Le syndrome du glucagonome - entité rare mais typique : un cas genevois et revue de la littérature

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" Le syndrome du glucagonome : entité rare mais typique. Un cas genevois et revue de la littérature"

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Glucagonoma syndrome: rare but typical disorder. A case in Geneva and review of the literature

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Résumé en français de la thèse rédigée en anglais sous le titre : « Glucagonoma syndrome : rare but typical disorder. A case in Geneva and review of the literature »

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Introduction

Environs 95% des cancers pancréatiques sont des adénocarcinomes canalaires, les tumeurs neuro-endocrines (TNE) constituant les 5% restants. Nous allons dans ce travail détailler ces derniers, et plus particulièrement le glucagonome avec son syndrome.

Les TNE sont occasionnellement encore désignées sous le nom d'apudomes pancréatiques. Ce terme souligne que les TNE naissent des cellules endocrines pancréatiques, c'est à dire du contingent pancréatique d'un « système » cellulaire endocrine vaste, les cellules APUD (amine precursor uptake decarboxylation).
Pearse, en s'inspirant du concept de « système endocrinien diffus » proposé par Feyrter en 1938, a avancé en 1966 cette célèbre hypothèse selon laquelle, ces cellules endocrines du pancréas et de l'intestin feraient partie d'un groupe beaucoup plus largue de cellules impliquées dans le contrôle physiologique de processus biochimiques et métaboliques. Elles partageraient un certain nombre de caractéristiques cytochimiques et ultrastructurales ainsi que des mécanismes particuliers de synthèse, de stockage et de sécrétions d'amines ou de peptides biologiquement actifs. On postulait aussi une origine embryonnaire commune, à savoir neuroectodermique. Ces cellules APUD peuvent être localisées, en plus du système digestif, dans la thyroïde (cellules C), la médullaire surrénale, le poumon, la peau et le système nerveux.
Plusieurs caractéristiques communes de ces cellules APUD ont été infirmées par la suite, mais cette théorie continue d'aider à conceptualiser certaines particularités des TNE, comme la survenue de néoplasie endocrinienne multiple ou la possibilité d'une tumeur sécrétant plusieurs hormones.

Le glucagon a été découvert en 1923, deux ans après l'insuline. En 1942 Becker et coll. décrivaient, sans le savoir, le premier cas d'une patiente, associant une tumeur insulaire pancréatique à un diabète, une tendance aux thromboses et une éruption cutanéo-muqueuse particulière.

Grâce au dosage radio-immunologique du glucagon en 1966, Mc Gavran et coll. ont rapporté dans le NEJM le premier cas prouvé sous le titre « A glucagon-secreting alpha-cell carcinoma of the pancreas ».
En 1974 Mallinson et coll. ont finalement identifié dans une publication dans le Lancet « A glucagonoma syndrome » à partir d'une série de neuf cas. A ce jour il y a environ 250 cas publiés.

Incidence, épidémiologie, classification, et anatomopathologie

L'incidence des TNE provenant du pancréas est estimée à 1,8/1'000'000 habitants par an. Le glucagonome comprend environs 2,5% de ces TNE ce qui équivaut à une incidence de 0,05/1'000'000 habitants par an.
Le glucagonome touche un peu plus souvent les femmes (55%) que les hommes, avec un âge moyen au moment du diagnostic de 65 ans (tranche d'âge de 19-84 ans). Il faut préciser, et c'est aussi vrai pour toutes les TNE, que ces chiffres se basent sur des patients présentant un glucagonome avec le syndrome. Le glucagonome avec syndrome est très rare, alors que les glucagonomes sans traduction clinique sont probablement parmi les TNE les plus fréquentes comme en témoignent les études nécropsiques.

Classification du glucagonome :

1. Glucagonome avec le syndrome du glucagonome

2. Glucagonome sans le syndrome clinique typique :

    a) tumeur pancréatique endocrine solitaire, qui, étonnamment, ne développe pas le syndrome clinique

    b) glucagonome faisant parti d'une autre TNE (p. ex. insulinome, gastrinome)

    c) glucagonome faisant parti du MEN 1

    d) microglucagonome découvert lors d'études nécropsiques

Le glucagonome se situe la plupart du temps au niveau de la partie caudale et corporéale du pancréas. La taille est de 7,8 cm en moyenne, avec des extrêmes allant de 0,4 à 35 cm. En microscopie optique, les cellules tumorales sont souvent disposées en cordons ou en travées. Ces cellules ont un cytoplasme clair et présentent des grains sécrétoires atypiques.
Les marqueurs immunohistochimiques incluent la synaptophysin, la chromogranin et bien sûr le glucagon ainsi que des peptides dérivées du proglucagon (la glicentine GLP 1 et 2).
Environs un tiers des glucagonomes produisent plusieurs hormones, le plus souvent le PP, suivi de la somatostatine, insuline, et serotonine.
L'aspect histologique ne permet pas, à lui seul, de distinguer la malignité ou la bénignité ; les seuls critères valables de malignité sont l'invasion locale ou capsulaire, l'invasion vasculaire par des cellules tumorales ou la présence de métastases. Entre 54-82% des patients présentent

des signes de malignité et il existe une relation assez linéaire entre la taille de la tumeur primaire et des métastases. Ces métastases sont le plus souvent hépatiques (79-84%), suivi d'une atteinte ganglionnaire (30-37,8%), et plus rarement spléniques, péritonéales ou osseuses.

Le syndrome du glucagonome

Les signes les plus fréquents de ce syndrome sont les manifestations cutanées sous forme d'une dermatose appelée **érythème nécrolytique migrateur (ENM),** un **diabète** modéré, une **perte de poids** importante, des **atteintes neuropsychologiques** et une tendance aux **complications thrombo-emboliques**.

La plupart des patients sont diagnostiqués par un dermatologiste, à cause de cet **ENM** caractéristique qui évolue typiquement sur 7-14 jours. La dermatose disséminée débute par des lésions maculo-papulaires érythémateuses. Avec l'extension de la dermatose apparaissent au centre des lésions vésiculo-bulleuses, suivies de croûtes nécrotiques. Finalement on observe une amélioration avec une pigmentation post-inflammatoire. Le patient souffre d'un prurit intense et de douleurs importantes au niveau intertrigo et des pieds. D'autres atteintes incluent des ongles dystrophiques, une chéilite, glossite et stomatite. La biopsie effectuée sur le bord de lésions fraiches montre une histologie évocatrice.
Cet ENM affecte en moyenne le patient pendant 3 ans ½ avant le diagnostic.
La pathogenèse du ENM n'est pas encore élucidée, mais le glucagon en soi, ainsi que l'hypoaminoacidémie et l'hypoalbuminémie semblent jouer un rôle.

Beaucoup de patients présentent un **diabète** modéré, en moyenne depuis 7,7 ans avant le diagnostic. Comme c'est le cas pour l'ENM, la survenue d'un diabète est nettement plus fréquente avec des tumeurs de 4 cm ou plus qu'avec des plus petites. Ce diabète est surtout dû à l'augmentation de la gluconéogénèse et glycogénolyse par action du glucagon, mais d'autres facteurs doivent y contribuer, puisqu'il n'existe pas de corrélation linéaire entre l'importance de l'hyperglucagonémie et celle du diabète.

Des **atteintes neuropsychologiques**, avant tout une dépression majeure, affectent régulièrement les patients. Certains auteurs vont jusqu'à décrire un syndrome paranéoplasique neurologique incluant une baisse des fonctions cognitives supérieures et de l'acuité visuelle, une faiblesse des membres inférieurs avec hyperréfléxie et parfois une ataxie. Une étude a montré que le glucagon augmente le cAMP dans des cellules gliales en culture, ce qui pourrait expliquer ces symptômes.

Les **complications thrombo-emboliques**, se présentant souvent sous forme de thrombose des veines profondes avec risque d'embolie pulmonaire mortelle, sont à rechercher activement. On sait que le glucagon augmente le facteur X ; mais le piège réside dans le fait que les paramètres habituellement déterminés dans le bilan de coagulation ne sont pas pathologiques.

L'action catabolique du glucagon et l'anorexie en relation avec le glucagonome sont responsables d'une **perte de poids** significative, allant de 12-20 kg.

Aspects biologiques

Le dosage du **glucagon** par méthode radio-immunologique apporte la certitude diagnostique. Chez des sujets en bonne santé et à jeun, la glucagonémie se situe autour de 50-200 pg/ml.
Les patients présentent une valeur moyenne de 2110 pg/ml, les extrêmes allant de 500-6600 pg/ml. L'analyse radio-immunologique identifie en réalité 4 fractions du glucagon, avec diverses activités biologiques ; ceci pourrait expliquer le fait qu'il n'y ait souvent pas de corrélation entre l'importance de la glucagonémie et la sévérité clinique et/ou métabolique.

L'**insulin**émie se trouve à des valeurs normales ou élevées, pour compenser partiellement la production accrue de glucose stimulée par le glucagon.

Le taux plasmatique des **acides animés** se trouve abaissé, souvent autour de 25% de la norme ; la glutamine et l'alanine, essentielles pour la gluconéogénèse, sont les plus fortement touchées.

Presque invariablement le patient présentera une **anémie** normochrome normocytaire. Cette anémie est entre autre provoquée par l'effet inhibiteur du glucagon sur l'érythropoïèse.

Souvent on notera aussi des concentrations abaissées du **cholestérol** (le glucagon diminue la synthèse hépatique des lipoprotéines de très faible densité (VLDL) et le taux circulant de lipides) et des **protéines/albumine** (action protéolytique du glucagon).

Localisation de la tumeur

La plupart des glucagonomes ayant atteint une taille importante (en moyenne 5-10 cm) au moment du diagnostic suspecté, ils sont assez facilement identifiables par les techniques d'imagerie habituelles. Le **CT scan** est l'examen initial de choix, qui détectera environs 95% des cas. L**'IRM** équivaut à ce taux de réussite, mais semble être supérieur dans l'identification de petites métastases hépatiques.
L'US transabdominal occasionnellement ne permet pas de visualiser le pancréas, en plus d'être opérateur-dépendant. L'ultrasonographie par voie endoscopique présente par contre une très bonne spécificité et sensibilité, même dans la détection de petites lésions.
Des tumeurs de plus petite taille pourront être identifiées par **artériographie cœliaque**, qui montrera une masse hypervascularisée.
Le **scintiscan** à octréotide pourrait, en plus, aider dans la détermination d'éventuelles métastases.

Traitement

L'indication à **l'intervention chirurgicale** sera posée dans la majorité des cas, en raison de nombreuses formes malignes et de la morbidité secondaire à l'hyperglucagonémie.
La guérison définitive n'est possible que dans 5% des cas. La chirurgie non-curative apportera pour les autres patients une amélioration clinique importante, sans évidence d'une

amélioration de la survie à long terme. La préparation préopératoire est essentielle pour diminuer les risques opératoires en relation avec les ravages cataboliques du glucagon et la tendance thrombo-embolique.
L'embolisation sélective de l'artère hépatique est un moyen efficace et relativement peu invasif pour des métastases hépatiques symptomatiques.
Le rare patient inopérable bénéficiera d'une **thérapie anti-sécrétoire avec l'Octréotide**. Ceci va améliorer la dermatose, la perte de poids et les plaintes digestives, mais semble sans effet sur la croissance tumorale.
La **chimiothérapie** palliative sera tentée chez le patient qui ne répond pas à l'Octréotide. Le choix et mélange d'agent cytotoxique se déterminera pour chaque patient empiriquement.

Les patients avec tumeur bénigne, où l'exérèse chirurgicale complète est possible, auront une espérance de vie normale, qui est fortement diminuée à une moyenne de 3 ans pour les cas à présentation maligne. Une revue note une survie de 50% à 16 mois et de 20% à 60 mois du moment du diagnostic.

Description du cas

Nous rapportons le cas d'un homme de race blanche de 67 ans au moment de son opération (novembre 1989), le seul patient ayant été identifié avec le syndrome du glucagonome dans la région lémanique.
Dans ses antécédents on note des lithiases rénales répétitives et des investigations en 1976 pour une possible lésion endocrinienne de l'hypophyse.
Notre patient avait consulté pour la première fois en octobre 1985 le service de dermatologie de l'hôpital universitaire de Genève en raison d'une éruption cutanée généralisée intensément prurigineuse. Devant des lésions maculo-papulaires sur fond érythémateux avec desquamation plantaire au niveau des pieds, une forme d'eczéma d'origine indéterminée était retenue et le patient traité symptomatiquement. Au cours des prochains jours l'affection cutanée avait disparue, suivie d'une récidive quelques semaines plus tard. Le patient présentait au cours des mois suivants d'autres poussées d'une durée de 2-3 semaines, débutant habituellement aux membres inférieurs pour ensuite intéresser le reste du corps, interrompues par des phases de rémission presque complète s'étendant sur 2-6 semaines. Les investigations incluaient des frottis-culture de peau, diverses analyses sanguines, et des biopsies cutanées, sans changer l'impression diagnostique d'eczéma chronique. Ensuite le patient a jouit de 2 périodes asymptomatiques prolongées, de 8 et 12 mois respectivement, interrompues par une seule poussée classique.
En mai 1988 notre patient, maintenant dans un état cachectique, souffrait à nouveau de la dermatose, très prurigineuse, avec aussi des douleurs importantes du aux lésions fissurées des plantes des pieds. Il avait des idées suicidaires. Lors d'une hospitalisation dans une clinique privée, la radiographie thoracique et l'US abdomino-pelvien ne montraient rien de particulier. Un CT scan abdominal mettait en évidence une masse rétropéritonéale polycyclique de 4x5cm, juste inférieur à la tête du pancréas, interprétée comme un groupe d'adénopathies. À la recherche d'une lésion tumorale primaire plusieurs autres examens ont été effectués, dont une colonoscopie, des investigations urologiques, un CT scan crânio-cérébral, et des marqueurs tumorales, tous négatifs. Les analyses sanguines relevaient une anémie marquée et une hypoprotéinémie. Finalement les médecins concluaient à une maladie carentielle et l'état

du malade s'améliorait avec l'administration de plasma et un régime riche en vitamines et protéines.

En raison d'une nouvelle péjoration, le patient était ré-hospitalisé en août 1989 en milieu universitaire à Genève. A l'examen clinique on soulevait un poids de 59,3 kg (pour un poids habituel de 73 kg) chez cet homme pâle et d'apparence chroniquement affligée. Les systèmes cardio-pulmonaires et abdominaux ne montraient rien de particulier. Neurologiquement on notait une certaine faiblesse des membres inférieurs avec hyperréfléxie des réflexes ostéo-tendineux, et un signe de Hoffmann positif. Les nerfs crâniens n'étaient pas affectés. Un bilan neuropsychologique révélait une diminution des fonctions cognitives supérieures, avec un manque de mobilité intellectuelle, un trouble de l'écriture et une défaillance de la mémoire à court terme.
Après qu'un nouveau CT scan thoraco-abdominal montrait une nouvelle fois la lésion abdominale connue, l'artériographie coeliaque séléctive (et mésentérique supérieure) confirmait finalement la présence d'une tumeur hypervascularisée.
Puisqu'un dermatologiste avait inclus dans son diagnostic différentiel, en plus d'une déficience en zinc et/ou de vitamines B2/B6, aussi le syndrome du glucagonome, le dosage du glucagon a été demandé ; la valeur pathologique de 800 pg/ml a permis de confirmer le diagnostic de glucagonome, avec son syndrome.
Une prolactinémie normale et l'image inchangée d'une selle turcique élargie ont écarté la possibilité diagnostique de MEN 1.
Les investigations biologiques complémentaires ont révélé une hypoaminoacidémie globale, et une intolérance pathologique au glucose.

Le patient a immédiatement reçu des injections sous-cutanées d'Octréotide, ce qui a été suivi de la disparition très rapide de la dermatose, et lui a permis de pouvoir marcher à nouveau.
Lors du congé accordé avant l'intervention chirurgicale notre patient a été ré-hospitalisé en urgence en raison de l'apparition de thromboses veineuses profondes bilatérales étendues, et un traitement avec héparine a été introduit. En raison de la mauvaise compliance des injections à domicile d'Octréotide la dermatose avait récidivé, et la reprise de ce médicament s'accompagnait à nouveau d'une réponse spectaculaire sur sa peau.

La sanction chirurgicale consistait en une duodéno-pancréatectomie céphalique, sans complication. Pas d'évidence de métastases. La tumeur excisée était encapsulée, mesurant 3,3 x 2,5 cm. L'analyse immuno-histochimique était positive pour le glucagon, VIP et la chromogranine.
Peu de temps après l'opération, la concentration plasmatique en glucagon était de 146 pg/ml ; des mesures ultérieures montraient des valeurs fluctuantes et inexpliquées, avec un maximum de 433 pg/ml en 1990 et un minimum de 59 pg/ml en 1992. Un CT scan abdominal ne montrait aucun signe de récidive.
Depuis l'intervention le patient n'a plus jamais eu de ENM, ni problèmes thrombo-emboliques. Ses fonctions cognitives supérieures se sont nettement améliorées. Il a rapidement retrouvé son poids antérieur.
La valeur de tous les acides animés s'est normalisée ; celle des autres paramètres biologiques également.

Le patient est décédé 19 ans plus tard, de vieillesse, sans aucun lien avec son glucagonome.

Conclusion

Le syndrome du glucagonome est une entité clinique rare mais caractéristique et donc identifiable.
Le clinicien devrait suspecter ce diagnostic chez un patient se plaignant d'une dermatose prurigineuse inexpliquée et récurrente, surtout si elle est associée à un diabète modéré, une tendance aux problèmes thrombo-emboliques et une perte de poids significative. Des troubles neuro-psychologiques seront souvent signalés. La certitude du diagnostique repose sur la démonstration d'une hyperglucagonémie, hypoaminoacidémie et d'un résultat positif pour le glucagon à l'étude immuno-histochimique du tissu tumoral.

