruction, hemorrhage in a pheochromocytoma, etc.).[345-347]

Orthostatic hypotension can occur in patients with hypertension (about 70% of the pheochromocytoma patients). Some patients show hypovolemia (reduced blood volume), especially those with sustained hypertension. The presence of a pheochromocytoma can lead to an elevated metabolic rate.

In general, there is no correlation between the catecholamine and metabolite excretion in the urine and the severity of the complaints. No distinct relationship has been found between the weight of the pheochromocytomas removed at surgery and the complaints of the patients. Some patients have a very distinct complaint pattern of paroxysms which is consistent with their abundant release and secretion of catecholamines and metabolites in the urine. But there are also patients with high urinary levels who report never having had an attack. In Hill et al.'s series,[348] 37% had distinct paroxysms. Carney[339] found that 60% of pheochromocytoma patients had symptoms. Theoretically, it is possible for patients to have pheochromocytomas with mainly minimal secretory activity.[349]

Our patients with the MEN-2 syndrome show relatively more beta-receptor stimulation; arrhythmia and tachycardia are more frequent in these patients. Blockade of alpha- or beta-receptors alone can be extremely dangerous in MEN-2 patients.[350-352] The biological behavior

of pheochromocytoma differs from family to family and is much more favorable in some families than in others.

4. Tumor Markers

In cases of pheochromocytoma, catecholamines are stored along with a variety of soluble proteins in the chromaffin secretory granules or vesicles. Catecholamine secretion from the adrenal gland occurs by exocytosis, the entire content of the vesicles, including soluble proteins, being released. The proteins within chromaffin granules have collectively been called chromogranines. Among these proteins are the peptide hormones calcitonin, somatostatin,[353] neurotensin,[354] VIP,[355] substance P,[356] ACTH, beta-MSH, beta-endorphin, lipotrophin, and preproenkephalin. Spark et al.[357] (1979) described a 47-year-old woman with Cushing's syndrome caused by ectopic ACTH secretion from a functioning pheochromocytoma, and Hoffman et al.[358] reported a case with Cushing's syndrome due to ACTH production. Dermody et al.[359] developed a cell line derived from a human pheochromocytoma, which produced calcitonin and ACTH, and Nakada et al.[360] in 1981 detected ACTH and beta-MSH in a human pheochromocytoma removed from a Sipple patient with no manifest clinical features of Cushing's syndrome. Bertagna et al.[361] determined the tissue concentrations of immunoreactive lipotrophin, beta-endorphin, and metenkephalin in ten pheochromocytomas. These peptides were present in all cases, with and without Cushing's syndrome. The authors found no correlation between the two classes of opioid peptides, i.e., proopiomelanocortin and preproenkephalin, which is consistent with the accepted theory that endorphin and enkephalin derive from different precursor molecules. Since metenkephalin has been shown to be present in the same chromaffin cells that contain the catecholamines and to be released with the latter after stimulation,[362,363] the plasma level of enkephalin might be a good tumor marker for the detection of pheochromocytoma.

Calcitonin seems to be present in the chromaffin secretory granules of most pheochromocytomas.[364-368] Mendelsohn et al.[369] demonstrated calcitonin in pheochromocytoma from patients with the MEN-2 syndrome whose pheochromocytoma also contained metastatic MTC.

5. Prognosis

There is only a small chance that a patient with the MEN-2 syndrome and only medullary hyperplasia or even tumors in both adrenal glands will ultimately die of invasive growth or metastases of a malignant pheochromocytoma. Life is endangered much more by the overproduction of catecholamines. For instance, in a series of 17 patients reported by Carney et al.[339] five died from the effects of pheochromocytomal activity. Other data collected from the literature[322] indicate that of the 149 patients reported to have MEN-2, 33 (22%) died from the functional consequences of pheochromocytoma (myocarditis, myocardial infarction, or a cerebrovascular accident). The tumors do not have to be large to be dangerous for the patient: the turnover of catecholamines is higher in small early pheochromocytomas than in large tumors.[342,370] In our patients with the MEN-2 syndrome small pheochromocytomas were shown to have a higher daily excretion of VMA and metanephrines per gram tissue than larger ones.

If the urinary secretion of catecholamines and metabolites has increased distinctly over a certain period in patients with MEN-2 (i.e., patients with proven C-cell hyperplasia or medullary thyroid carcinoma), bilateral adrenalectomy seems warranted and CT-scanning redundant. In practice, however, it is usually preferable to have some information about the size and site of the adrenal tumor.

On the other hand, there are individuals with pheochromocytoma who show very low secretory activity in the absence of stimulation.[349] In such patients with the MEN-2 syndrome, pheochromocytomas can be insidious and treacherous because they can grow to considerable

size without producing any clinical symptoms and/or positive test results. In such patients the first manifestation can lead to a fatal issue.[371] This sometimes occurs at a relatively young age.[19,372,373] In one of our families, five female members died during childbirth at an age between 26 and 37 years. On these grounds we consider that for patients with MEN-2 who have undergone surgery for MTC and whose catecholamine and metabolite excretion is in the normal range, CT scanning is a useful procedure.

6. Diagnosis

During the last decade the main biochemical diagnostic test for pheochromocytoma has been the determination of urinary vanillylmandelic acid (VMA), metanephrines, and catecholamines.

In MEN-2 patients we can expect to find pheochromocytoma especially in both adrenal glands; in other words, the hyperplastic or neoplastic medulla is found together with cortical tissue and therefore epinephrine in particular will be produced. In 1976, Coupland et al.[374] found that chromaffin tissue outside the adrenal gland did not contain a detectable amount of epinephrine. It is now generally accepted that the glucocorticoids secreted by the cortex enhance the activity of the medullary enzyme phenyl-ethanolamine-*N*-methyltransferase (PNMT), which converts norepinephrine into epinephrine. Because of the anatomical structure of the adrenal gland and its portal circulation, the medulla is exposed to relatively high concentrations of corticosteroids. When pheochromocytoma are present in the adrenal medulla, a relatively higher production of epinephrine is to be expected. A positive relationship between PNMT activity and epinephrine concentration has been established in pheochromocytomas.[375]

It is known from the literature that in MEN-2 patients the amount of epinephrine in pheochromocytoma tissue in the adrenal gland is often higher than 50% of the catecholamine content.[376] The epinephrine and norepinephrine in the tumor do not always correspond to the levels in the blood or urine. Sometimes norepinephrine is not secreted but only serves as precursor for epinephrine. Coupland et al.[374] showed that in the adrenal medulla the turnover of epinephrine is much higher than that of norepinephrine. Epinephrine-containing cells in the adrenal medulla incorporated initiated DOPA much faster and secreted newly synthesized epinephrine much sooner than norepinephrine-storing cells which took up and released their basic amines.

MEN-2 patients show relatively more beta-receptor stimulation. Arrythmia and tachycardia are more frequent in these patients. Blockade of alpha- or beta-receptors alone can be extremely dangerous in these patients.

From the foregoing it is evident that in this group of patients it is useful to determine epinephrine and norepinephrine separately to find out whether the ratio between them increases as an expression of tumor growth,[377-379] and to localize the site of the pheochromocytoma.[341,342] A high E/N ratio indicates pheochromocytoma tissue in association with adrenal medulla. An increase in this ratio in a given period sometimes constitutes a more distinct indication of the rate of progression from adrenal medulla hyperplasia to pheochromocytoma.

It should be kept in mind, however, that patients with accessory pheochromocytomas without cortical tissue and producing very large amounts of catecholamines, will show very low E/N ratios. This situation occurs especially in patients who have undergone bilateral adrenalectomy and still have very small pheochromocytomas without cortical tissue.

It is conceivable that the storage and release mechanisms function normally in a tumor that induces paroxysmal hypertension but abnormally in patients with sustained hypertension (constant release independent of physiological exogenous stimulation, e.g., by stress).

Since about 50% of the catecholamines in the urine are conjugated, especially in patients without paroxysms or sustained hypertension, the urine must be hydrolyzed before the total

content of these substances is determined. The determination of only free unconjugated catecholamines immediately after a paroxysm provides better indications in patients with paroxysms and without hypertension.

The most sensitive diagnostic tools available have been the methods for the determination of urinary metanephrines and VMA, with accuracy rates of 95 and 89%, respectively. In recent years, the measurement of fractionated urinary and plasma catecholamine levels has greatly increased the diagnostic accuracy.