Puisqu'il s'agit d'une tumeur à croissance lente et que les symptômes sont secondaires à la surproduction hormonale, l'on peut être soit complètement curatif en absence de métastases, soit efficacement palliatif avec le geste chirurgical et/ou des thérapies anti-sécrétoires/chimiques.

Notre patient avait été sévèrement atteint par le syndrome, avec tous les traits présents. Il avait bénéficié de manière spectaculaire de l'administration d'Octréotide pour faire disparaître l'ENM.
Quatre ans après les premiers symptômes cutanés la sanction chirurgicale était curative et le patient n'a plus jamais eu d'ENM ou d'évènement thrombo-embolique, est sans particularité sur le plan neuro-psychologique et a des valeurs biologiques dans la norme.

La rareté et hétérogénéité des TNE en générales et du glucagonome en particulier posent un énorme défi ; des études y relatives sont difficiles à réaliser et les centres hospitaliers inévitablement manquent d'expérience. La création de centres d'excellence et la collaboration étroite entre divers centres hospitaliers est souhaitable et important.
Sur le plan thérapeutique, une radiothérapie avec des analogues de la somatostatin est envisageable. Dans un proche avenir on devrait pouvoir développer des analogues peptidiques avec une affinité sélective pour le récepteur GLP-1 ; ainsi, une radiothérapie spécifique du glucagonome pourra être proposée.

1. History

The first known description of the most illustrious disease of the pancreas, namely diabetes, dates back to 1550 BC: the Ebers Papyres (Thebes, Egypt) describe polyuria and its treatment, preceding for more than 1000 years the discovery of the responsible organ by the Chalcedonian Herophile (1,2).
Then one had to wait patiently for Thomas Cawley who in 1788 was the first person to suggest a link between diabetes and the pancreas which was found to be filled up with calculi (3).

A. Research on pancreas anatomy and physiology

In March 1642, Johann Georg Wirsung (1600-1643), in the presence of the young Thomas Bartholinus (1616-1680), described the main duct of the human pancreas which bears his name and achieved medical immortality, after his student Moritz Hoffmann (1621-1698) reportedly told him of such a duct in the rooster a year earlier (1). Wirsung gave also the first description of a double excretory duct and of the pancreatic juice: "…I found a turbid fluid which acted like a corrosive fluid on the silver probe" (4).

The physiologist Claude Bernard (1813-1878), amongst many other contributions, studied pancreas juice from 1849 to 1856 and showed that it emulsifies fats, splitting them info fatty acids and glycerol and that it could convert starch into sugar. He proved that all digestion did not take place in the stomach, as previously believed, and that pancreas juice furthered the digestion of proteins that was begun in the stomach (1).
Bernard also contributed to the progress of endocrinology in general, when he differentiated in 1855 between the "sécrétion externe" of the liver, meaning the discharge of the bile, and the "sécrétion interne", by which he understood the giving-off of glucose into the blood by the same organ (3).

The young Paul Langerhans (1847-1888), while still a medical student, as part of his doctoral thesis described in 1869 islands of clear cells scattered throughout the pancreas. These islands were more richly innervated than the surrounding tissue. He suggested that these islands might be lymph nodes. Laguesse in 1893 generously called these the "islets of Langerhans", and actually conceived the idea that these islets produced an antidiabetic internal secretion (7).

B. Pancreatic pathologies since the 17th century

I. Introduction

Theories regarding the pathology of the pancreas preceded the scientific facts.
Although diseases of the pancreas were apparently unrecognized in antiquity, this neglect of the organ was more than amply remedied later by physicians of the 16th century, who ascribed most diseases to the new organ of interest: for instance Highmore considered it the seat of apoplexy, palsy and hysteria (1). Fernel (1542) regarded the pancreas as the source of a great number of disorders, such as fever, hypochondria and melancholia (2).

II. Pancreatitis

Although others had written on inflammation of the pancreas earlier, it was Reginald Fitz, the Harvard pathologist who in 1889 drew attention to the catastrophic type of acute pancreatitis with abdominal signs and symptoms and firmly established the disease entity with its gangrenous, hemorrhagic and suppurative aspects (1).

III. Diabetes mellitus

Thomas Willis (1621-1675) named this metabolic disorder the “Pissing Evil’. In 1674 he discovered that the urine tasted “wonderfully sweet as it was imbued with honey or sugar” (1).
In 1682, the Swiss Johann Conrad Brunner (1653-1727), while trying to demonstrate the pancreatic functions, removed the greater part of his dogs’ pancreas. He noted their increased appetite, thirst as well as their weight loss before dying. But Brunner did not associate these cardinal symptoms with diabetes (5).
By evaporating urine, Matthew Dobson (1713-1784) proved that its sweet taste was caused by sugar; he also found the sweet taste of the blood in diabetics and thus must be credited for having discovered hyperglycemia (3).
In 1788, Thomas Cawley published a “singular case of diabetes”. The pancreas of that patient was full of calculi and Cawley suggested a connection between diabetes and the condition of the pancreas, noting that the disease may follow injury to that organ; he was the first observer to do so (3).

A century later a revolutionary discovery was made when Oskar Minkowski and Joseph von Mering found in 1889 that total pancreatectomy of a dog caused diabetes (1).

Eugene Opie gave evidence that the islets might be the seat of the disease in 1901 when he found hyaline changes in the islets of diabetic patients (1).

From that time on, many scientists tried to extract a hypoglycemic substance from the pancreas, now known as insulin. At last the dramatic discovery occurred in the years 1921-22 when Frederic Banting (1891-1941), a surgeon, and Charles H. Best (1899-1978), a medical student, showed that an extract of the ligated pancreas decreased the hyperglycemia of dogs with diabetes. Collip, a biochemist, was then recruited to improve the extraction process, and it was such an extract that was successfully tried in a human diabetic, Leonard Thompson (1,7).

IV. Cancer of the pancreas, gastroenteropancreatic neuroendocrine tumors and the APUD concept

IV.1. Cancer of the exocrine pancreas

The earliest recognition of cancer of the pancreas has been attributed to Morgagni (died 1779) who mentions five cases previously published in the Sepulchretum of Bonetus in 1679 and other cases reported prior to his own (1,4).
At about the end of the 19th century, the clinical signs and symptoms of cancer of the head of the pancreas were well known, and many cases were verified histologically.

L. Bard and A. Pic distinguished in 1888 between duct and acinar cell cancers and even mentioned the possibility of islet cell cancer (1).

There were attempts to extirpate cancers by surgery, mostly unsuccessful, until the “classical surgical tour de force” of Whipple, Parsons and Mullins who in 1935 devised a pancreaticoduodenectomy for ampullary cancer.

IV 2. Gastroenteropancreatic (GEP) Neuroendocrine Tumors

IV.2.1. Introduction

GEP endocrine tumors secrete active hormones and may be associated with distinctive clinical syndromes. These uncommon tumors arise from the GEP neuroendocrine cells and constitute a clinical challenge, both diagnostically and therapeutically. The GEP collection of diffuse neuroendocrine cells is a large reservoir of cells that secrete amines and peptides for the purpose of regulating and

modulating normal physiological control of carbohydrate metabolism, the digestion of proteins and fats and the provision of an acid or alkaline pH appropriate for assimilation of nutrients. These cells influence physiological control by means of paracrine activity on neighboring cells, by traditional (humoral) endocrine function and by neurocrine activity through aminergic and peptidergic neurotransmitters.
The tumors that arise from these cells have been designated by a variety of terms, such as argentaffinomas, enterochromaffin cell tumors, neuroendocrine tumors, carcinoid tumors and APUDomas. This diverse nomenclature has generated a considerable amount of confusion and with the following historic review we shall try to explain how the present understanding of GEP neuroendocrine cell structure and function occurred, as well as the tumors that arise from these cells.

IV.2.2. Carcinoid tumors

Discussion of the carcinoid tumors is presented under a separate heading, because they arise predominently (85%) from the digestive tract, whereas most of the other GEP neuroendocrine tumors arise from the pancreas and as such will be detailed under the following heading.

Be it mentioned already that carcinoid tumors are the most common of the GEP endocrine neoplasms, accounting for 55 to 86 percent of all such tumors (8,9). The difficult question of the frequencies of all those tumors will be treated later.

Carcinoid tumors were first observed in 1888 by Lubarsch who described multiple small tumors at autopsy in the distal ileum of two patients (10). The first clinical description was given by Ranson who in 1890 described a patient with ileal carcinoma and multiple liver metastases experiencing diarrhea and dyspnea induced by eating (10).

In 1907 Oberndorfer introduced the term “Karzinoid” (“resembling carcinoma”) to describe a morphologically distinct class of intestinal tumors that behave less aggressively than the more common intestinal adenocarcinomas (10). Gosset and Masson, using silver impregnation techniques, demonstrated in 1914 that carcinoid tumors might arise from enterochromaffin cells (Kulchitsky’s cells) of the glands of Lieberkühn. They also suggested that these tumors were of endocrine origin (10).

In 1953, Lembec demonstrated the presence of serotonin in carcinoid tumors (10). A year later Thorson and colleagues described a series of patients with small intestinal carcinoids and hepatic metastases, establishing a clinical entity of the “carcinoid syndrome”: an endocrine argentaffin-positive, gastrointestinal tumor producing serotonin, and causing the typical symptoms of diarrhea, flushing, asthma, cyanosis, and right-sided valvular heart disease due to fibrosis (10).

IV.2.3. Pancreatic neuroendocrine tumors or Islet cell tumors

As mentioned before, Bard and Pic were the first to point out in 1888 (funny enough the same year that Lubarsch described for the first time carcinoid tumors) that the islet cell was a potential candidate for the development of cancer.

A. G. Nicholls recorded in 1902 an adenoma of the islet of Langerhans and in 1909 R. L. Cecil, in a study of the islets of Langerhans in diabetes, mentioned an “enormous adenomatous hypertrophy of an island of Langerhans” which probably was an islet cell adenoma (1). A. Vecchi in 1914 described a malignant islet cell carcinoma in a 63-year-old man (1).

In 1926 S. Warren collected 16 islet cell tumors from the literature and added 4 of his own. In none of these was there reported any clinical signs or symptoms suggesting that the tumor gave rise to functional effects (1).

The year 1927 was marked by the opening of a new era in tumor biology - that of the functioning neoplasm. The patient, a 40-year-o1d physician with cachexia and hypoglycemia was operated on by W. J. Mayo. At autopsy an islet cell carcinoma was present in the head of the pancreas and there were metastases in the lymph nodes and in the liver. Extract of the tumor demonstrated the presence of insulin. This was the first recognition of a functioning beta-cell islet tumor - namely an insulinoma (1).

In 1929 the first successful operation for a functioning islet cell adenoma was performed by R. R. Graham of Toronto. Whipple and his group later operated on a large group of patients and set clinical standards for the diagnosis of the tumor (the famous Whipple triad) (1).

In 1950, investigators reviewed 398 reported islet cell tumors, and a 41 percent incidence of “nonfunctioning” islet cell neoplasm was reported (11). However, at that time, any tumor not associated with hypoglycemia, that is, not an insulinoma, was considered nonfunctioning. Therefore, many of these nonfunctioning tumors probably represented functioning ones, which have since become recognized as gastrinomes, VIPomas, glucagonomas and others.

The association of a non-beta islet cell tumor of the pancreas and peptic ulcer disease was already reported in 1946, but it was not until 1955 that a report by Zollinger and Ellison eventually brought attention to the possibility that one of the non-beta islet cells might be hyperfunctioning (12). Two patients with ulceration of the jejunum had an associated non-beta islet cell tumor of the pancreas. Marked gastric secretion, resistant to conventional medical and surgical treatment, and an ulcerogenic

factor of pancreatic islet cell origin was postulated. In 1960, gastrin from tumor extracts was first isolated and pathophysiological proof of its role as the “ulcerogenic factor” was provided by Gregory and co-workers (1,12).

In 1957, the association of islet cell tumors and diarrhea was first reported, but this was originally considered a variant of the Zollinger-Ellison syndrome. A year later a separate syndrome associated with watery diarrhea, hypokalemia and achlorhydria was described by Verner and Morrison. Elevated levels of VIP were later found in the plasma and tumors from patients with this diarrheogenic syndrome in 1973 after the isolation of VIP had been reported a year before (12).

The first well-documented instance of a glucagon secreting islet cell tumor was described in 1966 (13); the detailed story of glucagonomas will be related below.

Pancreatic polypeptide was isolated in 1972. Since that time, only 38 patients with a PPoma have been reported as of 1994 (12).

Somatostatinomas were first reported in 1977 and the somatostatinoma syndrome defined two years later. Approximately 80 somatostatinomas have been reported as of 1991 (12).

Moreover there exist very rare types of functional GEP neuroendocrine tumors, all predominantly found in the pancreas: pancreatic adrenocorticotropinomas, pancreatic parathyrinoma, GRFomas, neurotensinomas and a single case of both GIPoma and secretinoma.

IV.2.4. Brief history of gut endocrinology and the APUD- concept

Already in 1870 a population of cells with granular cytoplasm distinct from other mucosal epithelial elements of the gastrointestinal tract was recognized by R. Heidenhain (12). These cells have been referred to as Kultschitzky cells because of the mistaken belief that they were first observed by Kultschitzky in 1897 (12). These cells were initially believed to represent a homogeneous population, and because they closely resembled the chromaffin cells of the adrenal medulla, they were designated as enterochromaffin cells. The endocrine nature of these cells was for the first time proposed in 1906 by Ciaccio (12). In 1914 investigators observed that these enterochromaffin cells had an affinity for silver salts (argentaffinity) and later it was found that, if histologic sections were treated with a reducing substance before being exposed to silver salts, a larger number of granular cells could be demonstrated. Cells stained after such treatment were designated as argyrophil cells (12).

The physiology era of gut endocrinology began in 1902 when Starling demonstrated that hydrochloric acid instilled into canine duodenum elicited secretion of alkaline pancreatic juice (12). This blood-

borne chemical was called ‘secretin”. Three years later Edkins described a potent gastric acid secretagogue subsequently termed “gastrin” (12). Some 20 years elapsed before Ivy and Oldberg extracted a substance (cholecystokinin) from the bowel mucosa that caused contraction of the gallbladder (12). And thus the subsequent discoveries of gut substances went on and on in an almost logarithmic fashion with well over 40 different kinds of pharmacologically active substances, biogenic amines and peptides identified (12).

Friedrich Feyrter (1895-1973), Professor of pathology in Danzig, postulated in 1938 that a group of cells distributed along the entire mucosa of the gastrointestinal tract, with characteristic clear cytoplasm (“helle Zellen”) and a distinct affinity for silver salts (argentaffin and argyrophil reactivity) comprised a “diffuse endocrine epithelial system” (14). He later refined his description and coined the term “paracrine” to describe hormonal activity directed not only at distant target tissue, but also at cells in the immediate vicinity of the secreting endocrine cells *(15).* Feyrter suggested furthermore that these cells arose from intestinal endoderm and that while the endocrine cells of the intestine remained scattered among the enterocytes lining the intestinal lumen, those of the pancreas migrated into their definitive location after a budding-off process termed “endophytie’ (16).

A. G. Everson Pearse, borrowing from the work of Feyrter, put forth in 1966 the famous hypothesis that the peptide - producing cells of the gut and pancreas belonged to a much larger group of cells, which include the adrenal medulla, calcitonin - producing cells of the thyroid, carotid body type I cells, and some of the cells of the anterior pituitary (17,18). All of these cells share many cytochemical, ultrastructural and functional characteristics. Subsequently he coined the acronym “APUD” to reflect the amine-handling (Amine Precursor uptake and Decarboxylation) properties of all these cells, postulating at the same time that they all were of neuroectodermal origin and constituted a diffuse neuroendocrine system acting in concert with the autonomic nervous system to control the functions of the intestinal organs. This theory was based on the concept that these cells differentiate from a common progenitor cell and some metabolic features are retained that can then be used to identify this relationship.

There are numerous neuronal markers shared by APUD cells, such as neuron-specific enolase, a specific acetylcholinesterase, synaptophysin, receptors to tetanus toxoid, protein S-100, chromogranins A, B, C etc (19). However, shared characteristics of APUD cells do not prove a common origin (cells of various origins are able to express a APUD-like phenotype) and so Pearses´ hypothesis has been revised. The term APUD is now considered inadequate because several cell types included in the system do not metabolize amines (20). Furthermore there is now evidence to suggest that some APUD cell types, in particular, the GEP neuroendocrine cells, are not of neural crest origin but are instead derived from enterocyte stem cells or pluripotent duct cells (of embryologically endodermal or foregut origin) (21).

Although the APUD-theory may not hold as far as the above mentioned aspects are concerned, this theory has nonetheless been responsible for the appreciation that endocrine cells in the GEP system and other endocrine organs have neuroectodermal predetermination and have characteristics like those of neurons in the CNS as well as in the sympathetic and parasympathetic systems. Functional and physiological similarities and complex neurohormonal interactions continue to support an integrated APUD-concept. Precursors of these APUD cells may have the potential to produce and secrete any polypeptide that is specific for the large number of mature neuroendocrine cells found in different organs (22).

The word ‘APUDoma”, indicating a tumor arising from cells with APUD-like characteristics, is now firmly established in the vocabulary and the APUD-concept has also provided the conceptual basis for understanding the occurrence of the multiple endocrine neoplasia (MEN) syndromes, as well as the potential for one tumor to be composed of several cell types and to secrete more than one hormone.

The polemic about the APUD-concept is of course far from being settled.

V. Glucagon, Radioimmunoassays and G1ucagonomas

Glucagon was the second pancreatic hormone to have been discovered, only two years after insulin. In 1923 J. R. Murlin’s group of Rochester, Minnesota, found, during their studies of the solubility of insulin in various solvents, that acetone is not only ‘not so dependable as ethyl alcohol as an insulin precipitant”, but “...it throws down a substance, soluble in 95% alcohol, which has hyperglycemic effect... This hyperglycemic substance has been given the name of glucagon…”. Murlin demonstrated that the extract had its maximum hyperglycemic effect when injected into the portal vein, rather than in the periphery (3).

The peptide hormone was isolated much later, in 1955 by Staub et al. (7). The amino acid sequence of porcine glucagon (29 residus, 3500 daltons) was reported by Bromer et al. in 1957 (7).

The discovery of radioimmunoassay, and specifically the radioimmunoassay of insulin by Yaloq and Berson in 1959 was of greatest importance also for the glucagon hormone (23). R. H. Unger et al. applied its principles and thus developed the first radioimmunoassay for glucagon (24), which ultimately was the basis for the discovery and proof of the first well-documented case of a glucagon-secreting islet cell tumor in 1966 (13). Following is a condensed account by Dr. R. H. Unger himself of this crucial period in the history of glucagonomas (from personal communication, April 18, 1997): “The story begins in 1959, when we developed the first radioimmunoassay for glucagon and began publishing papers on glucagon physiology. We were on the lookout for a possible glucagonoma, but non was found until Mc Gavran, a young pathology resident in the department of Dr. Paul Lacy at Washington University in St. Louis, Mo., became suspicious about a patient. The patient had, to the

surprise of all, survived well beyond the time consistent with the original diagnosis of carcinoma of the pancreas. Mc Gavran took a second look at the tissue sections. He was struck by the fact the cells resembled an endocrine alpha-cell tumor, rather than a tumor of exocrine pancreas. At that point, Dr. Lillian Recant, a diabetologist at Washington University asked me to fly up to St. Louis to examine the patient. I went over the tissue sections and brought back some tissue samples and plasma. It was quite apparent from the assays of both, that this lady had glucagonoma.

M. H. Mc Gavran, R. H. Unger et al. subsequently published their findings in 1966 in the New England Journal of Medecine under the title “A glucagon-secreting alpha-cell carcinoma of the Pancreas” (13). They described as suspicious clinical findings “the presence of a pancreatic neoplasm, with or without hepatic metastasis, the presence of diabetes and the presence of a chronic eczematoïd and / or bullous dermatitis” of which they were “uncertain whether this is an integral or incidental part of the syndrome” (13).

As a matter of fact, this unusual dermatitis had already puzzled Becker et al. as early as 1942 and they, without knowing it, reported the first case ever of a patient with the glucagonoma syndrome (25). They described a woman with diabetes, marked weight loss, normochromic anemia, an increased tendency for thrombosis and an unusual skin rash who was found to have an extensive pancreatic islet cell carcinoma at autopsy. They remarked that “… a comment on our first case seems to be impossible at present, and we restrict ourselves to the recording of it. Only the observation of other cases of pancreatic tumor will decide whether the cutaneous changes in our case were primarily due to the neoplasm” (25).
Five years later Hamperl reported the case of a 65 year old woman with diabetes, who at autopsy was found to have an islet tumor at the head of the pancreas, which on silver staining was positive for alpha-cells.
Zhdanov described in 1956 a middle aged woman who suffered from anemia, diabetes and a disseminated vesicular and pruritic skin rash; at autopsy a tumor was discovered in the tail of the pancreas, consisting of argentophilic alpha-cells.
In 1960, Goessner and Korting recorded the case of a 51 year old man whose initial complaint was a skin rash. Thromboembolic disease, respiratory failure, and death ensued. A large pancreatic neoplasm was found, staining positively for alpha-cells and an extract of the tumor mass produced hyperglycemia when injected into rabbits.
A few additional cases were reported, until, as we described above, Mc Gavran et al. documented for the first time hyperglucagonemia resulting from a functioning islet cell carcinoma.

The following investigators helped to further characterize glucagon-producing tumors. Wilkinson coined in 1973 the term “necrolytic migratory erythema” to describe the appearance and five-year course of a 52-year-old woman with cyclic episodes of annular and gyrate erythema of the legs, related to a carcinoma of the pancreas (26). This term incorporated the clinical and histologic features of both

the figurate erythemas and toxic epidermal necrolysis, which Wilkinson felt were the predominant characteristics of this eruption.

A year later Mallison and co-workers reported the first series consisting of nine patients with glucagon-producing tumors (27). Their series established and identified the glucagonoma syndrome as a distinct entity consisting of necrolytic migratory erythema, weight loss, anemia, and diabetes mellitus, in association with a pancreatic alpha-cell tumor and elevated plasma glucagon level (27).

The identification of this syndrome increased interest and awareness of A-cell tumors of the pancreas. Presently there are around 250 published cases worldwide which all contributed to a better understanding of this very rare tumor and a more detailed listing of the many clinical features associated with the glucagonoma syndrome.

2. Physiology of Glucagon Hormone

A. Generalities

Glucagon is a peptide hormone of 29 amino acids and has a molecular weight of approximatively 3500 daltons. It is secreted by the A-cells of the pancreatic islets. This hormone is one of a family of several peptides with similar primary structures that includes secretin, VIP, GIP, and GH-releasing hormone (28). Peptides related immunologically to glucagon are produced in the brain, salivary glands and intestine (28), but in this text we will concentrate on glucagon and glucagon-like peptides occurring in the intestine and pancreas only.

The pre-proglucagon gene, situated on chromosome 2, consists of 6 exons and 5 introns, and codes for the prohormone to glucagon, proglucagon (PU), which is made out of 160 amino acids (28). Multiple peptides are generated by cell-specific differential post-translational processing of this same proglucagon. We are not going to detail all the various peptides produced, which is unnecessary for the present purpose, but let us mention that the pancreatic A-cells produce predominantly glucagon, GRPP (glicentin-related pancreatic peptide) and PG (1-61). PG (1-61) is also called proglucagon 9000 (the number designates the molecular weight in daltons) and includes within its 61 amino acid structure the 29 amino acids which make the glucagon hormone (28). In the plasma one finds moreover big plasma glucagon (BPG: 30000 daltons), a heterogeneous material of unknown origin and function, and smaller immunoreactive component, glucagon 2000, a degradation product of glucagon (28,29). A radio-immunological method using Unger's 30K antiserum recognizes the C-terminal end of the molecule; chromatographic analysis of this plasma reveals a heterogeneous material comprising the following four IRG (lmmunoreactive glucagon) components: IRG 3500, IRG 9000 (= proglucagon), IRG 2000 and BPG (29).

The intestinal L- cells process proglucagon predominantly to glucagon-like peptides (GLP's). Glucagon is not formed in these cells; rather it remains in the form of two incompletely processed precursors, glicentin and oxyntomodulin, the biological activities of which are poorly understood (28).

By what biochemical mechanism does glucagon act on target cells? E.W. Sutherland et al. (3) pointed out that the study of the origin of glucagon-induced hyperglycemia has provided several interesting general biological considerations. They demonstrated the interconversion of the enzyme phosphorylase between an active and inactive form. When glucagon is secreted in response to for example hypoglycemia, it increases the conversion of inactive liver phosphorylase B into active phosphorylase A, which, in turn, increases the breakdown of liver glycogen. This is brought about by the actions of a kinase and a phosphatase which catalyse phosphorylation and dephosphorylation of the catalytic

enzyme. This was the first observation, in 1968, of a regulatory mechanism, which is not uncommon in metabolic systems. The most important result proved to be the discovery that glucagon enhances the production and / or release of cAMP, which is an essential co-enzyme for the kinase. Thus was born the “second messenger” concept: according to this idea, glucagon binds to a specific receptor on the cell surface and this hormone-receptor- complex activates the enzyme adenylcyclase. As a result, cAMP within the cell, the “second messenger”, increases and activates allosterically the protein kinase A which in turn phosphorylates target enzymes, which are either activated or inhibited, depending on the enzyme (30).

B. Regulation of Glucagon Secretion

There are many factors, such as nutrients and metabolites, hormones and neural mediators, which affect the secretion of glucagon positively or negatively. The quantitative contribution of each of these factors in regulating glucagon-secretion is poorly understood, but it is generally accepted that blood levels of glucose and amino acids are the most important regulatory components. Table 1 gives a summary of the major modulators involved (31).

TABLE 1. Effects of various major modulators on glucagon secretion

	Glucagon
Islet Hormones:	
Glucagon	▼
insulin	▼
Somatostatin	▼
Nutrients / Metabolites:	
Glucose	▼
Amino Acids	▲
Fatty acids	▼
Neural Mediators:	
alpha-Adrenergic	▲
beta-Adrenergic	▲
Cholinergic	▲
Gut Hormones:	
Gastric	▲
CCK	▲
GIP	▲
Secretin	▼
VIP	▲

C. Actions of Glucagon on Target Cells

Insulin and glucagon are antagonists. Insulin can be regarded as a plethora-hormone, whereas glucagon is a “fasting-state”-hormone. In the fasting periods, our bodies tend to stop storaging energy and start to draw on the reserves. We metabolize less glucose (which in “normal” times is the first choice of energy-giving substract), and instead of it more fatty acids. At the same time we synthesize more glucose, destined for the “noble” organs (30).The following section details the major actions of glucagon on target cells and already outlines some of the physiopathological implications for the glucagonoma syndrome.

I. Effects on glucose metabolism

Glucagon’s primary site of action is the liver where it increases glycogenolysis, gluconeogenesis, beta-oxidation of free fatty acids (cetogenesis) and lysosome formation, while it decreases glycogenesis, glycolysis and lipogenesis (30). It is vitally important in regulating glycemia, particularly in the postabsorptive phase (32). It is the main regulator of hepatic glucose output establishing a so-called “set point” (33). When glucagon is elevated, there is a transient increase of glycemia- mainly due to glycogenolysis- that wanes after 3-4 hours and then returns to the previous set point (34). When the liver is chronically exposed to pathologic doses of glucagon, it switches over from a glycogenolytic organ to a gluconeogenic one (35). In normal persons, increasing glucagon levels stimulate secretion of insulin, which reduces action of glucagon on liver. Thus, the absence or presence of more or less mild diabetes or glucose intolerance in glucagonoma patients depends on the relative concentrations of insuline and glucagon, which ultimately determine the net effect on hepatic glucose production. More will be said about the various hypothetical explanations of glucose imbalance later on.

II. Effects on amino acid and protein metabolism

Glucagon has several effects on amino acid metabolism. Normal physiologic levels of glucagon seem to help maintain plasma amino acid concentrations, because glucagon deficiency (after total pancreatectomy for example) increases them (36). Pathologic doses of glucagon produce a generalized- meaning glucogenic as well as nonglucogenic amino acids- hypoaminoacidemia. This it does in at least two ways. Glucagon induces hepatic gluconeogenesis from mainly glucogenic amino acids. In addition, glucagon activates two enzymes (carbamoyl phosphate synthase and arginosuccinate synthase) which are required in the urea cycle and aid in the metabolism of protein by increasing the liver’s ability to remove nitrogen (34),
Whereas acute hyperglucagonemia in healthy humans does not significantly stimulate proteolysis, a

study suggests that enhanced protein breakdown could be a long-term effect of prolonged high glucagon concentrations (116). Glucagon-induced hypoaminoacidemia could contribute to the increased proteolysis, since amino acids promote protein deposition and inhibit protein breakdown (116).
In summary, glucagon excess increases proteolysis, amino acid oxidation and urea production (54,116). Enhanced protein catabolism is associated with weight loss, decreased lean body mass and muscle-wasting (38). Also, as will be discussed later, the necrolytic migratory erythema (NIME) of glucagonoma patients could be in relation to this upset in protein and amino acid metabolism.

III. Effects on fatty acids

In the fatty tissues glucagon increases glycogenolysis, for internal use only. Synthesis of fatty acids is diminished. Lipolysis is activated leading to exportation of glycerol and free fatty acids, which now constitute the major source of fuel (30). Because glucagon also increases secretion of insulin, which diminishes lipolysis and tends to maintain normal free fatty acid concentrations, cetonemia usually doesn't develop (35). Chronic elevation of glucagon results in a decreased peripheral and circulating pool of lipids and this results in a changed metabolic equilibrium that may play a role in producing NME (39).

IV. Miscellaneous effects

Glucagon exercises many more actions. Not all of them are well understood, but some play a role in the symptoms of glucagonoma syndrome and therefore are described here. Many of these effects require pharmacologic doses of the hormone which are usually present in glucagonoma patients.

It has been shown in vitro that glucagon may increase arachidonic acid and its metabolites (prostaglandins and leukotrienes) in the epidermis (40), which lead to the hypothesis that subsequent local trauma may release these substances, causing the inflammatory lesions of NME.

Glucagon is involved in causing the often observed hypocholesterolemia in patients: the reason for this seems to be a reduction in very-low-density lipoprotein (VLDL) hepatic synthesis, without knowing the exact mechanism implicated (41).

A study of the effect of glucagon on erythropoiesis of normal rats and mice showed an inhibitory effect of glucagon on erythropoiesis; this effect was demonstrated by the depression of reticulocytosis, Fe-uptake, normoblast percentage, absolute normoblast counts, erythropoietic response to exogenous erythropoietin and reduced erythropoietic response to hypoxic stimulation

(42). This study strongly suggests the preponderant role of glucagon in causing the anemia as part of the glucagonoma syndrome.

Experiments in dogs showed glucagon to increase mesenteric blood flow (43), which could be a possible cause of villous hypertrophy of the small intestine rarely observed in patients. Another factor clearly established for causing such hypertrophy is the hormone enteroglucagon, known to be a trophic factor for the small intestinal epithelium (43).

Glucagon's effect on the small bowel mucosa in either reducing absorption or enhancing the net secretion of water and electrolytes seems to be responsible for diarrhea, and glucagon's smooth muscle-relaxing properties may explain constipation (in inhibiting gastrointestinal motility) and early satiety sometimes observed (44, 21).

The role of glucagon in causing the thromboembolic phenomenon is not entirely clear. Glucagon affects coagulation parameters, but the relationship to the clotting imbalance is not known. What is known is that factor X is raised in the glucagonoma syndrome and is produced by the alpha cells, thus partly explaining the high level of clotting problems (45).

Glucagon has been shown to increase cAMP in cultured glial (Muller) cells of the chick embryo retina (46). This could be the mechanism by which glucagon causes the reported neurological impairments in patients.

The discussion later on of the full range of symptoms of the glucagonoma syndrome will show the eminent role the hormonal secretion of glucagon plays in explaining many aspects of the physiopathology of the clinical picture.

3. Classification, Prevalence/Incidence, and Epidemiology of GEP Neuroendocrine Tumors

A. GEP Neuroendocrine Tumors

We will first present a classification of tumors derived from APUD cells (table 2), so-called APUDomas, adapting it from the one proposed by C. Weil (47). The APUD theory offers a conceptual explanation for multiple endocrine neoplasia, ectopic hormone production, multihormonal tumors and switches from one tumor syndrome to another.

The most recent WHO classification categorizes all GEP neuroendocrine tumors on the basis of clinical-pathological criteria (130,132)

TABLE 2: A classification of APUDomas (ectopic products not included)

Tumor location	**Tumor type**	**Entopic production**
Anterior pituitary	Usually adenoma	ACTH/MSH, GH, PRL
Thyroid	Meduallary carcinoma	CT
Para thyroid	Adenoma	PTH
Adrenal	Pheochromocytoma	Catecholamines
Paraganglia	Chemodectoma, ganglioneuroma, neuroblastoma	Catecholamines
Thymus	Thymoma	
Lung	Oat-cell carcinoma and carcinoids	5-HT, histamine
Skin	Melanoma	
Gl-tract	Various carcinoids Garstinoma Somatostatinoma	5-HT Gastrin Somatostatin
Pancreas	Insulinoma Glucagonoma Somatostatinoma PPoma Pancreatic carcinoid Gastrinoma VIPoma	Insulin Glucagon Somatostatin PP 5-HT Gastrin VIP

Let us concentrate now on the neuroendocrine tumors arising from the GEP-system, with special emphasis on those existing in the pancreas. One is immediately struck by the astonishing fact that, in spite of the presence of huge numbers of these neuroendocrine cells in the gastro-intestinal tract which secrete a great variety of different regulatory peptides, the majority of these (non-carcinoid) GEP neuroendocrine tumors originate in the pancreas. For unknown reasons, no tumors related to cells exclusive of the small intestine, such as secretin, CCK, motilin, GIP or neurotensin cells, have been identified so far (48).

The pancreas of a normal adult weighs around 70g and has 1-2 million ilets of Langerhans, each of them weighing approximatively 1 microg. Thus the total weight of these pancreatic endocrine cells equals l-2 grams.

Adapting from the work of E. Solcia et al. (48), we classify the endocrine tumors of the GEP-system in tumors of the pancreas and those oft the gastro-intestinal tract.

In one series, the combined incidence for all GEP neuroendocrine tumors (of which pancreatic endocrine tumors form only a portion) was 3,6 per million persons per year. Carcinoids were by far the most common, with an incidence approximatively equal to that of all the pancreatic endocrine tumors combined (15). From this study, which was conducted in a well-defined population in Northern Ireland over 15 years, we can deduce that the overall incidence for neuroendocrine tumors arising from the pancreas only, equals roughly 1,8 per million persons per year. These represent less than 5% of all the tumors (exocrine and endocrine) of the pancreas.

TABLE 3: Annual incidence per million and respective percentage of the most frequent **pancreatic** endocrine tumors.