The methods used for the measurement of the plasma catecholamine level has the advantage of being both simple and rapid. However, it is questionable practice to base a diagnosis on the result obtained in a single plasma sample, since many factors influence plasma catecholamine levels and, furthermore, there may be overlapping between the plasma catecholamine levels of normal subjects and those of patients with small pheochromocytomas. In addition, the half-life of catecholamines is less than 2 min, and random plasma measurements can be misleading in cases where secretion from tumors is paroxysmal with normal blood levels in the intervening periods. For these reasons, preference is often given to periodic measurements of catecholamines and metabolites in urine. Although the measurement of plasma catecholamines should be most informative in the group of patients with hypertension and continuous overproduction of catecholamines, when the patient is normotensive at the time of sampling, the plasma values may be false-negative.[322]

Plasma sampling during paroxysmal attacks may be helpful, but values obtained by direct venapuncture of normal patients under stress can also be very high.

7. Provocative Tests

Pharmacological tests are not specific for catecholamine-producing tumors and are of limited value.[380] An adequate pharmacological screening test to demonstrate pheochromocytoma is not available. Every test that can precipitate a hypertensive crisis (whether due to drug administration or some physical maneuver) or can lower the blood pressure appreciably involves a potential risk.

Pharmacological tests fall into two categories, one covering situations in which the blood pressure is relatively normal and the other covering patients with distinctly elevated blood pressure. Three tests can be used in patients with relatively normal blood pressure:

1. The histamine test,[381] which is based on the measurement of blood pressure increase after intravenous administration of histamine-base. False-positive (11%)[382] and false-negative[322,383] results have been reported. The side effects can be very serious.
2. The glucagon test,[384] which gives both false-negative and false-positive results and involves the risk of a hypertensive crisis.
3. The tyramine test,[385] which is less dangerous than the histamine test but cannot be considered reliable because of the high frequency of false-negative results.

When the blood pressure is distinctly elevated, an adrenergic blocking test is used; three types are available:

1. The phentolamine test, which often gives false-negative results.
2. The clonidine suppression test,[386] which offers several advantages in that it inhibits only neurogenically mediated catecholamine release and thus confers diagnostic specificity. The side effects are minimal.
3. The pentolinium expression test.[387] This ganglion blocker inhibits the release of norepinephrine and epinephrine from adrenergic nerve ending and the adrenal medulla in normal persons but not in patients with autonomic function of adrenal medulla tumors.

Due to the risks involved, the use of pharmacological tests is only justified in subjects complaining of severe paroxysms and without elevated excretion of catecholamines and metabolites in the urine. During the test plasma samples are taken and urine is collected for determination of catecholamine and metabolite level.

At present, no strict limits can be given as indications for surgical treatment of pheochromocytoma. Factors such as paroxysmal attacks, hypertension, age, and the family history may play an important role in the final decision. A waiting attitude has proved to be justified when subsequent half-yearly controls showed no further rise. If in time only a marginal increase in the excretion of VMA and metanephrines is detected, additional information about the presence of pheochromocytomas may be obtained by CT. Preventive surgery seems premature for individuals with the predisposition to develop pheochromocytomas, for example patients with positive results of C-cell provocation tests. Predisposition does not always lead to expression of the syndrome. Some patients with proven MTC can reach old age without developing pheochromocytomas.

8. Localization

The main radiological procedures utilized for localization have been nephrotomography, selective angiography, and, since 1976, CT. CT has shown the highest diagnostic accuracy, and it is a safe method provided glucagon is not administered during the investigation. In our experience, only very small nodules or medullary hyperplasia are missed. As a periodic screening test, however, CT has the disadvantage of exposing the patient to excess radiation.

In the unoperated patient with biochemically diagnosed pheochromocytoma, our initial localization procedure is CT of the adrenal glands; further studies are not required unless there is strong suspicion of metastatic disease.

The recently developed positive radioisotope scan for adrenal medullary tissue[388]can be expected to be helpful in suspect patients whose CT scans are negative and in patients with indications of malignant disease.

9. Treatment

Pheochromocytoma with unmistakably malignant characteristics have seldom been described in patients with the MEN-2 syndrome. As already mentioned, the chance may be considered small that a patient with the MEN-2 syndrome will die due to malignant degeneration and extensive metastases of pheochromocytomas; for these patients the overproduction of catecholamines is much more dangerous. In the above-mentioned series reported by Carney et al.[339] 5 of the 17 patients died from the effects of pheochromocytoma activity. A review of the literature by Manger and Gifford[322] in 1977 has shown that 33 out of 149 reported MEN-2 patients (22%) died due to pheochromocytoma complicated by myocardial infarction or a cerebrovascular accident. Furthermore, the pheochromocytomas in MEN-2 patients can be very treacherous because they can become very large in the absence of symptoms or positive test results and thus the first attack can be fatal. Fatal attacks can occur at a relatively young age. In such patients childbirth can lead to serious attacks.[19,372,373] On these grounds, Carney et al.[339] apply very strict criteria for surgery once the diagnosis adrenal medullary disease has been made in a patient with the MEN-2 syndrome: the only form of treatment accepted is bilateral adrenalectomy with extirpation of all extra-adrenal pheochromocytoma tissue.

The main objective of the operation must be the removal of as much of the catecholamine-producing tumor tissue as possible to reduce the risk of a myocardial infarction or a cerebrovascular accident. It therefore seems justifiable to extirpate the large pheochromocytomas in the adrenal gland together with the entire gland, and, if present, any extra-adrenal pheochromocytoma tissue in the celiac region and the side-chain regions. The possible presence of small cervical, thoracic, or sacral pheochromocytomas does not justify the risks involved

in mutilating radical explorations, partly because extra-abdominal pheochromocytomas have never been demonstrated in MEN-2 patients. If patients who have not been operated on are suspected of having hyperplasia of chromaffin cells (normal catecholamine excretion and negative adrenal gland imaging in a patient with MTC belonging to a MEN-2 family), it is justifiable to postpone surgery. But if the patient has paroxysms and if the catecholamine levels rise in consecutive tests, biadrenalectomy and exploration of the side-chains are indicated even if the adrenal glands have a normal appearance. If functionally active pheochromocytomas and/or roentgenographic signs are present, we prefer to perform bilateral total adrenalectomy rather than removal of one enlarged gland because of the bilateral predisposition, the high probability of simultaneous bilateral involvement of the adrenal medulla, the risk of local recurrence if both glands are not removed, and the high mortality associated with the presence and increasing activity of pheochromocytoma tissue. Avoidance of a second operation is an additional advantage.

The physician cannot guarantee life-long follow-up of these patients, although patients without adrenal glands require life-long substitution therapy under good medical care. This treatment involves little risk.

The above-mentioned indications for bilateral adrenalectomy hold except in cases in which extra risk is involved, for instance patients who have already reached an advanced age, or who have a reduced life expectancy due to widespread metastases of MTC. In these cases, alpha- and beta-adrenergic receptor blockade may be considered to suffice.

The surgical approach is based on the probability of simultaneous bilateral involvement of the adrenal medulla, on the risk of local recurrence if the entire adrenal gland is not removed, on the occurrence of accessory adrenal glands, and on the very high mortality due to the activity of pheochromocytomas.

The exploration of the celiac plexus is by preference performed after removal of the left adrenal gland via a costo-lumbal incision along the 12th rib on the left side. The rib is resected subperiostally and the diaphragm is left intact. This also facilitates exploration of the side-chain. The right adrenal gland is removed at the same session and via a similar incision, after the patient has been turned on to his other side.

In general, tumors of patients with the MEN-2 syndrome usually appear benign during operation. In agreement with the most recent reports, sodium nitroprussid seems to be the drug of choice for the treatment of significant hypertension. Propranolol is very useful for the treatment of supra-ventricular tachycardia. Patients with ventricular arrhythmia are treated with lidocaine.

10. Postoperative Follow-Up

It is imperative to maintain follow-up of MEN-2 patients after surgical treatment of pheochromocytoma to detect any recurrence of over-production of catecholamines. Persistent or recurrent pheochromocytomas in patients who have previously undergone surgery may result from:

1. Failure to find a lesion during the initial exploration
2. Development of a second primary tumor at a different site
3. Peroperative disruption of the tumor during the first operation, with implantation of tumor cells
4. The presence of metastatic disease

The management of patients with recurrence of persistent symptoms may be difficult. Once the diagnosis pheochromocytoma is confirmed, attempts must be made to localize the tumors. Methods of choice are CT of the adrenal and lungs, intravenous pyelography, chest radiography, pulmonary tomography, liver and bone scans, arteriography, ultrasonography,

and selective venous sampling for catecholamine determination. At present, the results of the use of ^{131}I-metaiodobenzylguanadine(MIBG) for the localization of pheochromocytoma are encouraging. Preliminary results show that even small extra-adrenal pheochromocytomas as well as malignant metastases can be demonstrated. Perhaps therapeutic doses of ^{131}I MIBG will deserve consideration in the future.