Tumor type	**Incidence**	**Percentage of islet tumors**
Insulinoma	~ 0.8	~40%
Non-functioning tumors	~ 0.6	~ 30%
Gastrinomas	~ 0.4	~ 20%
VlPomas	~ 0.1	~ 5%
Gucagonomas	~ 0.05	~ 2.5%
Somatostatinomas	~ 0.025	~ 1.25%
PPomas	~ 0.025	~ 1.25%

Insulinomas are the most common pancreatic endocrine tumors with an incidence of 0,8 to 0,9 per million persons per year (15). Next in order of frequency come the nonfunctioning pancreatic endocrine tumors, with an estimated incidence of 0,6 per million persons per year (12). Gastrinomas then follow, occuring in 0,4 per million persons per year (15).
ViPomas have an anticipated annual incidence of 0,1 per million persons per year (12). The incidence of the glucagonoma syndrome is estimated to be 1 in 20 million per year (21); thus this syndrome represents something like 2% of all endocrine tumors arising from the pancreas, and 1% of all GEP neuroendocrine neoplasms.
Somatostatinomas are even rarer, with an annual incidence of 1 in 40 million of the general population (12). Pure PPomas, meaning that they consist of at least 50 percent PP-secreting cells, probably have an incidence similar to somatostatinomas (12). The remaining funtional pancreatic endocrine tumors have been reported very rarely and therefore will only be listed in the following table 4, without any comment here.
The prevalence of pancreatic endocrine tumors during life is less that 1/100000 of the general population.

TABLE 4- Classification of pancreatic endocrine tumors

Entopic islet cell tumors (~45%)
– Insulinoma
– Glucagonoma
– Somatostatinoma
– PPoma
– Serotonine-producing pancreatic carcinoid
– Non-functioning, clinically silent
-incidentally found (for example at surgery)
-systematically searched for (autopsy studies)

Non-functioning locally symptomatic tumors (~30%)

Gut-related and ectopic tumors (~25%)
– Gastrinoma
– VIPoma
– ACTHoma = Pancreatic adrenocorticotropinoma
– Pancreatic parathyrinoma
– GRFoma
– Neurotensinoma
– GIPoma
– Secretinoma

Table 4 gives an exhaustive classification of all known pancreatic endocrine tumors (12,48). Gastrinomas and VIPomas are classified under ectopic tumors, as is classically done. This may be inappropriate because it is known that the fetal pancreas produces considerable amounts of gastrin, and that VIPergic neurons are also represented in the abundant network of peptidergic neurons found in the normal pancreas.

An interesting finding, published by Kimura et al., concerns autopsy studies and non-functioning, clinically silent tumors (49). In 800 unselected autopsy cases they performed a clinicopathological analysis of endocrine tumors of the pancreas and found the following prevalences using two different methods: 738 individuals had histological studies of only three random sections from the head, body, and tail of the pancreas with the resulting prevalence of 1,6%. This number confirmed prior autopsy studies (50). But the 60 individuals whose pancreas was sectioned at 5mm intervals showed an amazingly high prevalence of 10%. This study suggests that endocrine tumors of the pancreas are found at a surprising high rate when histological analysis is conducted thoroughly (49).

Endocrine tumors of the pancreas manifest themselves in 2 distinct epidemiologic groups, a sporadic form affecting the general population and an autosomal dominant inherited form occurring as part of the multiple endocrine neoplasia type 1 (MEN 1) syndrome (multiple tumors in the parathyroid glands, the anterior pituitary lobe and in the endocrine pancreas). The nonhereditary (sporadic) pancreatic endocrine tumors tend to occur in the fifth or sixth decade of life, but rare cases occurring in early childhood have been reported. There appears to be no obvious socioeconomic or ethnic predilection. There may be a small sex preference in favor of women (about 60%). Tumors are generally single. Pancreatic endocrine tumors in patients with MEN 1 are almost always multiple and tend to present at an earlier age, commonly during early adulthood. The first manifestations of MEN 1 are not usually from a pancreatic lesion.

B. Glucagonoma

Glucagonoma is a rare islet cell tumor. Yoshia Haga et al. noted in 1995 that of the 200 or so published cases in the English literature, 78 are probable cases and only 122 proven ones (51). Since then, about 50 more cases have been reported. The most often reported annual incidence is 1 in 20 million. Bloom et al. even went to propose an incidence of 1 in 30 million per year (52). Another group pointed out that the glucagonoma syndromes are an underdiagnosed clinical entity and suggested that their true incidence may well approximate that of insulinomas and gastrinomas (53).The above –mentioned autopsy study by Kimura et al. demonstrated the high prevalence of endocrine tumors; moreover it was shown that 85 % of these consisted of predominantly glucagon-producing cells, which would make glucagonoma tumors the most prevalent of all pancreatic

endocrine tumors (49). But this finding has clinically no significance.

Glucagonomas are slightly more prevalent in female (55%) than in male patients (29,117). The mean age at diagnosis is 65 years (range 19-84 years) (54), but many patients have a long history of unrecognized symptoms suggesting that the tumor might have been present up to a decade earlier (55). Most of the cases (75%) are being observed between 45 and 70 years (54). The syndrome has not been observed in children.

It must be pointed out that when talking about frequencies and clinics of any of the endocrine pancreatic tumors, almost only the tumors with a syndrome due to hormone hypersecretion have been considered. This is especially true for glucagonomas. Glucagonomas with their typical glucagonoma syndrome are very uncommon, while glucagonomas without clinical manifestations occur more frequently (49,56,117). We will solely concentrate on glucagonomas associated with the syndrome. In order to be complete, the following classification (table 5) adapted from the one proposed by Ruttman et al. (56), is included here.

TABLE 5: Classification of glucagonomas

1. **Glucagonomas associated with the typical clinical syndrome**

2. **Glucagonomas not associated with the typical clinical syndrome (characterized on morphological and / or biochemical grounds)**

   a) solitary malignant endocrine pancreatic tumors, which amazingly do not develop the syndrome, but are sometimes associated with diabetes (probably secondary to the tumor growth)

   b) glucagonoma as part of a single insulinoma, gastrinoma or other pancreatic endocrine tumor;

   c) multiple glucagonomas associated with MEN 1; 16,5% of glucagonomas are associated with MEN 1 or its variant (51);

   d) microglucagonomas as found in autopsy studies (frequency already pointed out).

4. Histopathology, Tumor Biology and Morphology of Glucagonomas

A. Origin

Endocrine tumors of the pancreas are often called islet cell tumors, a term almost automatically implying that these tumors originate from mature endocrine cells constituting the islets of Langerhans. This may not be true according to some authors.
It is known that during embryogenesis pancreatic islets form through budding off of endocrine cells from endotermal intralobular ductules. It is thought that the epithelia of pancreatic ducts contain some multipotent stem cells which can differentiate into acinar cells or endocrine cells. This finding has led to the suggestion these tumors are ductular in origin (57). Supporting this hypothesis that these tumors represent a dedifferentiation of an immature stem cell are the facts that they often contain ductular or tubular structures within or just around them (49), have at times cells containing both hormone granules and mucin granules, produce hormones not normally present in the adult pancreas and sometimes secrete multiple hormones (48).

Another hypothesis favors mature islet cells as starting point: experiments in mice rendered transgenic for a hybrid vasopressin / simian virus (SV) 40 oncogene showed that heritable insulinomas of such transgenic mice take origin from mature islet cells (58). These experiments may be applicable to inherited tumorigenesis in man. These authors are quick to admit that as far as the sporadic (non-hereditary) endocrine tumors are concerned, it still needs to be established whether islets or ductules or BOTH, are at the origin of their genesis (58). The following fact supports this hypothesis as well: tumor locations most often coincide with the physiologic location of the respective endocrine cells in the pancreas. This is especially true for glucagonomas which most often are located in the pancreas' tail or body (glucagon cells predominate in the tail and body) (51,117).

B. Morphology of Glucagonomas with and without Syndrome

I. Glucagonomas with syndrome

Their histological structure do not have a common, specific pattern and vary from tumor to tumor and even within the same neoplasm (59). Trabecular and solid or diffuse growth patterns are the most frequent; sometimes anastomosing thin ribbons of cells or glandular formations are found (12). Individual tumor cells have a granular and abundant cytoplasma. They are most often polygonal or, within gland-like structures, cylindric.
Malignant tumors present with frequent atypical mitoses and their nuclei are hyperchromatic, polymorph and of bizarre forms, and even some multinucleated cells are found (59). In contrast, the nuclei from benign tumors appear more normochromatic, round and uniform and there are no mitotic figures. Ultrastructurally atypical secretory granules are found which do not correspond to the normal alpha cells of the pancreatic islets (60).

II Glucagonomas without syndrome

There are some important differences. Here the tumor cells are ribbonlike arranged. Ultrastructually, these cells almost always contain typical alpha-granules. Thus they reflect more closely the morphology of normal islet alpha-cells (59), Very interesting is the finding that some of the largest tumors among these often multiple adenomatous glucagonomas without the syndrome showed morphological characteristics similar to those found in glucagonomas with the syndrome (60). This suggests that small, apparently non-functioning tumors may in some cases evolve into large neoplasms with the glucagonoma syndrome.

III General and specific endocrine tumor markers

The term “neuroendocrine” is used to group neuronal and endocrine cells which share a common phenotypic program (not necessarily a common origin!) characterized by simultaneous expression of a number of genes encoding certain markers and hormonal products.

III.1. General markers of neuroendocrine differentiation are:

NSE (neuron-specific enolase), PGP-9,5 (protein gene product 9,5), synaptophysin, chromogranin A, B and C, HISL-19, 7B2, and the epitope Leu-7 (62).

NSE, PGP-9,5 and synaptophysin are cytoplasmic proteins or constituents of small clear vesicle membranes. The staining intensity of these markers is therefore independent from the cells content of secretory granules or type of hormone produced. Of these, only synaptophysin, identified by immunohistochemistry, is useful for determining the endocrine nature of a neoplasm since this protein is not expressed in non-endocrine tumor cells (61).

Antisera against chromogranins, HISL-19, 7B2 and Leu-7 recognize components of neurosecretory granules and thus depend in their staining intensity on granule content of the cells. In alpha-cells, chromogranin A are intensely expressed and, to a lesser extent also chromogranin B and C. A study (63) showed that the staining patterns of chromogranin A, B and C in tumoral alpha-cells associated with the glucagonoma syndrome do not differ from those of tumoral alpha-cells in nonfunctioning endocrine pancreatic tumors: thus it seems that production and secretion of chromogranins are unrelated to glucagon biosynthesis and release in neoplastic alpha cells. In the surrounding nonneoplastic alpha-cells, however, their decreased glucagon immunoreactivity, due to the inhibitory effect of the tumoral hypersecretion of glucagon, is correlated with a decrease in both chromogranin B and C, but not A (63).

III.2. Specific markers

The specific markers necessary for precise typing of neuroendocrine tumor cells are: cell-specific hormonal peptides and biogenic amines. In most functioning tumors, the hormone causing the syndrome can be detected immunocytochemically. There exists no correlation between the staining intensity or the number of positive cells and the severity of symptoms (62).
Glucagonomas with the syndrom often stain weakly for glucagon (60), probably due to excessive hormone secretion (62). In the tumors unreactive to glucagon antisera, reactivity for peptides deriving from proglucagon (glicentin GLP 1 and 2) can frequently be demonstrated (64). This finding may also suggest that glucagonoma cells have a decreased capacity to convert proglucagon-forms to glucagon (60).
In addition and depending on whether a battery of antisera against pancreatic and ectopic hormones is used, multiple secretors (those producing two or more gastrointestinal hormones) can be identified. One statistical evaluation detected them in 32,5 % of patients (with and without the

syndrome) for whom immunohistochemistry was carried out. PP was most often identified (51,1 %), followed by somatostatin (34,7 %), insulin (22,7 %), gastrin (19,0 %), serotonin (16,0 %), VIP (10,4 %), ACTH (5,6%) and Calcitonin (4,2 %) (117).
In tumors not associated with the syndrome, a very large number of cells reacting with antiserum against glucagon is found, again showing that they closely resemble normal alpha-cells (49,120).

IV. Malignancy

Histological features alone do not permit to distinguish between malignant and nonmalignant islet-cell tumors. The only valuable criteria for malignancy are local or capsular extension, vascular invasion by tumor cells or the presence of metastases. When glucagonoma syndrome is diagnosed, evidence of malignancy is present in 82% of the cases (29). According to another review of literature, exactly 54,7% had metastasis (51).
The liver is most often involved (79,9% - 84,1%), followed by lymph nodes (30,2% - 37,8 %), spleen (4,8 %), peritoneum (4,8 %), bone (3,2 % - 8,1 %) and very rarely kidney, colon, adrenal gland, lung and duodenum (51,117). There exists an almost linear positive correlation between tumor size, beginning with 2 cm up to 35 cm, and metastasis: tumors sized 2-4 cm metastatise in 38,5% of cases, whereas tumors of 8 cm or larger metastatize in almost 60% of cases (51).

5. Glucagonoma Syndrome: Clinical Presentation, Diagnosis and Management

A. Clinical features

The most prominent clinical features of patients consist of cutaneous manifestations including a necrolytic migratory erythematous rash, stomatitis and glossitis, a mild diabetes, anemia and weight loss.
The syndrome also goes by the acronym “4D syndrome”, which stands for *d*ermatosis, *d*iabetes, *d*eep vein thrombosis, and depression.

I. Cutaneous and mucosal lesions

I.1. Distribution and appearance

The majority of patients owe their initial diagnosis to a dermatologist, because of this caracteristic rash, called necrolytic migratory erythema (NME), present in most cases of glucagonoma syndrome (29,56,65,66,124,126,127,134). The average duration of NME before diagnosis is 3.5 years (51), lasting as long as 10 years sometimes. Tumors of 4 cm or greater have a significantly higher incidence of NME (94,9%) than smaller tumors less than 4 cm (27,6%) (51). The skin lesions are widespread, most commonly involving the lower abdomen, buttocks, groin, perineum, thighs, the face and distal extremities (29,67). The dermatitis typically evolves over 7 to 14 days: the individual lesions begin as small and often irregular erythematous macules that enlarge, while becoming papular. As the erythema spreads peripherically, a superficial central blister appears, followed by erosions, as the upper third of the epidermis undergoes necrosis (29,68). At this time the lesions can become secondarily infected with Candida or Staphylococcus species (21,66). These erosive lesions may assume linear, annular, or serpiginous configurations, either by extension of a single area or by confluence or several (68). The lesions then tend to heal in the centre with a raised erythematous edge. Healing is followed by a postinflammatory hyperpigmentation (29,68). At a given time, different lesions may be in varying stages of development, or lesions may progress in synchronous waves (68). Typically, the skin changes wax and wane, but trauma plays an important part in their severity and site. The intense pruritis associated with NME is a major cause of patient’s morbidity.

Other dermatologic manifestations are dystrophic nails, thinning of hair, angular cheilitis, painful atrophic glossitis (raw, red, beefy, sore tongue with preservation of papillae), and stomatitis (65,29,66)

I.2. Histology

The characteristic histological features may be missed unless biopsies are taken from the edge of a fresh lesion. Often multiple biopsies are necessary. The characteristic histology is not seen in older lesions where progressive changes and superinfection result in a nonspecific dermatitis. The following histopathologic characteristics (not all of which are always present) of NME can be summarized (68,69,122):

- Remarkable pathologic changes in the upper 1/2 of epidermis, while very little or absent in the lower 1/2 of epidermis.
- Parakeratosis and sometimes neutrophil leucocytes invasion in the stratum corneum.
- Irregular acanthosis with spongiosis (absence of acantholysis).
- Subcorneal and midepidermal clefts, sometimes giving rise to vesicles and even pustules when neutrophils aggregate.
- Irregular necrosis of the upper epidermal cells resulting in pallor of the upper prickle-cell layer.
- Fusiform keratinocytes with pyknotic nuclei.
- Capillary proliferation in the papillary dermis.
- Mild perivascular inflammatory cellular infiltration in the upper dermis.
- Negative immunofluorescent findings for lg’s and complement.

I.3. Pathogenesis

Many theories exist as to the pathogenesis of NME, and they do not exclude each other, pointing to the concept of multifactorial upset as the cause.
One theory suggests that glucagon itself induces the cutaneous lesions; often spectacularly rapid resolution of NME after surgical removal of the glucagon-producing tumor and improvement of NME after treatment with somatostatin analogs that temporarily inhibit glucagon secretion favour this idea (55,70). But it does not work in all patients. Some patients show no response to such treatment despite no evidence of residual disease (39).
Glucagon-induced hypoaminoacidemia, leading to depletion of epidermal protein, is present in most cases and a dramatic improvement in skin lesions has been observed in some cases (but not in others) after parenteral administration of amino acids (71,121).

Marinkovich et al. (72) proposed that the often observed hypoalbuminemia may potentiate the release of arachidonic acid and its inflammatory metabolites (prostaglandins and leukotriens) by glucagon in the following way: albumin has been shown to sequester fatty acids released from tissue membranes, making them inaccessible to further degradation to metabolic products such as prostaglandins. In consequence low albumin levels would allow even small increases of glucagon concentrations to raise considerably high levels of prostaglandins that would induce the inflammatory changes of NME.

Another popular hypothesis concerns the fact that, as seen before, chronic elevation of glucagon results in a decreased circulating pool of lipids; thus some reported successful response to intravenous infusion of essential fatty acids (73,74,121), but again, in other cases this did not help at all. A role for zinc deficiency has been proposed because of similarity of glucagonoma skin lesions and those observed in acrodermatitis enteropathica. However most cases show normal plasma zinc concentrations (29).
Glucagon has been shown to stimulate the production of “acute phase” proteins (75). Raised levels of one of these, fibrinogen, could possibly alter skin blood flow leading to skin changes. Thus, the exact cause of NME remains uncertain at present, but clearly, glucagon hormone is responsible either directly or indirectly.

I.4. Differential diagnosis of NME

NME associated with hyperglucagonemie >800 pg/ml is pathognomic of the glucagonoma syndrome. But when glucagonemia is not assayed the following skin diseases and internal pathologies rarely associated with NME-like skin disorders should be considered (66,68,72,76).

skin diseases:

Pemphigus foliaceous
Chronic mucocutaneous candidiasis
Seborrheic dermatitis
Psoriasis vulgaris
Pustular psoriasis
Subcorneal pustular dermatosis (Sneddon-Wilkinson disease)
Chronic benign familial pemphigus (Hailey-Hailey disease)
Acrodermatitis enteropathica
Erythema annulare centrifugum
Chronic erythema multiforme
Toxic epidermal necrolysis
Pellagra

skin disorders associated with internal pathologies:

Hepatic cirrhosis
Hepatopathic hemochromatosis
Hepatocellular carcinoma
Jejunal adenocarcinoma
Pancreatite
Idiopathic malabsorption
Coeliac sprue

II. Diabetes mellitus (DM)

Typically, diabetes associated with the glucagonoma syndrome is described as mild to moderate; these patients are not prone to ketoacidosis (except in very rare instances) or microvascular complications of diabetes. This may well reflect the short duration of the disease (21). Average duration of diabetes prior to diagnosis is 7,7 years (51), which is longer than it was for NME. Wermers et al. underlined this point in an article which reported the largest single-institution experience comprising 21 patients: they observed that when DM and NME coexisted, the former always occurred first (66). Following the same train of ideas Vinik et al. (21) suggested that any patient over 60 years developing diabetes, without a family history, should be investigated; at the same time they conceded that screening of all adult onset diabetics with glucagon levels may probably not be cost-effective, even though one autopsy study of diabetic patients had shown a 0,8% occurrence of glucagonomas.
Again, as was the case with NME, there exists a significantly higher incidence of DM in tumors of 4,0cm or greater, that in smaller ones (51).
Diabetes is usually easily controlled by dietary measures or oral hypoglycemic agents, and around 22% of patients will require insulin (51,54).

II.1 Pathogenesis of this DM

Classically, this DM was thought to be primarily caused by the gluconeogenic and glycogenolytic actions of glucagon. In normal individuals, however, increasing glucagon levels stimulate the secretion of insulin, which greatly reduces glucagons action on the liver. Moreover, there is a poor correlation between hyperglucagonemia and hyperglycemia: some patients with very high serum glucagon levels (up to 9700 pg/ml) did not develop diabetes, whereas others with far lesser glucagon levels (as low as 120 pg/ml) presented with diabetes (77).
Nevertheless, an important pathogenetic aspect certainly lies within the relative concentrations of insulin and glucagon, which ultimately determine the net effect on hepatic glucose production. One research-article demonstrated the fact that hyperglucagonemia causes in pancreatic B cells a drop in

immunoreactive insulin content along with ultrastructural features of enhanced secretion and notably an increase in the amount of immature secretory granules, together with a decrease in mature ones (78). Also, some patients may present a pre-existing state of peripheral insulin resistance, as the high prevalence of DM type II in adults is well known. Of course, replacement of normal islands of Langerhans by tumoral tissues as well as the frequent presence of hepatic metastases, which could moderate the gluconeogenesis stimulated by high glucagon levels, will influence glucidic metabolism (79). The secretion of different molecular sizes of glucagon by the tumor with reduced biological activity, may explain varied effects on glucose metabolism (77,80). Concomitant secondary increases in hormones such as ACTH, VIP, somatostatin and human PP may contribute to the onset of diabetes (66). Finally, pancreatic malignant lesions and malignant disease in general have a well-known association with impaired glucose metabolism (81).

III. Weight loss

The hypercatabolic effect of glucagon and anorexia resulting from the tumor burden cause weight loss in almost all cases. A mean loss of 12-20 kg is reported, with a maximum of 30 kg (29,65). It is not associated with any pejorative prognosis, since it has been observed in benign as well as malignant alpha-cell tumors (54). Also the weight loss does not correlate with tumor size.

IV. Anemia

Many patients have a normochromic, normocytic (rarely micro- or macrocytic (54)) anemia at some stage in the disease, with a mean hemoglobin concentration of 9,4g/100ml (29). The fall in hemoglobin seems to correlate with advanced disease and severe skin involvement. Although some patients have low plasma iron concentrations, these improve on oral iron without normalisation of the hemoglobin (27).
The marked inhibitory effect on erythropoiesis by glucagon has already been discussed in a previous chapter on glucagon-physiology.

V. Thromboembolic complications

These disorders are far more common in patients with glucagonoma than in patients with other islet cell tumors, underlining the probable role of glucagon hormone in its pathogenesis, as we described in the chapter on glucagon-physiology. One group reported that in their experience over 50% of patients died from pulmonary embolism rather than from direct problems associated with tumor bulk (52); consequently, thromboembolism should be carefully searched for in all patients, and if

found, aggressively treated.
The most common problems are deep vein thrombosis and pulmonary embolus, but cerebral and renal artery thrombosis have also been reported (80).
Coagulation parameters are normal.

VI. Neuropsychiatric disorders

Alterations in mental status frequently accompany endocrine tumors and glucagonomas are no exception.
Depression is the most prevalent feature and whether it is specific to high glucagon levels or merely the result of a chronic wasting disease is not clear presently (55,133).
The rarely observed occurrence of scotoma, long before glucagonoma is associated with its syndrome, has been reported (80,82,84,85), and so it has been proposed that scotoma may be a key for earlier diagnosis of these pancreatic tumors by ophthalmologists (84). There exists an ophthalmological entity called the orogenital syndrome, caused by malnutrition, which includes scotoma and is associated with degeneration of the bipolar sensory neurons (84); hyperglucagonemia's catabolic state closely "imitates" a malnutrition (84).
Other disorders include personality changes and anxiety neurosis (51), disorientation, nervousness and insomnia (80). A few authors even described a neurologic paraneoplastic syndrome associated with glucagonoma patients (82,83,85). These reports overlapped in the following symptoms: decrease in cognitive functions (affecting for instance short-term memory, abstract thinking), reduced visual acuity (optic atrophy), ataxia and lower limb weakness and spasticity, associated with hyperreflexie. Various additional individual symptoms comprised nystagmus, abnormally labile affect, syncopic disturbance of consciousness, micturition difficulties and fecal incontinence. More reports will be needed to better define this possible paraneoplasic syndrome.
A possible role of glucagon in these neurological impairments was discussed in the chapter on glucagon-physiology. Cure of the underlying tumor results in a remission of most symptoms.

VII. Other features

Bacterial or fungal infections sometimes accompany glucagonoma, such as mucocutaneous infections of skin, gum, vulva and vagina, pneumonia, and sepsis (13,27,51).
Some of the cases with peptic ulcer and / or chronic diarrhea are associated with multiple hormone release (gastrin, VIP) by mixed cell populations within the tumor, or co-existence of other hormone-producing tumors (51).
The possible physiopathology of the rarely observed coarse intestinal folds has previously been discussed. Abdominal pain has no particular characteristic.

Table 6 lists all known clinical features of glucagonoma. Various reviews, as will be quickly noted by the reader, differ on the observed frequency of almost any given symptom, sometimes with great disparities. The problem is probably twofold: depending on the cases reviewed, not all the parameters (clinical and biochemical) were explored, because of lack of experience with the syndrome; furthermore, the 250 or so well-documented cases still are too few in number to allow us to give definitive information on the respective frequency of every symptom.
Biochemical findings and their frequency are discussed in the following chapter.

TABLE 6. Clinical manifestations of glucagonoma syndrome (29,51,52,54.56,66,122,127)

Necrolytic migratory erythema	67%	-	94%
Glucose intolerance / Diabetes	74,8%	-	90%
Weight loss	56%	-	96%
Anemia	42,6%	-	90%
Stomatitis / cheilitis	33,9%	-	67,5%
Glossitis	28,7%	-	67,5%
Thromboembolism	11%	-	50%
Neuropsychiatric disorders	5%	-	24,3%
Chronic diarrhea	14%	-	29%
Peptic ulcer	17,4%		
Various infections	14,8%		
Abdominal pain	12%		
Dystrophic nails	9,6%		
Nausea / vomiting	8,7%		
Coarse intestinal folds	7,8%		
Alopecie	5,2%		
Muscle atrophy	3.5%		

B. Biological Findings

I. Plasma glucagon

Diagnosis of glucagonoma syndrome can be easily made with radioimmunoassay of plasma glucagon. In normal subjects in the basal fasting state, plasma glucagon levels average 50-200 pg/ml. In one large review of glucagonomas, in only two patients was the plasma glucagon level 200 to 500 pg/ml, in four it was between 500 and 1000 pg/ml, and in 52 patients levels exceeded 1000 pg/ml (29). Another large study closely agrees with these results, showing a mean plasma glucagon concentration in 73 cases of 2110 pg/ml with a range of 550 to 6600 pg/ml, 30 percent being 550 to 1000 pg/ml, and the remainder > 1000 pg/ml (86).

The radioimmunoassay actually recognizes four IRG components, as discussed in the glucagon-physiology chapter, comprising: IRG 3500 (= true glucagon), IRG 9000 (proglucagon), IRG 2000 (degradation product of glucagon) and BPG.

Comparison of glucagonoma patients with normal subjects shows noteworthy differences in the respective plasma immunoreactivity percentages of these four entities, proglucagon being increased, whereas IRG 2000 and BPG share a lower percentage as can be seen on table 7 (29,65).

Secretion of prohormones having greater molecular weights than that of the normally secreted hormone is also observed in other islet-cell tumors. Maybe these tumors have a decreased capacity to convert prohormones to hormones as compared to normal endocrine tissue, or it may be that the turnover of hormones is so rapid that prohormones are secreted or released because of cell necrosis prior to conversion (65).

TABLE 7. Molecular heterogeneity of plasma glucagon immunoreactivity in normal individuals and in patients with glucagonoma syndrome.

Entity	**% plasma immunoreactivity**	
	Normal subjects (n = 8)	Glucagonoma patients
BPG	29	11.5 – 14.7
Proglucagon	17	41.5 – 50.1
Glucagon	37	24.9 – 47
IRG 2000	17	8 – 12.3

The biological activity of each molecular species does vary and could explain why the concentration of total glucagon measured in the plasma of these patients often does not correlate with their clinical or metabolic abnormalities.
Hyperglucagonemia is reported to occur in chronic renal insufficiency (87), diabetic ketoacidosis (88), prolonged starvation (89), acute trauma (90), severe burns (91), bacteremia or septicemia (92), hepatic insufficiency (93), and familial hyperglucagonemia (94). Plasma glucagon levels in these conditions do not exceed 500 pg/ml (29), except in cirrhosis of the liver, in which values of 1000 pg/ml or more have been reported (93). In the syndrome of familial hyperglucagonemia, where exceptionally plasma glucagon levels of > 1000 pg/ml have been reported, plasma fractionation indicates an excess in BPG only (94).
Provocative testings (for example with glucose, arginine, secretin, etc.) in search of repeatable secretory behaviour of glucagon from tumors do not provide reliable information and therefore are of little or no help in the diagnosis.

II. Plasma insulin

Plasma insulin levels are often normal or elevated, as long as the patient's insulin reserve is intact (27,66,86), thus partly compensating for the hyperglucagonemia and increased hepatic glucose production. However, no correlation between circulating plasma insulin and glucagon levels exists (86). One review reported increased insulin levels in 6 of 13 patients tested, ranging from 21-81 microU/ml (normal <20 microU/ml) (94).

III. Plasma amino acids

Plasma concentrations of amino acids are frequently less than 25% of normal, with glycogenic (alanine, glutamine) amino acids most affected, whereas branch-chain amino acids are reportedly less affected (95).
Amino acid levels often increase after tumor resection to above normal levels (55). Again, the reader is referred to the chapter on the physiology of glucagon for the physiopathology leading to hypoaminoacidemia.

IV. Other peptides secreted by glucagonoma

Many other peptides have been found to be elaborated by these tumors, most often without clinical significance. These include pancreatic polypeptide (in some series as many as 50% have high plasma pancreatic polypeptid levels), insulin, VIP, gastrin, calcitonin, adrenocorticotropic hormone, neurotensin and somatostatin (66).

V. Further laboratory findings

Hypocholesterolemia is often noticed, with a mean value of 130 mg/dl, the lowest reported value at 67 mg/l (29). Also hypoalbuminemia is frequently observed, with values that average 30g/l, the lowest value reported being at 18 g/l. Table 8 lists some more laboratory findings and summarizes all the ones we have described, with the respective frequency of occurrence (29,51,54,80).

TABLE 8. Laboratory findings

Entity	**Frequency (%)**
Hyperglucagonemia	99
Hypoaminoacidemia	32.2 – 97
Hypoalbuminemia	18.3 – 81
Hypocholesterolemia	27.2 – 80
PP hormone	50
Hyposiderinemia	36
Hypoproteinemia	13
Hyperinsulinemia	Rarely
Elevated ESR	Rarely
Hypozincemia	Rarely

C. Localisation of Tumors

Because most glucagonomas are malignant, it is important to attempt to localize the primary tumor and surgically resect it if it has not metastasized, or to establish the presence of metastases so that unnecessary surgery can be avoided.
Since most tumors are large (mean 5-10 cm) at the time of presentation, they can easily be detected by most imaging techniques.

I. CT scan and transabdominal US

Computed tomography scanning is the initial procedure of choice (21), because this study will detect 95% of primary pancreatic endocrine tumors more than 3cm in diameter and 95% of metastatic pancreatic endocrine tumors in the liver (96).

Other authors proposed transabdominal US as the first choice of noninvasive imagery, showing pancreatic tumors as a low echoic mass (51). But US does not always satisfactorely visualize the pancreas, especially in obese persons and patients after surgery of the upper abdomen, and is operator-dependant as far as the detection rate is concerned.

II. Arteriography

Some of the smaller primary tumors may not be detected by CT or US, and then selective coeliac and superior mesenteric arteriography is the procedure of choice (21,29,51,117). Glucagonomas are characteristically highly vascular and typically show a pronounced tumor “blush” (21); also displacement of major vessels can be shown. Arteriography has also proven to be more sensitive than CT in demonstrating small liver metastases (21).

III. Transhepatic portal venous sampling

When angiography has failed to localize the primary tumor in the presence of a biologically and clinically proven glucagonoma, then this technique is indicated (54). Percutaneous transhepatic approach to the portal vein has made selective catheterisation of the pancreatic veins feasible and allows localisation of pancreatic glucagonomas by hormone gradients: a 3-18-fold gradient between pancreatic efferent veins and peripherical veins have been observed (29).

This technique is not without complications (perforation of the gall bladder, hepatic hematomas) and so it is rarely used and fortunately not often required.

IV. Other more recent techniques

MRI has roughly the same detection rate as CT scan, but reportedly is superior in identifying small hepatic metastasis (51).

More recently, small glucagonoma tumors have been detected by endoscopic US and could have the highest rate of detection of pancreatic tumor (97,117,123).

Glucagonomas express somatostatin receptors in more than 80% of cases (119). A new method applies radiolabeled somatostatin analog (^{111}1n-octreotide, ^{123}1-Tyr-octreotide, ^{111}In-pentreotide) scintiscans for neuroendocrine tumors, with a detection sensitivity of 60-100% and rarely reported false-positive results (52,98,128,131). In addition metastatic spread is more likely to be detected by scintigraphic whole-body screening (115,118).

D. Diagnosis

Of course everything we have described so far allows the specialist to suspect the diagnosis of glucagonoma syndrome. We will not summarize here all the clinical and biochemical aspects which characterize the tumor, but propose a practical diagnostic work-up, based on the one proposed by Guenther Boden (99), which can be used by any medical doctor:

Diagnostic Work-up

Suspect the diagnosis in patients with
- unexplained chronic and therapy-resistant dermatitis, particularly if associated with thromboembolism and/or glucose intolerance and/or increased ESR, along with important weight loss (in spite of normal appetite)
- MEN 1 syndrome (familial or sporadic)
- known “asymptomatic” pancreatic tumor

▼
▼
▼

Biochemical diagnosis rests on the demonstration of
- elevated basal (overnight fast) plasma glucagon levels (highly suspicious if > 1000 pg/ml), and
- decreased plasma amino acid levels (highly suspicious if plasma levels of 1 or more glycogenic amino acids such as alanine, glycine, serine are < 25% of normal)

▼
▼
▼

Localization of tumor
- CT scan, ultrasound, selective angiography, somatostatin-receptor scintigraphy

E. Treatment

I. Surgery

Surgery will be indicated in the treatment of many glucagonoma patients. Vinik et al. even suggested that virtually all patients are candidates for some type of operative procedure, since these tumors are life threatening because of their hormonal secretion and their malignant potential (21). When the tumor is diagnosed early and thus often still localized to the pancreas, surgical resection will completely reverse all the clinical manifestations of the syndrome and result in a definitive cure. Unfortunately, complete cure will only be possible in less than 5% of cases (52). The great majority of patients present with tumors that are malignant at the time of diagnosis and regional or distant micro- or macroscopic metastases are already present (99). In these cases, noncurative surgical debulking may result in prolonged clinical remission, even though there is no evidence that this improves long-term survival (52). These tumors and their metastases are usually very slow-growing and their clinical symptomatology is mainly due to elevated hormonal plasma levels. If these can be reduced to less than half, clinical improvement usually follows (55). Dramatic responses follow both curative and major palliative surgery: the rapid post-operative fall in glucagonemia is accompanied with a rapid improvement of the dermatitis within 24 to 48 hours of operation (21).
There is also marked improvement in all the other symptoms within days (80). Besides controlling symptoms by reduction of hormone concentrations, the theoretical possibility exists that the beneficial effects of other therapeutic modalities can be enhanced by reducing the total tumor burden (80).
One group reported debulking procedures with partial pancreatectomy and splenectomy with or without resection of hepatic metastatic lesions in 12 patients (66). Four of the 12 had postoperative complications; the remaining 8 patients saw their serum glucagon levels decrease by a mean of 1442 pg/ml postoperatively (range: 214-8780 pg/ml). Of these, 6 patients (75%) had remission in NME, with complete resolution of the rash in 3 patients. Only 2 of the 8 patients have died, the other ones are stable, one of them now 38 months post-op (66).
Excision of a primary tumor with liver transplantation for metastatic disease confined to the liver have been attempted in 4 glucagonoma patients; one died following rejection but the other three are alive and disease free at 3 years (52). Liver transplantation for metastatic disease seems to be a possible therapeutic option for these patients, because metastases are limited, involving often the liver only, so that the usual problem of multiple metastases due to the immunosuppressed state as encountered in non-endocrine tumors seems to be of less concern. Guillausseau et al. reported in their review the various surgical interventions applied in 42 patients: left pancreatectomie was performed in 29 cases (69%) (glucagonomas are located to the left part of the pancreas in about two-thirds of the cases), a cephalic duodenopancreatectomy in 4 cases (9,5%), and a nearly total

pancreatectomy in 5 cases (11,9%). In 5 of these cases resection of hepatic metastases were also performed (29).
Preoperative management of glucagonoma patients is very important (21,66); they used to be poor operative risks because of the catabolic effects of glucagon (severe weight loss, hypoaminoacidemia, anemia, infected skin rash) and predilection for thromboembolism. In order to improve the patient's metabolic status, hyperalimentation for weight gain, adequate parenteral nutrition for correction of hypoaminoacidemia, transfusions for significant anemia, adequate control of diabetes, antibiotics, steroids and somatostatin analogues to improve the skin rash and prophylactic low-dose heparin treatment to prevent venous thrombosis are essential (21,66).