When possible, the residual tumor is removed completely. If a curative operation is not possible, a tumor-reducing resection is recommended. Patients with unresectable metastatic disease may have prolonged survival if maintained on alpha- and beta-adrenergic receptor blockade. The results obtained with cytotoxic agents in extensive disseminated solitary pheochromocytoma are not encouraging. Sometimes the synthesis blocker alpha-methyl-parathyrosine has beneficial effects. Streptozotocin in combination with adriamycin or cytoxan, vincristine or BCNU have been reported to induce a partial response in occasional patients.

B. Medullary Thyroid Carcinoma

In 1901, Walther Bürk[389] delivered his inaugural address at Tübingen University. In this lecture entitled *Ueber einen Amyloid Tumor mit Metastasen* he described a malignant tumor of the thyroid which contained amyloid and produced metastases in the cervical lymph nodes, mediastinal lymph nodes, the lungs, and the pleura. Thyroid carcinomas of this type have been distinguished from the follicular and the papillary thyroid carcinomas since Hazard et al.[21] In 1959, it was not yet suspected that MTC has an unusual histogenesis. Since MTCs were regarded as unusual carcinomas, attempts were made to find the cell of origin. Nonidez[51] introduced the term parafollicular cells and demonstrated that these cells in the canine thyroid gland were argyrophilic. These parafollicular cells have since been described by several investigators under different names.

The next relevant discovery was made by Copp et al.,[390] who in 1962 identified a new hormone which they called calcitonin (human calcitonin, HCT) because it was able to reduce the calcium level. The definitive evidence of the presence of calcitonin in the parafollicular cells was supplied in 1966/1967 by Bussolati and Pearse[52] on the basis of cytochemical immunofluorescence studies. Pearse[53] then gave these follicular cells the appropriate name of C-cells. In the same year, Williams[28] found in histopathological studies that MTCs were composed of cells whose structure resembled that of the parafollicular cells of the normal thyroid, and therefore suggested that MTC originated from these follicular cells. This hypothesis was verified a few years later in studies on the ultrastructure of the tumor cells.[391]

In 1970, Tashjian et al.[392] established that virtually all patients with an MTC had increased serum HCT levels. Moreover, calcium infusion led to a rise of the serum HCT level in patients with an MTC.[393,394] This suggested that small MTCs might be detected by a calcium stimulation test. Tashjian's hypothesis was confirmed by the results of a study done by Melvin et al.[395] in a large family with the MEN type 2 syndrome. It was thus demonstrated that HCT is a biochemical tumor marker for MTC. Periodic determination of the serum HCT levels has since been performed regularly and has proven to be indispensable for the detection and follow-up of individuals in high-risk groups and thyroidectomized MEN-2 patients.

1. Incidence

In most cases MTCs are solitary and sporadic. This unique tumor accounts for 3.5 to 11.9% of all thyroid gland carcinomas.[1,28,327,348,396-401] Most authors state that MTC is familial in about 10 to 20% of the cases, but according to some[3,379,402] this percentage is closer to 30%. The percentage of familial occurrence is probably even higher, as may eventually be confirmed when more is known about the MEN-2 syndromes and screening procedures are

used more widely to investigate suspect families. The sex ratio for the familial form is 1:1. The absence of sex difference is in marked divergence from the pattern in papillary and follicular thyroid carcinomas (F/M = 2:1). Anaplastic carcinomas show the same distribution as MTC.[396]

The age at diagnosis of the sporadic and familial form of MTC varies considerably. The age limits reported in the literature range from 2 to 60 years (mean: 21 years) for the familial cases and from 10 to 82 (mean: 36 years) for the nonfamilial cases.[3,401,403,404] Chong et al.[3] reported 51 years as the age at diagnosis of the sporadic form and 29 years for the familial form. Raue et al.[404] reported 44 years as the age at diagnosis for their entire MTC group. In the families we screen we find a mean age of 25 years for the manifestation of MTC. It is reasonable to continue screening procedures until the age of 35.[405] However, development at a later stage is possible. We have even seen the disease skip a generation.

In the MEN-2 syndrome, the age at which MTC becomes manifest (age at onset) is generally lower than that for pheochromocytoma.[1,379,406] The reverse is seen in a much smaller number of cases.[19,407]

2. Pathology

Sporadic vs. familial occurrence — C-cells can be easily detected in tissue sections by immunocytochemical demonstration of stored calcitonin, with the use of specific calcitonin antisera and either immunofluorescence or immunoperoxidase staining techniques. In the normal human thyroid gland, C-cells are dispersed within individual follicles sandwiched between the basement membrane and the follicular epithelium, either as single cells or small groups. The C-cells are not distributed evenly throughout the gland, but are concentrated deep within the lateral lobes along the upper two thirds of the central axis of the lobe. In adults, approximately one in a thousand cells in the entire glandular epithelium is a C + cell,[408,409] whereas neonates and children may have more prominent C-cell populations.

In the familial variety of MTC, neoplasms develop bilaterally and multicentrically on a background of pre-existing C-cell hyperplasia. In familial MTC, C-cell hyperplasia is probably the morphological reflection of the first mutational event, which is genetically and polyclonally determined, and occurs bilaterally in all patients. Initiation of monoclonal proliferation could be considered as the second mutational event, resulting in the development of adenomas and carcinomas. The progression from hyperplasia to real malignancy could be dependent on errors in DNA synthesis or repair. Perhaps there is a derepression of an oncogene. As the proliferative monoclonal process progresses, the C-cells completely encircle, compress, and displace the follicular epithelium, ultimately producing solid intrafollicular aggregates of C-cells with complete replacement of the epithelium and colloid, forming nodular hyperplasia.[408,409]

During the early phase of the development of MTC, C-cells break through the basement membrane and invade the thyroid interstitium. In a later stage the tumor consists of solid fields of monomorphic polygonal, spindle-shaped or round cells arranged in sheets transversed by connective tissue septae that divide the tumor into nests of variable size. This compartmental organoid pattern together with the presence of amyloid constitutes the diagnostic microscopical features of the tumor. The amount of amyloid varies. Cellular heterogeneity in MTC tissue is associated with a distinct biochemical pattern and its presence, whether in a primary or in a metastatic lesion, is indicative of a virulent neoplasm associated with a grave prognosis.[410] Sometimes amyloid is totally absent and the polymorphism of cells and nuclei is so great and mitosis so frequent that the tumor cannot be distinguished from anaplastic thyroid carcinoma. Zeman et al.[411] and Nieuwenhuijzen Kruseman et al.[412] described well-differentiated MTCs with anaplastic dedifferentiated metastases. These tumors are histologically classified into a spindle-cell type, a giant-cell type, and a small-cell

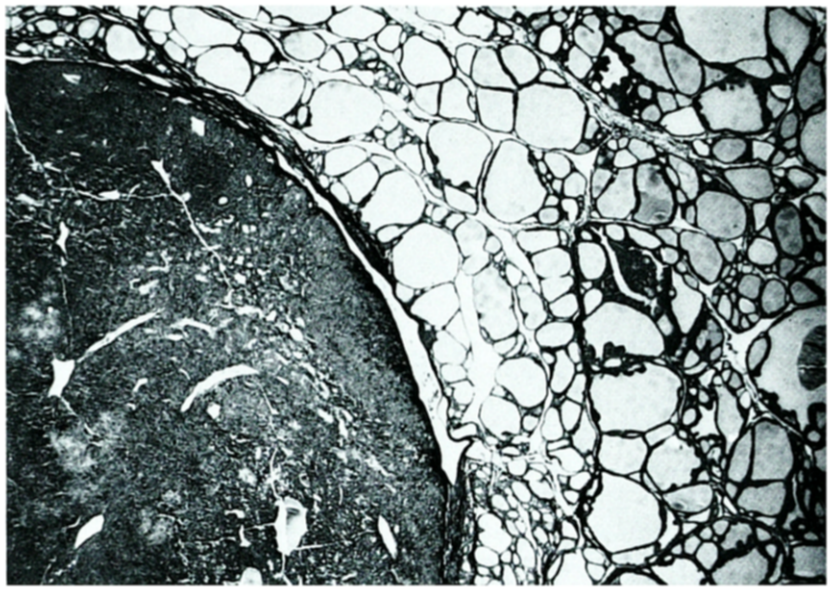

FIGURE 6. A sporadic solitary medullary thyroid carcinoma of the right lobe (diameter 1.2 – 0.7 cm) found in a female patient, 29 years old. After hemithyroidectomy the calcitonin levels were normal after provocation with calcium.