II. Hepatic artery embolisation

Hepatic artery embolisation is the therapeutic alternative to surgery. This procedure is very effective for symptomatic metastases because it decreases plasma glucagon and thus brings about a rapid remission of the rash and other symptoms or, if the patient suffers from right-upper-quadrant pain, it relieves dragging feeling of a very large metastases-filled liver (52,66). Again, this form of palliation seems not to be accompanied by survival benefits (100). The most serious complication of the procedure is the development of infections in the necrotic tumor; therefore, antibiotic coverage is obligatory (55).
This method has the advantage of being relatively noninvasive, performed under local anaesthesia, and repeatable if necessary.
Hepatic artery embolisation without major harm to the liver itself is possible and effective, because the liver derives only about 25% of its blood supply from the hepatic artery and 75% from the portal vein, and because most endocrine islet cell tumors are vascular with an arterial supply.

III. Antisecretory therapy and future radiation therapy

Boden et al. first showed in 1986 that somatostatin was beneficial in decreasing the symptoms and glucagon level in a patient with an inoperable recurrent glucagonoma (101).
Octreotide (= somatostatin analogue = SMS 201-995) has become very popular with many specialists concerned with neuroendocrine tumors (21,52,54,55,66,83,125,129,135) because of frequent spectacular responses and few and easily managed side effects such as occasional acute abdominal pain and distension, with or without diarrhea, during the first days of treatment (55); mild steatorrhea and some deterioration in glucose tolerance due to the concomitant inhibition of insulin release may occur with long-term treatment (55).
One article reviewed the use of octreotide in 43 cases (54), and reported a mean octreotide dosage of 300 microg/24h with 2 or 3 subcutaneous injections / 24h ; glucagon plasma levels returned within normal range in only 4 cases, but were reduced in 36. Cutaneous lesions improved in 27 cases

(62,8%), but no effect on tumor growth was observed (54,102).
Another team wrote that they used in their unit doses from a total of 150-1500 microg per day (52). In their experience the rash starts to improve within 48 hours and resolution occurs within 1 week. Chronic therapy causes the rash to occur with less frequency and less severity; in long-term therapies (years), however, the dose usually has to be increased as resistance to octreotide occurs. They found a total resistance occurring on the average after 2 years (long-term application without decrease in effect up to 3,5 year has been described (66)), with death following within 6 months or so (52). Often there is no correlation between octreotid's effect on glucagonemia on the one hand and improvement in the rash on the other: this strongly suggests a direct effect of octreotide on somatostatin receptors in the epidermis (52,83).
Octreotide generally also improves the symptoms of weight loss, abdominal pain and diarrhea, but not of diabetes mellitus (103).
Some patients do not respond at all to octreotide. Therefore the effects of this form of intervention need to be evaluated individually.
Andersson et al. with their research point toward a new use of octreotid for a potential future radiation therapy: they demonstrated that cultures of glucagonoma cells incubated with ^{111}InDTPA-D-Phe^{1}-octreotide actually bound them on their cell surface and even internalized them, both in the cytoplasm and nucleus (104,135). A recent paper talks of the profit to be gained in improving peptide analogs targeting other tumor-related receptors, such as glucagon-like peptide-1 (GLP-1) receptor. Known under the name peptide-receptor radionuclide therapy (PRRT), this could be a promising new treatment of glucagonomas (131).

IV. Chemotherapy

When surgery is not feasible, hepatic artery embolisation has been performed or is impossible because the portal vein is obstructed and symptoms become unresponsive to pharmacologic agents such as somatostatin, chemotherapy is indicated as palliation (55,66).
Murray-Lyon et al. first noted the potential of streptozotocin in treating malignant islet-cell tumor (105). Since then, Moertel et al. showed that 5-fluorouracil and streptozocin together are superior (rate of response 63%) in the treatment of islet cell carcinoma to streptozocin alone (rate of response 36%) (106,107). Renal toxicity can occur, so renal protein output must be carefully monitored. If output rises, streptozocin therapy must be discontinued; the dose must be considerably reduced when treating patients with impaired renal function.
Dacarbazine (=DTIC) can be considered the agent of choice for glucagonomas resistant to streptozocin (66,84). Another author advocated precedence of dacarbazine over fluorouracil and streptozocin (83); he moreover used alpha-interferon without being able to evaluate its therapeutic potential. Still another group reported dramatic clinical improvement of a patient with metastatic glucagonoma when treated with a combination of 5-fluorouracil and alpha-interferon (108).

One aspect of chemotherapy is certain; there is no exact schema applicable to every glucagonoma patient. The effective form and combination of palliative cytotoxic chemotherapy will have to be evaluated and decided empirically for each patient individually.

V. Symptomatic treatment

Symptomatic treatment of NME with topical and systemic antibiotics is only helpful when secondary infection is a problem. Steroids have been tried with only short-term beneficial effect. The rash sometimes responds to simple measures such as topical and oral zinc supplementation (52,55,74). Similarly, zinc coupled with EFA (essential fatty acid) infusion has been observed to be successful, as well as EFA infusions alone (73,74,121). Infusion of amino acids, high-protein diet and careful control of plasma glucose levels may also be helpful (52,55,73,121). Results will again be inconsistent.
Prophylactic measures and treatment of increased incidence of venous thrombosis and pulmonary embolus include aspirine, heparin and more chronically oral warfarin (52).

F. Prognosis

Patients with benign tumors where surgical cure is possible will enjoy a life expectancy similar to other ‘healthy” persons (117).
The prognosis for those patients with metastases at diagnosis is obviously less favourable, estimated by Wynick et al. to be around 2 1/2 to 3 years (52).
D. Grama et al. report a 50% survival at 16 months and 20% at 60 months from the time of diagnosis (109).
Wermers et al. in their single-institution experience of 21 patients, all of which had metastatic disease, reported death occurring in 9 of their patients at a remarkable average of 4,91 years after diagnosis (66). Twelve of their patients were still alive, with an average follow-up of 3,67 years. The longest survivor to be documented is a lady who has survived for 24 years since the diagnosis of her glucagonoma (absence of metastases at presentation) and for a further 18 years since she became symptomatic from presumed metastatic disease, considered unresectable (114).

6. Case report

We describe a patient with a large, locally resectable glucagon-producing tumor in the head of the pancreas who presented with a 4-year history of severe dermatitis (NME) prior to diagnosis. The patient, a 67-year-old white man at the time of surgery (November 1989), is the only known person so far in the entire Geneva area to have been identified as having a glucagonoma syndrome. Native of the United States of America, our patient retired from his activities as translator/administrator in 1987. He is the father of 3 children.
He had been smoking twenty a day the past 30 years, and no ethylism was noted. His past medical history was notable for repeated renal lithiasis and investigations in 1976 concerning a possible endocrine lesion of the pituitary gland (an enlarged pituitary fossa and hyperprolactinemia of 44,5 mg/ml had been observed). Family history was remarkable for the absence of diabetes and his parents had died from causes unrelated to the patient's present condition.

The patient's medical history begins in October, 1985, when for the first time he consulted the Department of Dermatology at the University Hospital of Geneva.
He had over the previous 2 weeks progressively developed a pruritic rash, which had started at the lower limbs to involve his entire body, with the exception of the face. He had never had before any dermatological complaints, did not suffer from asthma or hay fever, and was not aware of any allergy.
On physical examination the patient was noted to have lichenified lesions on the back of the feet and in the fold of the knees. The feet moreover were involved with desquamation of the sole and of the interdigital spaces. Scaly and erythematous lesions were noted on both thighs, around the inguinal folds and at the scrotum. The trunk presented with disseminated nummiform lesions. Excoriation, maculopapulor and erythematous scaly lesions characterized the medial part of the upper extremities. Eczema of unknown external cause was "diagnosed" and the patient sent home with a prescription including triclocarban, sulfadiazin and miconazol. Within the next 7 days the skin rash resolved almost completely. 3 weeks later the patient was back at the Department of Dermatology, with essentially the same rash and prurit. This cycle repeated again and again over the next 7 months with eruptions always starting gradually, at the lower extremities, subsequently involving most of the body and evolving over 2 - 3 weeks, interrupted by phases of almost complete remission which would last for 2 - 6 weeks. The patient's feet sometimes had a fissured aspect and an important edema of the lower limbs would be present. Various allergenes were searched for, but none could be incriminated as the cause for the dermatitis. Fungal cultures were all negative, except for one which yielded trichophyton, located in the interdigital spaces of the patient's feet.
Laboratory studies included measurement of levels of cyanocobalamine, folate and pyruvate, in

search of a deficiency disease, but levels were within normal range.
Further laboratory tests, comprising complete blood picture, blood chemistry, and coagulation parameters, were all within normal range, except for a hemoglobin concentration of 12,1g/100 ml and an hematocrit reading of 36,1 %, revealing a mild normochromic / normocytic anemia, and an erythrocyte sedimentation rate of 11 mm/hr.
A biopsy was performed which showed lesions constituted of mild epidermal hyperplasia and hyperkeratosis accompanied by irregular parakeratosis; the epidermis was moreover characterized by diffuse spongiosis along with an exocytosis of rare round cells from a mild perivascular infiltrate of lymphocytes and histiocytes. The diagnostic read: chronic eczema.

From June 1986 to January 1987 our patient did not suffer from the rash. Then much the same rash developed again; this time the dermatitis was accentuated on the genitals. The lesions were oozing, but neither vesicles nor pustules were observed. A few weeks later the rash underwent an almost complete remission which lasted for over a year, until the beginning of May 1988.
At this time he consulted a dermatologist in his private practice because of intense pain due to fissurated soles of his feet, which were highly incapacitating for the patient. Mycotic cultures yielded Trichophytum rubrum and the lesions resolved completely with topical antimicotic treatment (ciclopirox),
In August, 1988, the dreaded erythematous and scaly dermatitis recurred, resembling the rash he had had in the past, involving this time the left face also. Edema of the lower limbs was noted and investigations excluded venous insufficiency as the cause. Lesions improved with topical steroids, sulfadiazin and dexchlorpheniramin (anti-histaminic), but complete remission would not be achieved until the cause was discovered.
A trip to the United States of America ended with a consultation of an American dermatologist, because of aggravation of the rash, diagnosed intuitively as subcorneal pustular dermatosis of Sneddon-Wilkinson. Sulfones per os and intramuscular injections of steroids helped.
Back in Geneva a biopsy was performed again, revealing a similar histological aspect as the previous biopsies. Chronic eczema was diagnosed and the proposed clinical differential diagnosis included psoriasis, Sneddon-Wilkinson disease and parapsoriasis en plaques, the latter being the only one retained. Whole body PUVA-therapy was tried, without lasting success.

Hospitalization in a private clinic in Geneva was subsequently decided at the end of May 1989, because of the ever-present and now rapidly exacerbating rash, the patient’s cachectic state and emaciation. It was around this time that the patient considered suicide because of incapacitation, generalized prurit and pain, the latter being especially associated with fissurated soles of his feet. On physical examination important intertrigo of the groin and perianal area, erythematous patches on the lateral part of the nose and angular cheilitis were noted besides the classically affected and

previously described parts of his body.
A radiography of the thorax and abdominal and pelvic US were first performed, which revealed nothing noteworthy.
Unspecified impaired superior mental functions, mild polyneuropathy detected by electromyography studies and bloody stool led to the suspicion of a paraneoplastic syndrome, and, as a consequence, a possible tumor was searched for.
An abdominal CT scan showed a poly-lobulated retroperitoneal mass of 5x4 cm, located at the root of the mesentery, just below the head of the pancreas, interpreted as a group of adenopathies. The radiologist suggested a search for an underlying digestive neoplasma, but coloscopy and investigations of the small intestine were negative, as were the tumoral markers CEA and alpha-fetoprotein. Urological investigations equally ruled out a tumor in this area and a cranio-cerebral CT scan only yielded discrete signs of diffuse cortical - subcortical atrophia. Thus the idea of a tumor associated paraneoplastic syndrome was abandonned for the time being. In the field of infectious etiologies only tuberculosis was thought to possibly cause such an emaciated state, but cultures were negative and the rash did not correspond to an infection by Mycobacterium tuberculosis. One culture was positive for Candida albicans, located around the patient's mouth.
A further skin biopsy closely resembled the previous ones, with the addition of disseminated subcorneal necrotic keratinocytes; this histological aspect was identified this time as psoriasiform.
Laboratory tests included the following: hemoglobin level between 8.8 - 10.6 gm/100ml, hematocrit reading between 28,5 - 32.8 %, RBC between 3.3 - 3.87 mio/mm3, plasma iron level of 6.8 mcmol/l, an erythrocyte sedimentation rate of 56mm/hr, venous blood glucose at 6.23 mmol/l and total blood protein levels between 52 - 62 g/l. Laboratory values were notably normal for blood levels of cholesterol, triglycerides, zinc, liver function enzymes and coagulation parameters. The remaining laboratory tests were all within normal range.
Finally, the 'diagnosis" of some form of deficiency disease was agreed upon. This prompted the administration of plasma and a special diet rich in vitamins and proteins, all of which improved the patient's mental and cachectic state considerably. Unfortunately, hypoproteinemia and anemia were only slightly improved.
The rash rapidly decreased with an antimicotic per os and topical steroids. Our patient left the private clinic on June 2lst, 1989, and his long list of medication comprised multivitaminic supplements, magnesium, iron and ketoconazol, as well as triclocarban, chlorhexidin, sulfadiazin and corticoids to be locally applied.

Because of the patient's worsening general condition and a recurring widespread symmetrical eruption, his hospitalization in the University Hospital of Geneva was decided on August 29th, 1989. His feet and hands were erosive and fissured this time, which incapacitated him greatly. Moreover, a mildly atrophic and sore tongue of scrotal aspect was noted. A skin biopsy specimen

from the right armpit had the same features as the previous ones, with this time hypochromatic areas replacing parts of the granular layer and the occurrence of intra-cellular edema in the more superficial keratinocytes; this histological aspect was again interpreted as being rather specific for a deficiency disease, but the diagnosis of NME was also considered on the grounds of this biopsy.
The examining dermatologist proposed a differential diagnosis which included deficiency in zinc, vitamins B2 and B6, and glucagonoma syndrome!
The history taking by an internist revealed the following: considerable weight loss in the absence of a decrease in appetite; no insomnia; no visual or sensorimotor impairments; vesperal edema of lower extremities.
Examination revealed a thin, pale and chronically ill-appearing man in moderate distress. He presented with a weight of 59,3 kg (his ideal weight was around 73 kg), heart rate at 90/min and blood-pressure of 120/80 mmHg. He was apyretic and anicteric, and had no lymphadenopathy. Generalised amyotrophy was noted. The spleen and liver were not palpable, and no other unusual mass was felt.
Because of our patients “bradypsychic” behaviour he was seen by a neurologist who noticed impaired higher functions, manifested by a decrease in cognitive functions and diminished faculty to understand things. Cranial nerves were unaffected. There was evidence of symmetrically myotatic hyperreflexia of the extremities and a positive Hoffmann phenomena.
The patient tired easily during a subsequent neuropsychological set of tests and appeared “slowed down” when he was asked to handle a more complex task. He was described as having a certain lack of intellectual mobility, an impaired handwriting and an important decrease in his short-term memory.

Pertinent laboratory values

	At admission	Subsequent values
Blood glucose levels	5.4 mmol/l	A subsequent glucose tolerance test revealed a pathologic peak value of 11.7 mmol/l; other than that fasting glucose levels were always within normal range
Proteinemia	62 g/l	Lowest value on 6.10.89: 55g/l
Albuminemia	< 30 g/l	
Iron blood level	8 mcmol/l	
Folic acid	54.2 nmol/l (normal range: 7-39)	
Cyanocobalamin (vitamin B12)	153 pmol/l (normal range: 150-700)	This value increased steadily under polyvitaminic treatment
Vitamin B2	24.63 microkatal/l (normal value above 38 microkatal/l)	
Vitamin B1 and B6		Were shown to be within normal range
Zinc	8.5 mmol/l (normal range: 10-23 mmol/l)	
Erythrocyte sedimentation rate	39 mm/hr	
RBC count	3.5 Mio/mm3	Lowest value on 26.09.89: 2.8 Mio/mm3
Hemoglobin levels	9.5 gm/100ml	Lowest value on 25.09.89: 7.1 gm/100ml
Hematocrit reading	29.6 %	Lowest reading on 26.09.89: 23.9%
Reticulocyte count	6‰	Highest value on 2.10.89: 25%0
Normal WBC count and picture		
Normal urinary sediment		
Remaining tests were normal		

Thoracoabdominal whole-body scan showed a mass in the inferior part of the head of the pancreas, about 4 cm in diameter; needle biopsy was considered to be impossible in view of the anatomical localisation. No metastases were detected, particularly none in the liver.
Selective coeliac and superior mesenteric arteriography confirmed the presence of a hypervasculated tumor. A large-sized gastro-duodenal artery was noted, who's branches supplied the tumor, and the superior mesenteric artery presented with a very high blood flow of over 10 ml/sec.
A retrograde cholangiography showed hypertrophic folds in the mucosa of the duodenal bulbus and when biopsied revealed a heterotopic histology of gastric mucosal differentiation.
The pathologic *glucagonemia of over 800 pg/ml* firmly established the diagnosis of a glucagonoma tumor, associated with the syndrome.
In view of the patient's past history of a possible endocrine lesion of the pituitary gland, the diagnostic possibility of a MEN 1 (multiple endocrine neoplasia) was considered; a normal prolactinemia of 8.7 ng/ml and an unchanged enlarged pituitary fossa excluded this.
On September 22nd, 1989, an antisecretory therapy with subcutaneous injections of octreotide (0,05 mg) twice a day was started which resulted in a very rapid disappearance of the patient's entire rash, who then could walk again.
Improved diet and hypervitaminic supplements resulted in some weight gain and improved mental state.

On October 6th our patient took a leave from his hospital stay to visit with his family prior to the decided surgery, only to be readmitted again on October 24th 1989 because of diarrhea and the occurrence of important swellings of both lower limbs, due to extensive bilateral deep vein thrombosis, as seen with phlebography. A prothrombin time of 65 % and hyperfibrinogenemia of *5* gram (normal range: 2 - 4 grams) were observed and an anticoagulatory treatment with heparin started.
At admission the patient complained about recurrence of his rash, which was probably due to his non-compliance with the octreotide-injections and the administration of 0.1 mg of octreotide twice a day had again the same spectacular effect on his dermatitis.
Laboratory tests showed similar values as the ones previously observed and are therefore not reported here. Analysis of amino acid levels showed a remarkable panhypoaminoacidemia, the chronological development of which is reported in table 9.
During neuropsychological examinations by the same team as before our patient was described as having an improvement of his higher functions, namely a better short-term memory, greater perseverance and success when given a complex task, more vigorous in his actions and a close to normal handwriting.

Because of important thromboembolic risks - due to the tumor itself and also the post-thrombotic status - rapid surgical excision of the tumor was decided and the patient transferred to the department of digestive surgery on November 21st, 1989, where he had a filter (of Guenther, 30mm) placed in his vena cava inferior.

Table 9: Time course of the patient's amino acid levels

(glycogenic amino acids are italicized)

	29.08.89	25.10.89	17.11.89	11.12.89	14.03.90	09.10.90	Normal range mcmol/l
	Prior to surgery			After surgery			
Taurine	110	25	-	51	75	41	32-104
Aspartate	32	-	-	-	-	-	0-6
Threonine	45	-	26	-	170	187	100-168
Serine	166	-	41	-	152	141	67-193
Asparagine	-	-	-	-	69	49	196-628
Glutamic acid	118	-	-	241	23	47	27-88
Glutamine	118	170	233	-	1328	747	420-760
Proline	-	-	-	-	184	391	147-286
Glycine	232	59	161	215	379	295	173-295
Alanine	224	67	44	121	461	550	258-415
Citruline	-	-	-	-	45	34	11-45
Valine	117	87	86	119	185	283	196-316
Cystine	-	55	57	-	106	101	31-140
Methionine	-	-	-	traces	19	29	13-39
Isoleucine	36	18	17	39	58	82	45-99
Leucine	93	48	28	64	96	153	71-175
Tyrosine	24	13	-	31	40	83	33-82
Phenylalanine	110	31	18	35	39	61	42-212
Histidine	60	20	30	56	81	91	65-130
Tryptophane	-	-	-	25	54	43	
Ornitine	-	-	15	83	99	109	45-90
Lysine	134	79	93	45	190	249	143-205
Arginine	138	20	-	88	-	75	57-137

The intervention consisted of a classical cephalic duodeno-pancreatectomy, without complications. There was no evidence of metastasis, neither in the peripancreatic lymph nodes, not in the liver. Gross appearance of the resected tissue showed an encapsuled egg-shaped tumor of elastic consistency, 3.3 x 2.5 cm. Sections of the tumor area revealed a yellowish-beige tissue with a few anastomosing whitish ribbons and some hemorrhagic zones which were partly cystic. Closeby a cyst with a mucoid content was found, mesuring 15 x 9 x 3 mm. The surrounding pancreatic parenchyma had a lobulation of normal aspect, macroscopically.
Microscopically, polygonal cells formed large strands and ribbons which were often anastomosing with pseudo-cystic structures. Individual cells were moderately eosinophilic, polygonal and often voluminous, with a delicately granular and occasionally vesiculous cytoplasma. Nuclei varied a great deal in size, had bizarre and atypical forms (sometimes gigantics), and rare mitoses were observed. The tumor was well circumscribed by a fibrous capsule which in some areas, was infiltrated by tumor nests that still respected the capsule. Parenchymatous tissue immediately adjacent to the tumor had an atrophic aspect while the rest of the pancreatic parenchyma appeared to be normal. Immunohistochemistry showed cells that stained moderately to strongly for chromogranin, VIP, and glucagon, establishing without any doubt a pluri-hormonal-secreting glucagonoma tumor of the head of the pancreas.

The patient experienced no post-operative complications at all and recovered well during the subsequent 6 week hospital stay prior to going back home.
Shortly after his surgery the glucagon blood value read 146 pg/ml (cf. table 10). All the other laboratory values were within normal range, except for 9 amino acids which were still moderately reduced (cf. table 9). Our patient weighed 60,5 kg when he left hospital and reached his ideal weight by the end of l990 (cf. table 11).
A check-up in March 1990 revealed nothing noteworthy except for a surprising and unexplained glucagonemia of 433 pg/ml and 4 amino acids still slightly reduced.
The following check-up in October 1990 showed a glucagon level of 344 pg/ml and only 1 amino acid (asparagine) still below normal range.

An overall control in 1992 revealed a man in good health, weighing his 73 kg and having a glucagon blood level of 59.24 pg/ml. An abdominal CT scan showed no sign at all of a recurrent tumor. Since the surgery our patient has never had a recurrence of NME (information obtained through personal visit by the author).

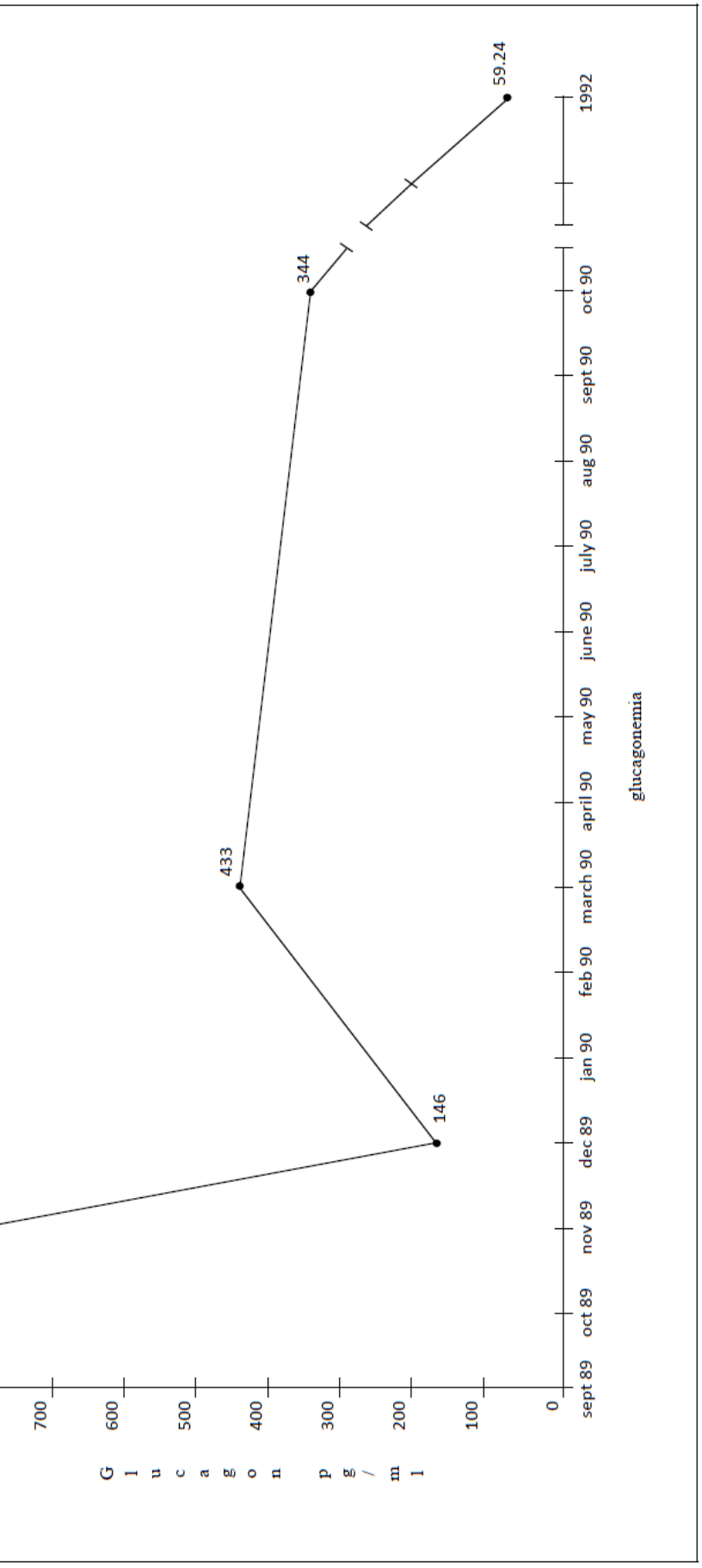
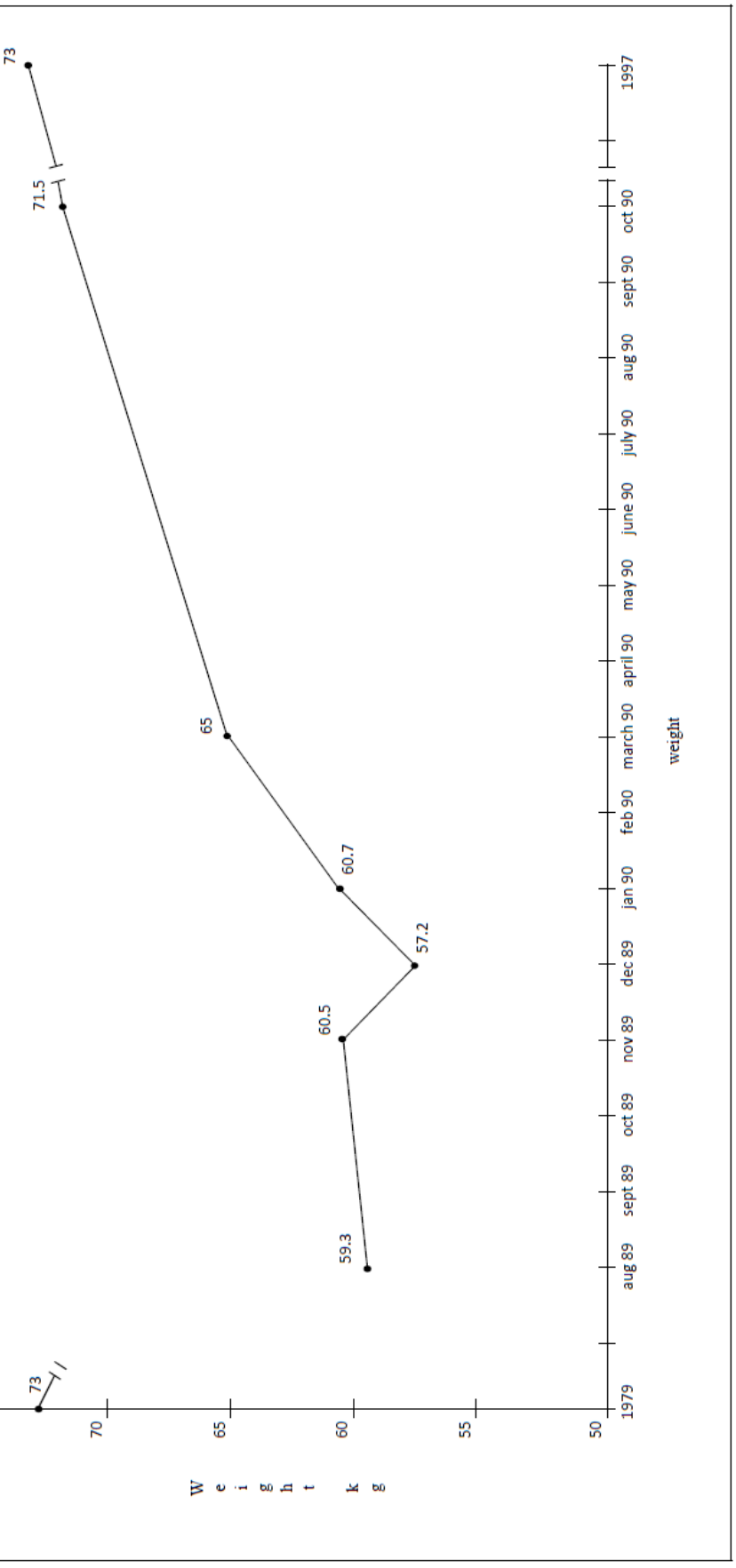

Glucagon pg/ml
700
600
500
400
300
200
100
0
sept 89
oct 89
nov 89
dec 89
jan 90
feb 90
march 90
april 90
may 90
june 90
july 90
aug 90
sept 90
oct 90
1992
146
433
344
59.24
glucagonemia

Table 10


Weight kg
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65
60
55
50
73
71.5
65
60.7
57.2
60.5
59.3
73
1979
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sept 89
oct 89
nov 89
dec 89
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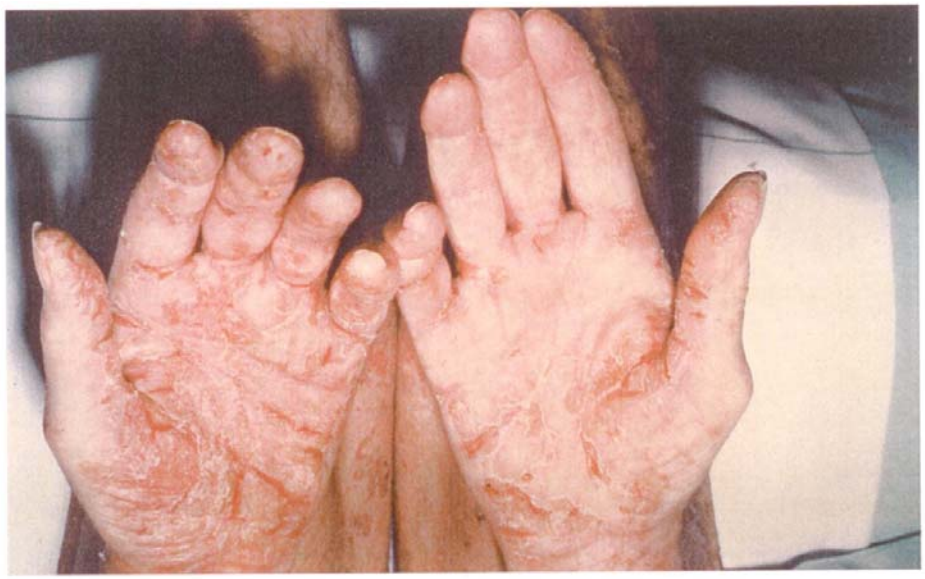
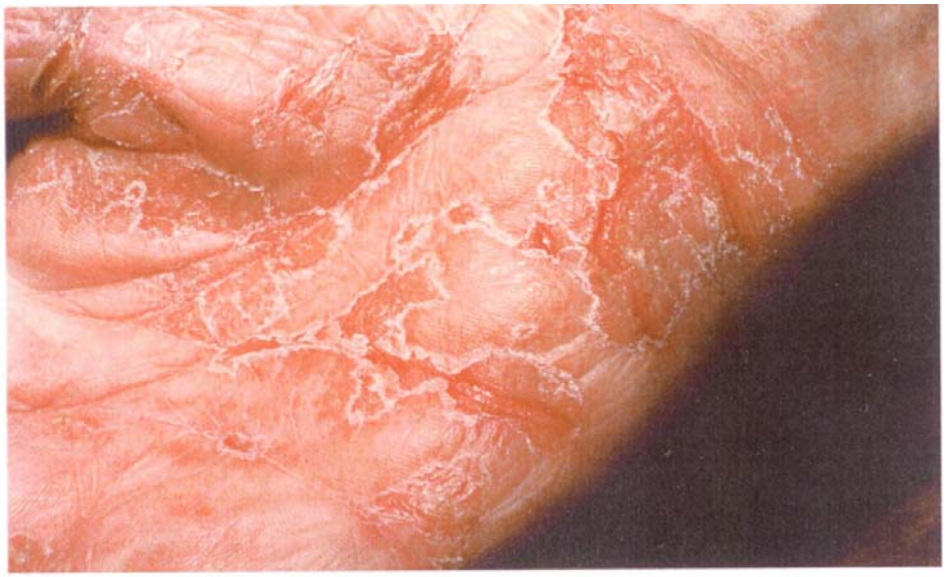
july 90
aug 90
sept 90
oct 90
1997
weight