type. In contrast to classical MTC, the anaplastic form is clinically characterized by a poor prognosis. However, anaplastic thyroid carcinomas with morphological and histochemical characteristics of MTC have been described. Criteria for diagnosis are argyrophilic granules and calcitonin immunoreactivity.[412] C-cell hyperplasia is always present beyond the primary tumor in all patients with the hereditary type of MTC. In contrast to the familial multicentrically developing MTC, solitary sporadic MTC is restricted to one lobe, has well-defined boundaries, and is often encapsulated. Furthermore, there is no C-cell hyperplasia in the vicinity of the tumor (Figure 6).

3. Symptomatology

The complaints of a patient suffering from MTC depend on the tumor mass, the extent of infiltration and metastasis, and sometimes the production of bioactive peptides. In general, MTC is extremely poor in symptoms. Patients without a family history therefore tend to be found at a later stage. In 1973, Hill et al.[348] described 73 patients with MTC among whom only 16% had symptoms; 7% (n = 5) of these had a feeling of pressure in the neck, difficulty in swallowing, and other local complaints, and 9% (n = 6) had complaints due to distant metastases or had diarrhea. Diarrhea was described in 30 patients with MTC by Bernier et al.[413]

The mechanism responsible for the diarrhea has been under discussion for many years. At present, five hypotheses are favored: Gray et al.[414] ascribe it to the high calcitonin levels in the blood, which lead to increased excretion of water and electrolytes into the jejunum. Williams et al.[415] who measured the prostaglandin levels in the venous drainage of the tumor, concluded that the high concentrations they found were responsible for the diarrhea. According to Bernier et al.,[413] serotonin and/or other derivatives of tryptophan play an important role in the development of diarrhea. However, Feldman et al.[416] found normal 5-HIAA excretion in MTC patients with diarrhea. Furthermore, MTC tumors might synthesize kal-

likrins, a group of enzymes capable of splitting kinins, e.g., bradykinin, from kininogene (a plasma globulin), and these vasoactive substances could cause both diarrhea and flushes. Khairi et al.[406] described 41 patients with MTC; 14 of these patients (34%) showed diffuse intestinal ganglioneuromatosis (MEN-2B syndrome) and 9 of these 14 (64%) had diarrhea as well. These authors thought that intestinal neuromatosis, giving rise to neuromotor disturbances in the gut, played an important role in the occurrence of flushes and diarrhea in MTC. In addition, the production of vasoactive intestinal polypeptide (VIP) by MTC has been described. In Wermer's syndrome, production of VIP by pancreas islet-cell tumors could cause the Verner and Morrison syndrome (watery diarrhea, hypokalemia, and achlorhydria).

Ectopic ACTH production by MTC might lead to Cushing's syndrome. Steiner et al.[1] reported a case of MTC and Cushing's syndrome with elevated plasma ACTH and urinary 17-hydroxycorticosteroids (17-HCS) excretion. The application of dexamethasone caused a striking decrease in 17-HCS excretion. Birkenhäger et al.[417] described a 45-year-old woman with MTC associated with Cushing's syndrome and galactorrhea. The plasma immunoreactive ACTH and cortisol were partially suppressed by dexamethasone. Apparently the suppressibility does not rule out ectopic hormone production. Williams[418] estimated the incidence of Cushing's syndrome in patients with MTC to be about 3%. Keusch et al.[419] described a MEN-2A patient with Cushing's syndrome and MTC developing a serious osteoporosis. They reviewed 22 patients from the literature. Among these 22 patients 18 died with average survival time of only 4.5 months after diagnosis of Cushing's syndrome was established.

In a case of MTC associated with Cushing's syndrome Bussolati et al.[420] demonstrated two different types of endocrine cells by immunofluorescence; one arranged in clusters, producing calcitonin, the other mostly arranged in duct-like structures containing ACTH. These observations were confirmed by Kameya et al.[421] who analyzed 18 cases of MTC immunohistochemically for calcitonin and ACTH-containing cells and found 14 ACTH-positive. In addition, they found that some ACTH-containing cells were also positive for calcitonin. In contrast, Sundler et al.[422] found no ACTH-reactive cells in their series of 10 cases of MTC.

Jolivet et al.[423] studied a 34-year-old female with Cushing's syndrome and MTC. The original tumor was composed of two cell lines, one containing both calcitonin and ACTH localized within the same cell, and another containing only CT-reacting cells. The cervical metastasis showed a marked decrease in both cell lines with fewer than 1% of cells reacting to ACTH and only 5% to CT. Disappearance of ACTH may represent a consequence of tumor progression.

Chin et al.[424] reported a patient presenting with a neck mass and Cushing's syndrome. They extracted mRNA from the tumors, which directed the translation of both calcitonin and ACTH precursor in a cell-free system.

A high degree of coincidental localization of both ACTH and calcitonin has been demonstrated in MTC, which suggests a close biosynthetic relationship between the two hormones. However, the high incidence of ACTH and beta-endorphin is in contrast with the few reported cases of ectopic Cushing's syndrome with MTC. This suggests either the identified ACTH is produced as an endocrine inactive precursor or if biologically active, is only released infrequently or that a higher incidence of ectopic Cushing's syndrome occurs in association with MTC than has previously been suspected.

4. Tumor Markers

Besides calcitonin, a large number of different substances have been described as produced by MTC, including peptide hormones, such as somatostatin, ACTH, beta-endorphin, MSH, VIP, CRF, and neurotensin, prolactin-stimulating factor, bombesin, and cholecystokinin (CCK). In addition, kallikrein, glycoproteins, and carcino embryonic antigen (CEA) have

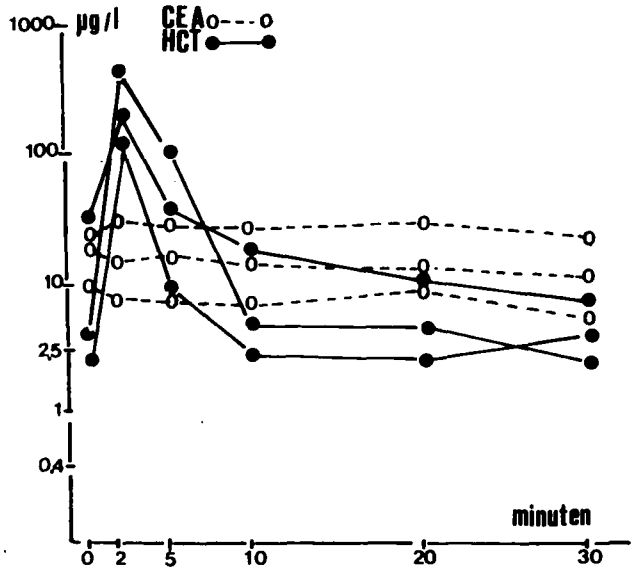

FIGURE 7. Short calcium stimulation test in three patients with MTC. During the test there is no increase in the CEA levels. Normal value for calcitonin until 0.3 ng/mℓ; for CEA until 2.5 ng/mℓ.

been found. In some cases serotinin and such biogenic amines as dopamine, norepinephrine, and epinephrine are produced. Prostaglandins are released in varying amounts by MTC. Not all of these substances are useful as tumor markers for the screening of families because they are not always produced and/or the blood levels are not always elevated in an early stage of the disease.

Enzymes such as histaminase, phenylethanol-amine-*N*-methyl transferase (PNMT), and DOPA-decarboxylase are produced by MTC. CEA is always elevated in the well-differentiated forms of MTC. The peak level of calcitonin after stimulation shows very good correlation with the CEA level. After C-cell provocation to release calcitonin by the administration of calcium and/or pentagastrin, the CEA level does not increase appreciably (Figure 7). CEA is a membrane-bound antigen which is not present in the micro-vesicles. During hormone release by C-cells there is no simultaneous release of CEA into the bloodstream by a process of decay and exfoliation of the tumor-cell membranes. Immunohistochemical analysis of MTC metastases showed no correlation between positive anti-CEA and anti-calcitonin reactions.

5. Prognosis

The prognosis of MTC depends on the origin (sporadic or familial) and extent of the disease as well as the differentiation grade of the tumor. Metastasis of MTC to regional cervical lymph nodes occurs in an early stage. Hazard et al.[21] found lymph node metastases in 58%. Williams[28] in 60%, Freeman and Lindsay[397] in 70%, Block et al.[425] in 66%, Catalona et al.[343] in 50%, and Fletcher[403] in 56% of their patients. General metastasis to the lungs, liver, and bones is thought not to occur until a later stage.[21,396] With respect to mortality, the 5-year survival rates in the various series are as follows: 75% according to Woolner et al.,[396] 48% according to Fletcher,[403] and 80% according to Chong et al.[3] Chong et al. reported a 10-year survival rate of 86% in the absence of cervical lymph-node metastases at the first operation and 48% in the presence of such metastases. For 10-year survival, Gordon et al.[426] reported 54% and Fletcher 23%. The prognosis of MTC varies widely. Weiler et al.[399] and Ibanez et al.[398] concluded that fatality in individual cases is unpredictable.

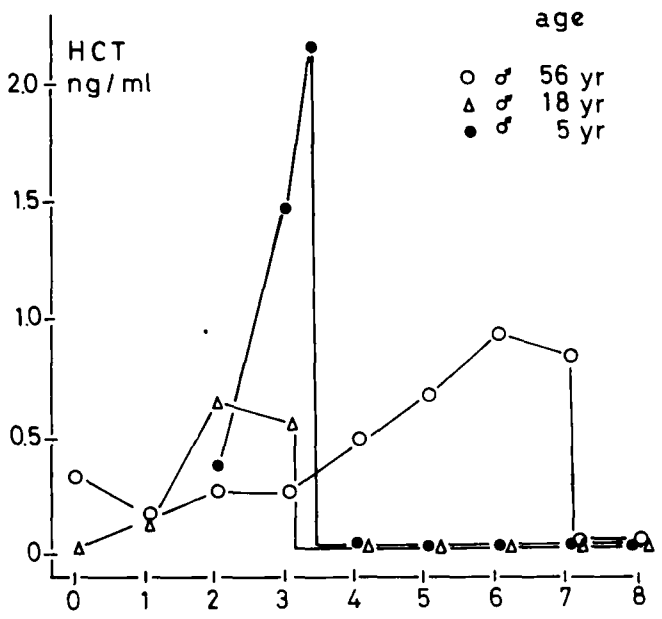

FIGURE 8. Serum calcitonin peak levels after provocation (short calcium test) in three MEN-II patients in consecutive years before and after surgical treatment. The importance of periodic control in close family members of different ages is clearly shown. Postoperative levels remain normal if operated on in an early stage indicating curative treatment.

The life expectancy associated with MTC as part of the MEN-2 syndrome is probably better because family screening can lead to treatment in an early stage. Block et al.[427] found that the average ages at death for patients with MEN-2A were 44 years and 50 years in families with or without concomitant pheochromocytomas, respectively. In the MEN-2B type mucosal neuroma syndrome the thyroid neoplasm is definitely malignant and provisions must be made for early diagnosis and subsequent thyroidectomy.[428] In our experience, the prognosis varies and is not predictable in individual cases.

Some investigators have questioned the rationale of early removal of MTC, stating that familial MTC can often be a rather indolent lesion and the outlook favorable without surgical treatment.[429-431] However, it should be emphasized that a considerable number of patients with hereditary MTC can develop either virulent disease with widespread metastases leading to death or highly aggressive local disease with invasion of cervical structures.[379,427,432-437] There are no prognostic tests available to separate patients with benign course from those with an aggressive course in future. The great variability of the clinical course (usually benign but occasionally and unpredictably aggressive) make early diagnosis and removal of MTC imperative.

6. Diagnostic Procedures

Several types of tests can serve to identify affected family members of MEN-2 patients. First, a careful check should be made to elicit signs or symptoms suggesting pheochromocytoma or hyperparathyroidism. Second, thorough physical examination will often lead to the identification of patients with palpable abnormalities of the thyroid gland. Once such a physical finding is obtained, thyroid scans can identify the "cold" nature of any MTC lesions present. Third, individuals with palpable MTC almost always have elevated basal levels of circulating calcitonin. In MEN-2 families, screening of family members by provocation of the release of C-cells is always required to identify the abnormal calcitonin secretion in an early stage (Figure 8).

In 1970, Care and Wosilait[438] put forward the hypothesis that calcium absorption in the gut leads to the secretion of a humoral factor into the bloodstream that acts as a biological multiplier of the calcium signal and suggested that pancreozymine might be such a factor. In 1971, these authors published the results of a detailed study on the role of digestive-tract hormones in calcitonin depletion. Cooper et al.[439] found in 1971 that pentagastrin gives a 40-fold increase of the serum concentration of calcitonin. This study demonstrated the great sensitivity of the C-cell for the active component of gastrin. In 1973, Hennessey et al.[440] were the first to perform a clinical study in a group of patients with MTC. They compared the effect of a bolus injection of pentagastrin 0.5 μg/kg with that of IV administration of calcium 15 mg/kg over a 4-hr period. Since 1975 we have used, in addition to the pentagastrin test, a new short calcium test in which a bolus dose of calcium is injected intravenously. This test will be referred to here as the short calcium test.

There is an essential difference between the pentagastrin test and the short calcium test. It may be assumed that receptors for gastrin and/or pentagastrin are present on the surface of the C-cell. After the hormone joins the receptor, probably by an endocytic process, they are incorporated into the C-cell and this leads to the ejection (emiocytosis) of micro-vesicles containing calcitonin.[441] However, C-cells have no receptor for calcium ions. These ions enter the cell passively through the cell membrane and, after reaching the cytoplasm, induce the contraction of micro-filament bundles and micro-tubules as a result of which the micro-vesicles are transported in the direction of the cell surface, where emiocytosis occurs. The presence of active myosin and tropomyosin in the micro-filaments is thought to play a role in this transport of micro-vesicles through the cytoplasm.

In the pentagastrin test the fasted subject is recumbent throughout the procedure. Venous blood is drawn for determination of the basal calcitonin concentration. Via the same cannula, a pentagastrin solution (0.5 μg/kg body weight in 2 mℓ 0.9 NaCl) is injected within 10 sec. Blood samples are taken 2 and 5 min after the injection. The drawbacks of this test are that many patients become nauseated, most have abdominal discomfort or even pain (esophagus spasm?) and a few vomit.

In the short calcium test the subject is in the same position. After blood sampling for determination of the basal calcitonin level, elemental calcium in a dose of 2.5 mg/kg body weight is injected intravenously within 30 sec. Blood samples are taken 2 and 5 min after the injection. The short calcium test has fewer side effects than the pentagastrin test. Most of the subjects experience a sensation of warmth over their entire body.

The third method, which most patients consider very acceptable, consists of alcohol provocation (50 mℓ whisky or vodka). This seems to be the most pleasant test for the patient.[442]

The group of Baylin and Wells[443] evaluated the use of combined pentagastrin and calcium infusion, which almost always gives higher values than those obtained with either the short calcium or the pentagastrin test alone. The morbidity was not higher than that associated with single tests. However, it is not certain whether the amounts of pentagastrin and/or calcium injected were sufficient to stimulate all of the C-cells maximally.

All of these tests have proven to be safe and can be performed on an out-patient basis for family screening purposes. None of these C-cell stimulation tests is free of side effects, however, and this presents some problems when periodic application is necessary.

7. Treatment

Criteria for surgery — For the recognition of an indication for surgical treatment of the neoplastic C-cells of the thyroid gland, the results of the calcium and/or pentagastrin provocative tests are of the utmost importance. When, after stimulation, the value of pure extractable calcitonin increases by more than three times the base line value and when the peak level after provocation exceeds 0.4 μg/ℓ calcitonin, diffuse C-cell hyperplasia is likely

and total thyroidectomy is indicated because virtually all patients with hereditary MTC have bilateral and multifocal involvement of the thyroid gland.

Before a thyroidectomy is performed in cases of either familial or sporadic MTC, it is imperative to exclude the presence of pheochromocytomas because the induction of general anesthesia may induce a hypertensive crisis and the patient may die during surgery. If pheochromocytomas are detected, adrenalectomy (if necessary bilateral) must be performed first and the thyroidectomy is postponed until the patient has recovered from this operation.

8. Surgical Procedures

In an attempt to cure the disease, we have adopted an active treatment policy for family members who show elevated peak levels in response to C-cell provocative tests. All patients with MTC are given at least a total thyroidectomy. In experienced hands, thyroidectomy can be performed with little surgical risk and without serious impairment of the quality of life, whereas living with a thyroid tumor that may become malignant, will cause anxiety and perhaps have a lethal outcome.

If patients are found to have a clinically occult MTC that is not grossly visible in cross-section preparations of the gland, only total thyroidectomy is performed. When the results of the stimulation test are strongly pathological (peak level higher than 2 $\mu g/\ell$ calcitonin), macroscopically visible lesions are usually present in cross-sections and in such cases we consider it recommendable to resect the lymph nodes in the central region of the neck, i.e., all nodes from the hyoid bone to the sternal notch, and laterally to the jugular veins. If macroscopically visible metastases are present in these nodes, a modified neck dissection is performed (selective lymph-node dissection) with sparing of the sternocleidomastoid muscles, the jugular veins, and the accessory nerves unless they are directly involved by tumor growth. The value of more extensive local surgery has never been documented. Refraining from total thyroidectomy is only justified if the operation involves extra risk and/or the advantages do not outweigh the unfavorable effects, e.g., in an elderly patient whose life expectancy is limited or in cases with extensive invasion and metastases of MTC.

9. Post-Operative Follow-Up

Management of patients with clinically evident residual disease and distant metastases — In most patients operated on for MTC in an advanced stage of the disease, the provocative tests continue to give positive results postoperatively. In these cases the CEA levels in the blood are also elevated. These phenomena could be due to inadvertently incomplete thyroidectomy in cases with tumor in remnants of thyroid-gland tissue. There is also a considerable chance that metastases are present in lymph nodes in the neck and mediastinum, as well as in the lungs, liver, and bones. Metastases have been described in the kidney, testes, spleen, pancreas, and skin.

A third possibility is that ectopic C-cell foci are present in the mediastinum or along the embryological migratory pathway from the neuroectoderm to the thyroid gland.

In a small proportion of the cases, tumor proliferation in the neck can be established by computerized axial tomography. If a pathological response to the C-cell provocative test is found post-operatively, various approaches can be used depending on whether demonstrable and surgically resectable metastases are present. In general, the management of patients with metastatic MTC is frustrating. However, most of the surgically treated patients feel quite well, and no objective positive signs — except the provocation test results — can be found by routine clinical evaluation.

Aggressive forms of chemotherapy are not indicated, in view of the apparently indolent course of the disease and the lack of an effective chemotherapeutic regimen. If there is a change from an indolent to a virulent course, chemotherapy can be considered. An indication for chemotherapy is found in aggressively growing, poorly differentiated, anaplastic, and

widespread metastases and in inoperable MTC. In most cases the administration of chemotherapeutic agents, either singly or in combination, to patients with extensive metastatic MTC is not beneficial. The grade of malignancy of the tumor does not always correspond with the degree of responsiveness of the neoplastic C-cells to provocation. Anaplastic dedifferentiation can occur without a proportional elevation of the calcitonin or CEA levels in the blood. All this means that periodic complete follow-up of surgical patients is necessary. In patients with diarrhea or resistance to other treatment, surgical tumor debulking may be useful.

10. Radiotherapy

Unfortunately, MTC cells are relatively insensitive to radiotherapy with a cobalt source. Radiotherapy may only be beneficial in some patients with inaccessible tumor deposits. Radioactive iodine is probably not very useful because this isotope is not organified by MTC tissue or metastases. However, follicular differentiation at the primary site as well as in cervical lymph-node metastases have been described and these tumors were found to contain calcitonin as well as thyroglobulin.[444] If carcinoma is present in remnants of thyroid-gland tissue, irradiation might destroy the adjacent MTC cells.

In the future, some help can be expected from radioimmunodetection methods. For example, when monoclonal antibodies against CEA coupled to ^{131}I are injected intravenously, the anti-CEA antibodies labeled this way attach themselves to MTC cells, and 24 hr after injection a concentration of radioactivity in the metastases can be localized with a tomoscan scintigraphic gamma-camera.

Hyperparathyroidism has been described in the MEN-2A syndrome many times.[1,26,343,446,447] Patients show indications of hyperparathyroidism, for instance hypercalcemia, nephrolithiasis, and radiographic signs of elevated bone resorption, especially before total thyroidectomy for MTC. Nevertheless, hyperparathyroidism is often difficult to diagnose because the elevation of the PTH levels is not constant; furthermore, correlation between the immunological activity and the increased biological activity of the PTH is sometimes lacking, the course is frequently asymptomatic, and the hyperparathyroidism is often normocalcemic.

Some authors[352,449,459] state that the hyperparathyroidism in Sipple's syndrome is primarily determined genetically. According to Pearse,[57] this is not unlikely because both the parathyroid glands and the neuroectoderm derive from the same embryological epiblast as the chromaffin cells and the parafollicular C-cells of the thyroid glands do and thus from the same system as that in which the primary genetic abnormality is localized.

Hyperparathyroidism might also occur secondarily due to adrenergic beta-receptor stimulation by the high catecholamine level in the blood.[450,451] Support for this hypothesis was supplied by the finding that after the removal of pheochromocytoma tissue, both the hypercalcemia and the hypophosphatemia disappeared in some patients.[452-456]

A high calcitonin concentration in the blood can lead to a compensatory stimulation of the parathyroid glands. This could be the result of a direct action of calcitonin on the parathyroid glands,[456] i.e., a stimulation occurring independent of the calcium level of the blood, but it could also be the result of stimulation occurring later, secondary to the hypocalcemia induced by the inhibitory effect of calcitonin on bone resorption. Moreover, there are indications that pheochromocytoma can synthesize ectopic calcitonin.

An action of calcitonin on the parathyroid glands is also suggested by the finding that hyperparathyroidism occurs in MTC patients without pheochromocytoma.[1,343,457,458]

Hyperparathyroidism in MEN-2A patients is frequently latent. Histological evidence of hyperparathyroidism is often found in material obtained at surgical exploration of the neck in patients with MTC, many of whom are young normocalcemic individuals.

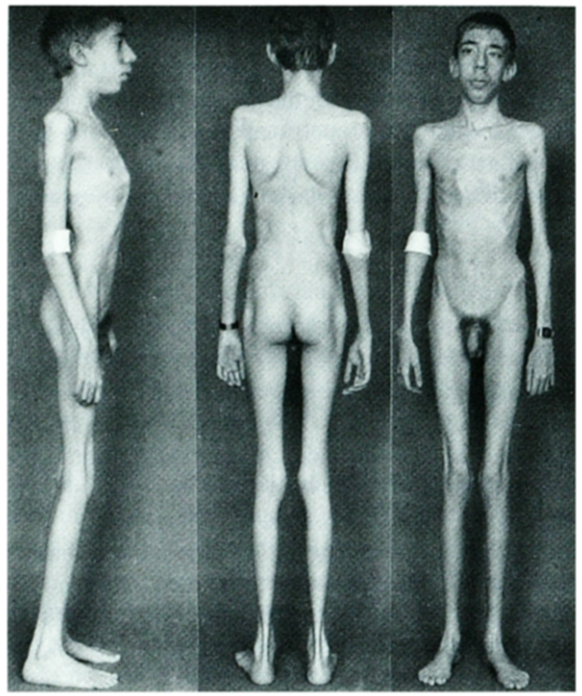

FIGURE 9. Clinical appearance of a MEN-IIB patient aged 16 years, body length 176 cm, body weight 35 kg. Note the marfanoid habitus, the characteristic facies, the thick lips, the pectus excavatum and genu valgum.

D. Multiple Endocrine Neoplasia Type 2B

The MEN-2B syndrome is a rare form of endocrine neoplasia which was clearly defined more than 15 years ago.[30] Since then, more than 100 clases have been identified.[102,406] The syndrome occurs sporadically but is generally transmitted genetically in an autosomal dominant manner. It has a characteristic phenotype expression which is unique and should promote early diagnosis. A slender habitus is combined with reduced body fat, poor development and hypotonicity of body musculature, and arachnodactyly. Unlike Marfan's syndrome, there is no ectopia lentis or aortic abnormalities. Mitral prolapse may be more common. The majority of patients have exhibited severe muscular wasting, especially of limbs, simulating a myopathic state. Abnormal electromyography and myopathic degeneration found at histological investigation are indicative of neurogenic myopathy.

A long list of skeletal alterations have been described, such as skeletal asymmetry, funnel chest, kyphosis, severe lordosis, scoliosis, aseptic necrosis of the lumbar spine, pes cavum, asymmetry or bizarre form of the skull, genu valgum, valgus deformity of the toes, and dislocated hips (Figure 9). Increased mobility of the joints has also been described.

Gonadal hypoplasia and delayed puberty noted in several patients are significant since the patients will become abnormally tall and have a low body weight. The facial appearance with blunt features and thick "bumpy" lips is even more specific and may resemble acromegaly by a prognatic jaw and macroglossy. The tongue and digestive mucosa are covered with small polypoid lesions which are submucosal ganglioneuromas. Ocular neuromas appear on the edges of the eyelids, and the visible presence of fine hyperplastic nerve fibers in the cornea is pathognomonic.

Multiple oral and ocular mucosal neuromata are sometimes grossly apparent but may even be diagnosed unexpectedly by the surgeon performing soft-tissue reduction of the lips for

affected patients, many of whom consult an oral surgeon or orthodontist because of unsightly labial deformity or gross malocclusion.

Often, the earliest sign of the syndrome is the multiple mucosal neuromata noted in early childhood.

1. Pathogenesis

An abnormal sensitivity to or an elevated level of some hormonal factor that promotes neural growth might be involved in the pathogenesis. Williams[459] suggested that a hormone-like nerve growth factor (NGF) could be involved.

In vitro, some tumors of neural-crest origin show better growth initially in the presence of NGF,[460] and differentiate morphologically as nerve cells.[461] A single observation of an elevated level of serum NGF in a patient with MTC has been reported.[462]

In 1972, Baum and Adler[463] reported abnormal skin-test results in an MEN-2B patient after intracutaneous injection of histamine. There was no flare around the weal. Two hypotheses have been put forward to explain this absence: (1) an abnormal axon reflex due to a neural lesion and (2) a rapid degradation of histamine by some substance such as histaminase secreted by MTC.

Carney et al.[464] found hypertrophy of cutaneous nerves in MEN-2B patients. However, persistence of abnormal results of histamine skin tests after removal of MTC suggests a different origin.

2. Pathology

The most pronounced sign is the increase in the number and/or size of nerves, many of which are tortuous and highly branched. Some of these nerves are qualitatively normal but many are distinctly abnormal (the latter exhibit a disorderly arrangement of axons resulting in an appearance mimicking plexiform neurofibroma). Variable proliferation of endoneural cells together with accumulation of myxomatous material between groups of axons is common. Frequently, a prominent regular or eccentric thickening of the perineurium is present. This is usually fibrous but occasionally it is due to proliferation of perineural cells. In occasional cases groups of axons are found to be intermingled with the fibers of the perineurium and to extend from the nerve into the connective tissue with loss of their perineural sheath in the process. Sometimes ganglion cells are present within enlarged lingual nerves (hamartomous proliferation of nerve elements, axons, and schwann cells and also ganglion cells).

3. Digestive-Tract Abnormalities

The ganglioneuromas which predominate in the digestive tract are present at birth and may extend from the oral cavity to the rectum and sometimes even involve the pancreas and gallbladder. It exists in diffuse nontumorous hyperplasia of the autonomic nervous system. There is a remarkably irregular thickening of neural tissue containing mature ganglionic cells in a fibrillar stroma, primarily involving Auerbach's plexus and, to a lesser extent, Meisner's plexus. Diffuse ganglioneuromatosis of the esophageal and gastric myenteric and submucosal plexus give clinical problems infrequently.

Barium-enema findings include an abnormal haustral pattern, thick mucosal folds, and colonic diverticula. Clinical features may include constipation, diarrhea, and abdominal cramps. The pathophysiological basis for constipation, megacolon, and diverticulosis has never been established. The ganglioneuromatosis of the alimentary tract might be expected to result in some motor abnormalities of the tract. Earlier studies by Bartlett et al.[465] and Schimke et al.[32] suggested that ganglioneuromatosis causes defective peristalsis in the esophagus, poor contractility of the colon, and irregular response of the internal sphincter to balloon distension. Neostichnine had no effect, whereas vasopressine resulted in immediate

contraction of the colon. These findings point to a neural rather than a muscular abnormality of the colon.

Alimentary-tract involvement in von Recklinghausen neurofibromas should not be confused clinically or pathologically with that in the syndrome under discussion, although the colon involvement can be very similar.

In neurofibromatosis the usual symptoms are different and do not appear until adulthood. The alimentary form of neurofibromatosis consists of neural and myomatous tumors differing from the diffuse nontumorous hyperplasia of the autonomic nervous system seen in MEN-2B.

In some cases colectomy is required for diverticulitis complicating diffuse diverticulosis.

Megalo-ureter has also been observed in association with megacolon. Both the colon and bladder receive their parasympathetic nerve supply from the second to fourth sacral nerves. The same hyperplasia of ganglion cells observed in the neuroenteric plexus has also been seen in the bladder. Diarrhea may be induced by humoral substances such as VIP, calcitonin, serotonin, or prostaglandins. A mucosal neuroma in a MEN-2B patient reported by Voelkel et al.[466] showed high concentrations of calcitonin.

4. Prognosis

The prognosis is often poor, since it is related to the evolution of the MTC, which especially in this syndrome is malignant and becomes manifest at an early stage.

IV. MIXED TYPE MULTIPLE ENDOCRINE NEOPLASIA SYNDROMES

Apart from the distinct principal phenotype groups, i.e., the MEN type 1 and type 2A and 2B syndromes, several mixed forms are described in the literature. Patients with features of both MEN-1 and MEN-2 and other tumor syndromes can also be divided into familial and sporadic forms.

A. Familial Occurrence of Mixed Form

The existence of a true overlap syndrome has seldom been established in MEN kindreds.

1. Overlapping of Men-1 and MEN-2 Characteristics

Cameron and Spiro[467] reported a patient with a gastrinoma and MTC who belonged to a typical MEN-2 kindred. Carney et al.[468] described three families in which bilateral pheochromocytomas occurred together with MEN-1 features. In the first of these families both the mother and the daughter had multicentric pheochromocytomas as well as islet-cell tumors. In the second family the index patient, a 13-year-old girl, was operated on twice for pheochromocytoma of the right adrenal gland; in addition, she had a paraganglioma in the left cervical region. Her mother died at the age of 43 years after an acute hypertensive attack. Post-mortem examination revealed bilateral multicentric pheochromocytomas as well as a pancreas islet-cell adenoma and nesidioblastomas. An uncle of the index patient had been operated on for a multicentric left adrenal pheochromocytoma. The third family proved to have three individuals with bilateral multicentric pheochromocytomas and two with a unilateral pheochromocytoma. In one of these patients the diagnosis neurofibromatosis was also made because of the presence of numerous café-au-lait spots and axillary freckling. A paternal aunt of the index patient died at the age of 62 from metastatic islet-cell carcinoma.

Janson et al.[469] reported bilateral pheochromocytomas in a mother and daughter, one of whom also had a nonfunctional multicentric islet-cell tumor and the other a pituitary tumor of unknown type. Because the mother also had renal adenomas and cysts, the diagnosis of von Hippel Lindau's disease could not be excluded. Mori et al.[470] described a patient with bilateral pheochromocytomas and an islet-cell tumor of the pancreas. Warner and Baustein[471] observed a patient with pheochromocytomas coexisting with a carcinoid of the ileum.

2. Familial Pheochromocytomas in von Hippel Lindau's Disease

Von Hippel Lindau's disease is an autosomal dominant disease characterized by the association of retinal hemangioblastomas with cystic cerebellar hemangioblastomas. Hemangioblastomas may also occur in the cerebral cortex, the medulla oblongata, spinal cord, kidney, pancreas, and bladder. Other associated findings include adenomas of the kidney, epididymis, liver, and adrenal cortex. Hypernephroma, pheochromocytoma, and paraganglioma of the sympathetic side-chain can also occur. The occurrence of pheochromocytoma in von Hippel Lindau's disease is not uncommon. Hoffman et al.,[472] who reported a case with intrathoracic and multiple bilateral pheochromocytoma and a paraadrenal pheochromocytoma, collected 61 cases from the literature, all occurring in the abdomen; of these, 21 were bilateral and in one the localization was in the organ of Zuckerkandl. In the family they studied, one von Hippel Lindau patient also had a hypernephroma and one member without von Hippel Lindau features had MTC. Other conditions common to von Hippel Lindau's disease are islet-cell tumors of the pancreas,[473,474] malignant carcinoid,[475] and neurofibromatosis.[476-478]

3. Familial Occurrence of Carotid Body Tumors and Pheochromocytomas

The simultaneous familial occurrence of pheochromocytomas, a functioning paraganglioma, chemodectoma, and a nonfunctioning paraganglioma of the carotid body, has been reported.[322,479-481] Nonfamilial cases of carotid body tumor and pheochromocytoma have also been reported, but are rare although the embryologic origin is similar.[482-485]

Paraganglioma derive from the neural crest and may occur in paraganglia associated with plexus along the aorta and in the chemoreceptor tissue of the bladder, prostate, and ovaries. All functional tumors, including cervical and intrathoracic functioning paraganglioma, are best termed pheochromocytoma.

4. Neurofibromatosis (von Recklinghausen's Disease) and Pheochromocytomas

The first report of the coexistence of neurofibromatosis and pheochromocytomas appears to have been made by Suzuki in 1910.[486] The incidence of pheochromocytomas in von Recklinghausen's disease may be less then 1%. Neurofibromatosis is an autosomal dominant ectodermal dysplasia with a frequency of 1 in 2500.[487] These patients have a considerably increased risk of malignancy, mainly due to an excess incidence of specific neural tumors such as gliomas and meningiomas.

Neurofibromas occur in three main forms: (1) peripheral fibromatosis involving primarily the peripheral nerves, (2) central neurofibromatosis involving primarily the central nervous system, and (3) visceral neurofibromatosis, mainly with involvement of visceral and autonomic ganglia in combination with neurofibromas, schwannomas, and ganglioneuromas.

Furthermore, neurofibromatosis with or without café-au-lait spots occurs in about 5% of the patients with either sporadic or familial pheochromocytoma.

B. Sporadic and Solitary Mixed Forms

Hansen et al.[488] described a 65-year-old female with a small cell bronchogenic carcinoma combined with MTC, neurofibromatosis, adrenal cortical adenoma, and parathyroid-gland adenoma. They collected seven patients from the literature and described these cases as mixed MEN syndromes, although none of the individuals belonged to a MEN kindred. Five of the seven patients had carcinoid tumors; in one of them as the sole manifestation of MEN-1 in association with MTC, in two in association with unilateral pheochromocytomas, and in three combined with neurofibromatosis. Two patients had pituitary tumors, one associated with an islet-cell tumor of the pancreas and neurofibrosarcoma and one associated with MTC and bilateral pheochromocytoma.

Nathan et al.[489] described a patient found to have a malignant gastrinoma who had been operated on previously for unilateral pheochromocytoma. Zeller et al.[490] saw an 18-year-

old female with bilateral pheochromocytomas and a symptomatic adenoma of the pancreas. Myers and Eversman[491] reported a 53-year-old woman with an acidophilic adenoma of the pituitary gland, recurrent parathyroid adenomas, a left adrenal pheochromocytoma, and a thickened nodular adrenal cortex. Earlier, Kahn and Mullon[492] had reported the association of acromegaly and pheochromocytoma in one patient. Morriss and Tymms[493] described a case in which an oat-cell carcinoma of the lung occurred in association with a pheochromocytoma and two gastric carcinoids.

Several explanations have been proposed for the concurrence of various types of tumor in the same patient. The association could, of course, be merely fortuitous, but the rarity of each tumor makes it statistically improbable that they should occur together.

The frequent simultaneous occurrence of a number of unusual neoplasms as mentioned above, led Bolande[494] to propose that all of these processes originate from an aberration in the migration, growth, and differentiation of neural crest tissue cells. He also coined the term neurocristopathies to designate the constellation of embryogenetically related disease entities including pheochromocytoma, neuroblastoma, MTC, neurofibromatosis, carcinoid, non-chromaffin paraganglioma (chemodectoma), MEN syndromes, and neurocutaneous melanosis. This explanation is acceptable and consistent with Pearse's unifying concept, which presumes a common origin of all APUD cells. However, other possibilities are also possible (see under *APUD concept*).

Finally, it has been proposed that some elements in various endocrine tumor syndromes may represent secondary acquired phenomena. For example, islet-cell hyperplasia progressing to discrete adenomas may occur in response to hyperglycemia induced by excess catecholamines secreted by a pheochromocytoma. However, such explanations remain speculative. In practice, the overlapping of elements of the classic MEN syndromes should alert clinicians to the chance of such associations in any particular patient.

V. ETHICAL AND SOCIAL ASPECTS OF SCREENING OF FAMILIES WITH GENETIC PREDISPOSITION TO CANCER

Periodic screening of patients with multiple endocrine neoplasia syndromes and their immediate relatives (parents, brothers/sisters, and offspring) probably improves both prognosis and life expectancy. Potentially life-threatening tumors such as pheochromocytoma can be traced and removed in an early stage. Periodic screening of large groups, however, puts an additional load on physicians and requires special administrative and outpatient facilities. The responsibility for the continuity of such investigations rests only upon the attending physician. Experience has shown that periodic control cannot be adequately guaranteed in this way by individual physicians. Interruption of the continuity due to conclusion of short-term research programs, loss of funds, or departure or death of the leading physician may result in unnecessary morbidity and even mortality and is extremely disturbing to the families in question. Patients and their close relatives may feel neglected and come to suspect having been used for experimental purposes only. They may be unwilling to cooperate in future.

One way to solve the practical problems involved might be to have the administrative work organized centrally and to have the family doctor perform the main screening work.

Centrally monitored screening has obvious advantages. Optimal coverage of both patients and, when necessary, close relatives, could be realized locally if investigation and therapy have been standardized according to well-designed protocols. This would ensure that treatment of relatives would begin at a stage when cure is still possible. If the attending physician should discontinue his work, retire, or die, the registration center would ensure that screening of the patients and/or relatives would be continued by a successor or at a nearby medical

center. People would retain their free choice of physicians and specialists. In addition, one of the important tasks of a registration center would be assistance in genetic counseling. Finally, the data collected at the registration center might be valuable in research. This, however, should be a secondary task and measures should be implemented to protect the privacy of the individuals concerned. For instance, epidemiologic studies should be possible without identification of personal data.

Apart from the obvious advantages, central registration of families with a genetic predisposition for cancer would raise some problems.

The privacy of groups of patients and their relatives would be at risk. Security measures should be adequate and stored data should be accessible only to a limited number of people. Individuals to be registered should have their rights protected such as the right to be informed about registry conditions, the right to refuse registration of medical data, and the right of access to their nonmedical data. Registered individuals should have the right to order the destruction of their medical data. A supervisory board has to be set up, preferably composed of, for the most part, members with a nonmedical background. This board has the primary task to see that the rules and regulations of the central registration concerning the protection of the privacy are strictly observed.

Accommodation for a data bank could be provided in a national or regional registration center for families with a genetic predisposition for cancer. For security reasons the data bank should preferably be located in a large clinical center.

Besides the problems associated with the protection of their privacy, the registered individuals' sense of security may be disturbed by the knowledge of the screening and the registration. The question arises as to whether it would be justifiable to conceal the fact of the screening/registration in a cancer-candidate file, particularly during a medical examination required for life or health insurance, in connection with a new job, etc.

For this reason, we believe that screening of families with genetic predisposition to cancer is only justified if both a predictive test for diagnosis is available and effective treatment is possible and thus screening would improve the quality of life and the life-expectancy. Only in this case some loss of privacy is acceptable. If treatment for an inherited cancer syndrome is possible, insurance companies will come to realize that registered individuals being screened annually, have at least a lower or a normal risk-percentage, and a higher life-expectancy than those individuals not being screened, which results in a positive effect on the acceptance of close relatives of patients for insurances.

Once screening of families has been started, the continuity of periodic screening and optimal coverage is guaranteed in the best way by central registration and coordination of decentralized investigation. The advantages of this system clearly counterbalance the disadvantages, the most important of which would be the risk of invasion of the privacy of both patients and relatives.

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