Table 11

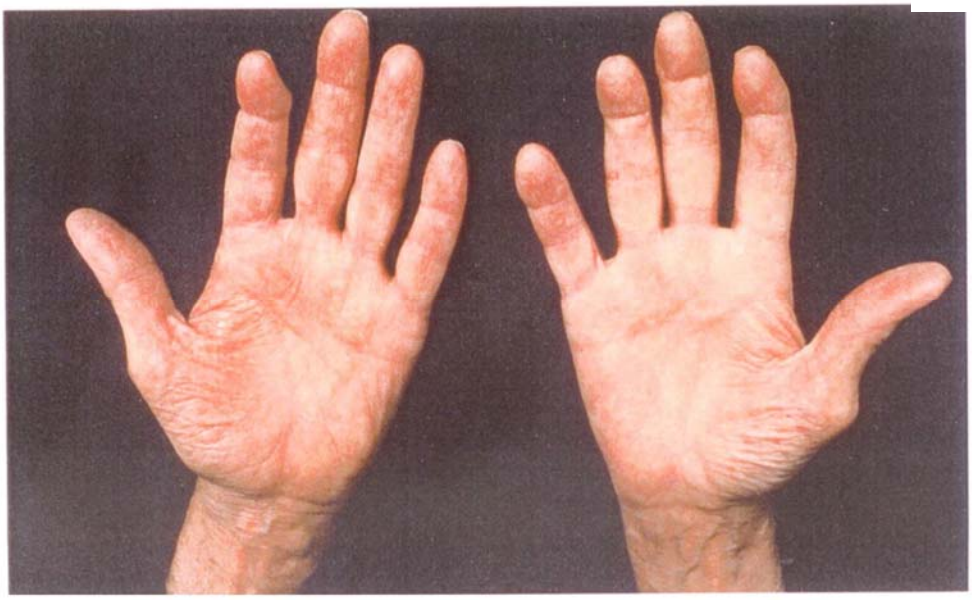


(a)

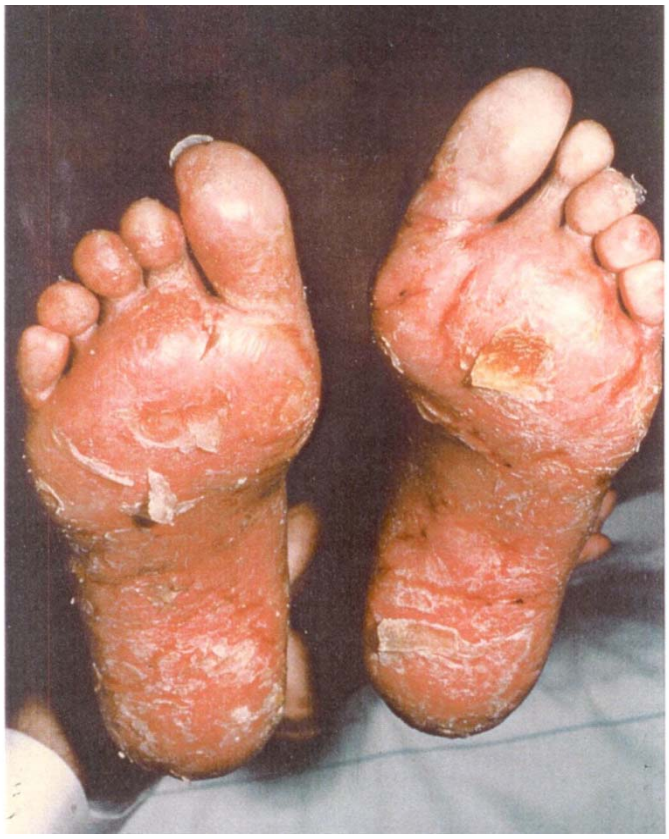


(b)

(c)

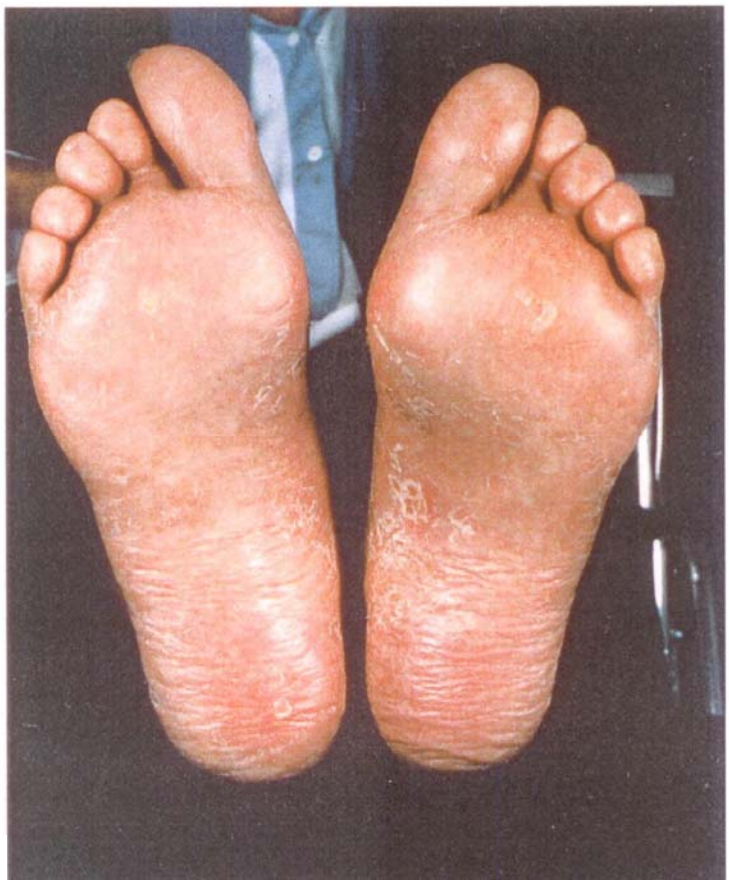


Skin rash before (a) (b) and after surgery (c). Note the excoriated aspect and the deep fissures (b).

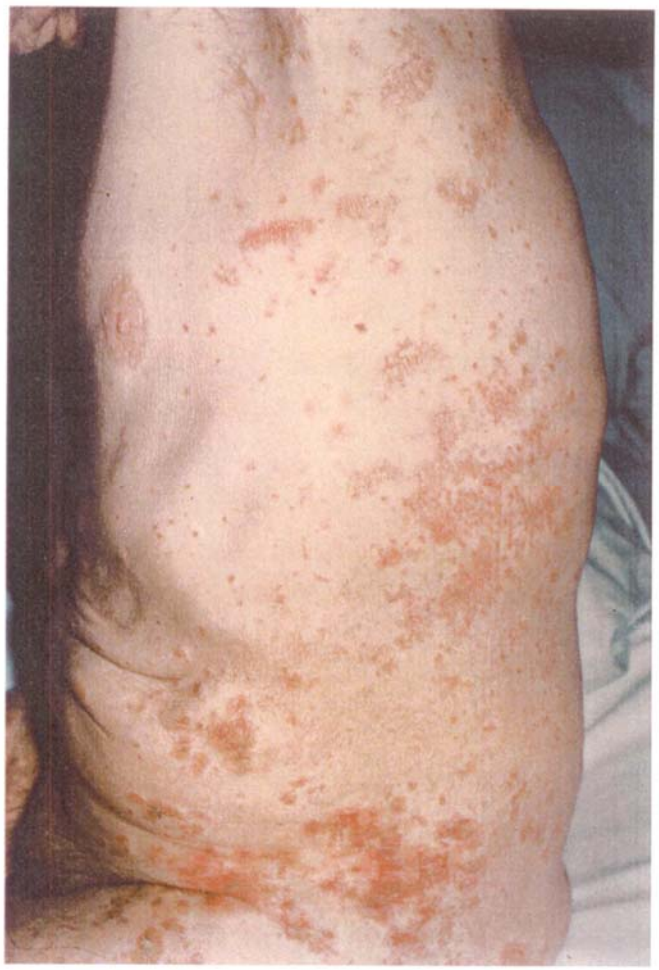


Skin rash of the patient before (a) and after resection of the glucagonoma (b). Note the erosive and fissured aspect along with desquamation of the sole and interdigital spaces.

(a)

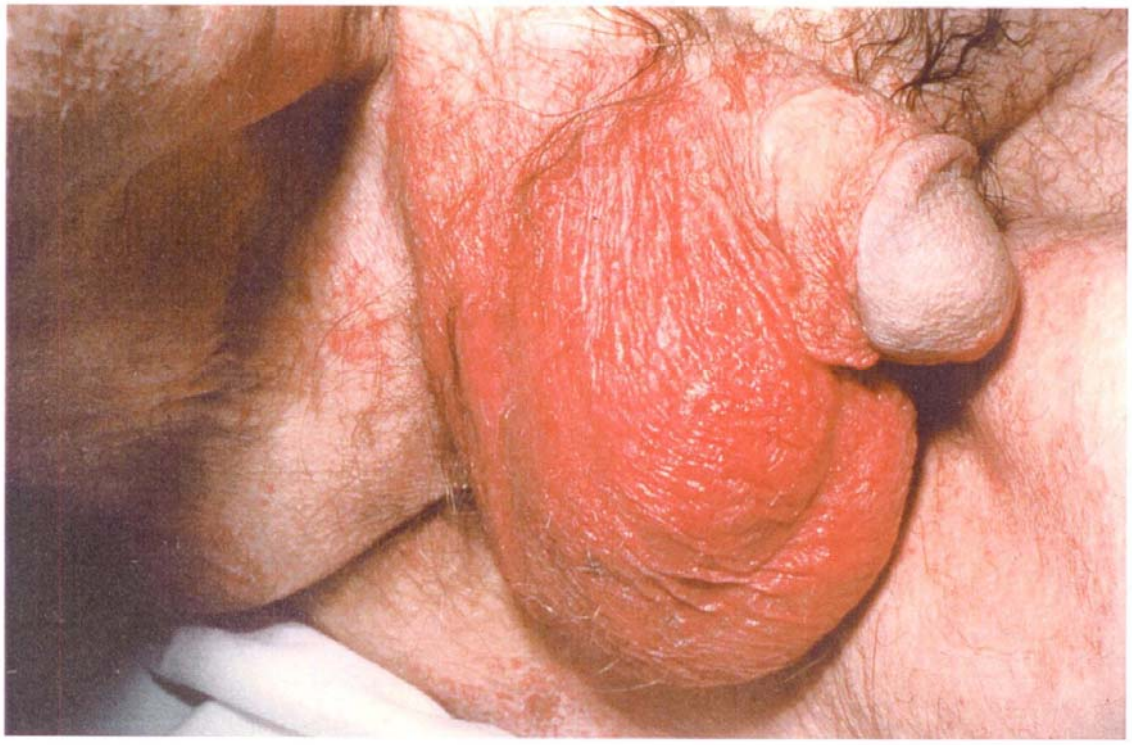


(b)



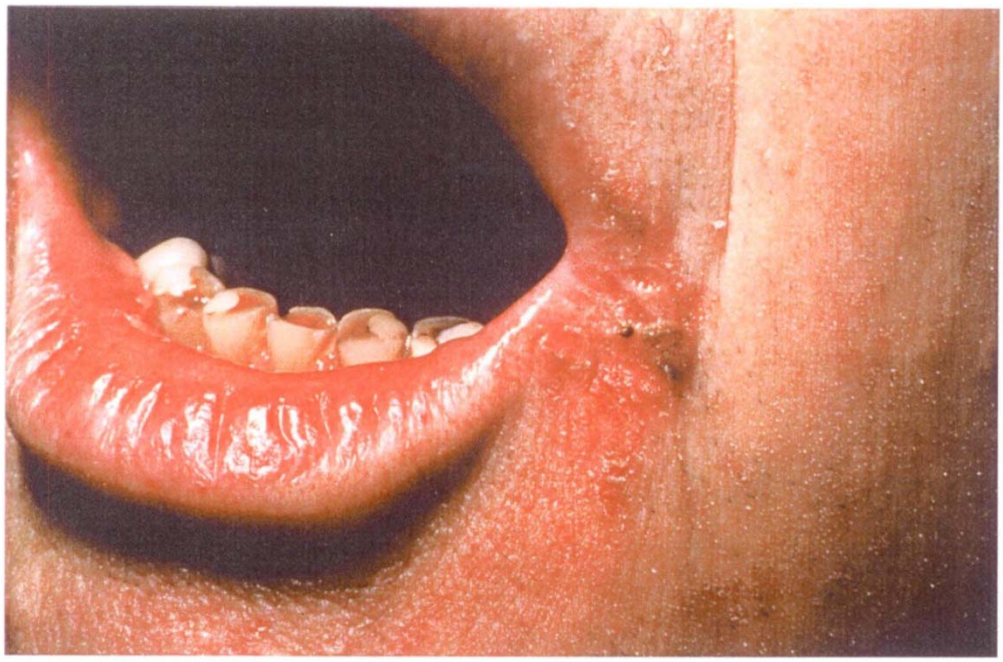
(a)

Lateral view of chest and abdomen of the patient (a). Large area of confluent lesions in various stages of development.

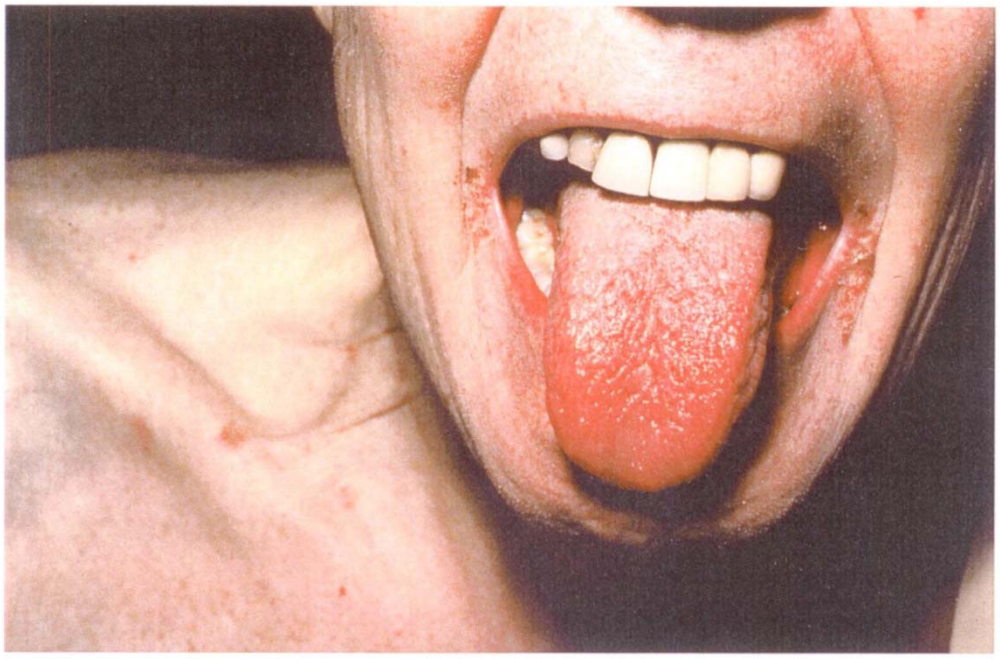


(b)

Accentuation of the dermatitis on the genitals (b)

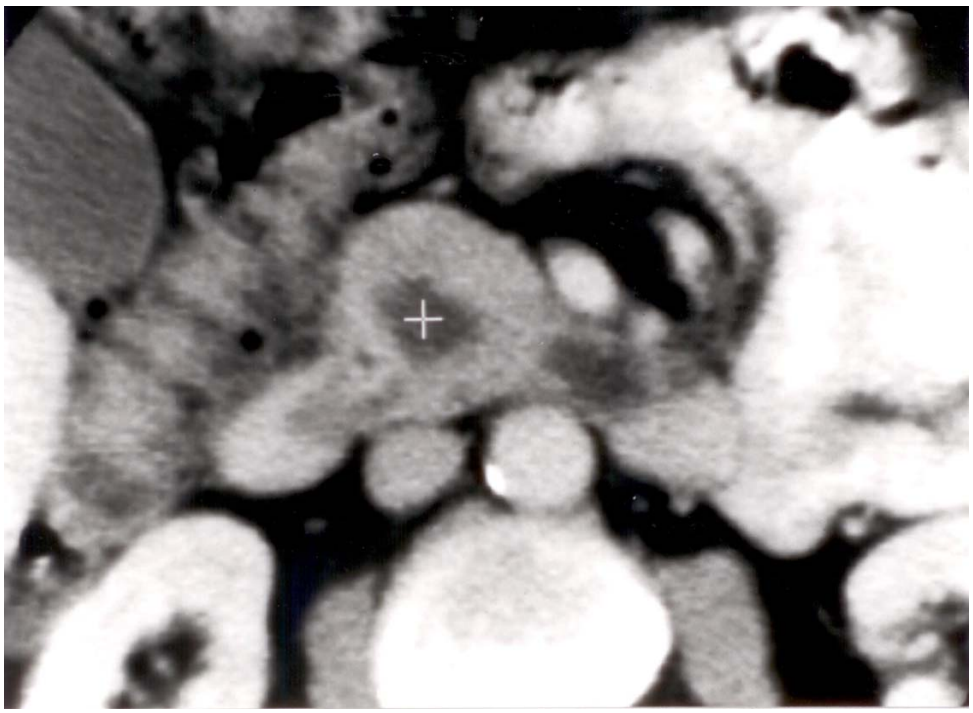


(a)



(b)

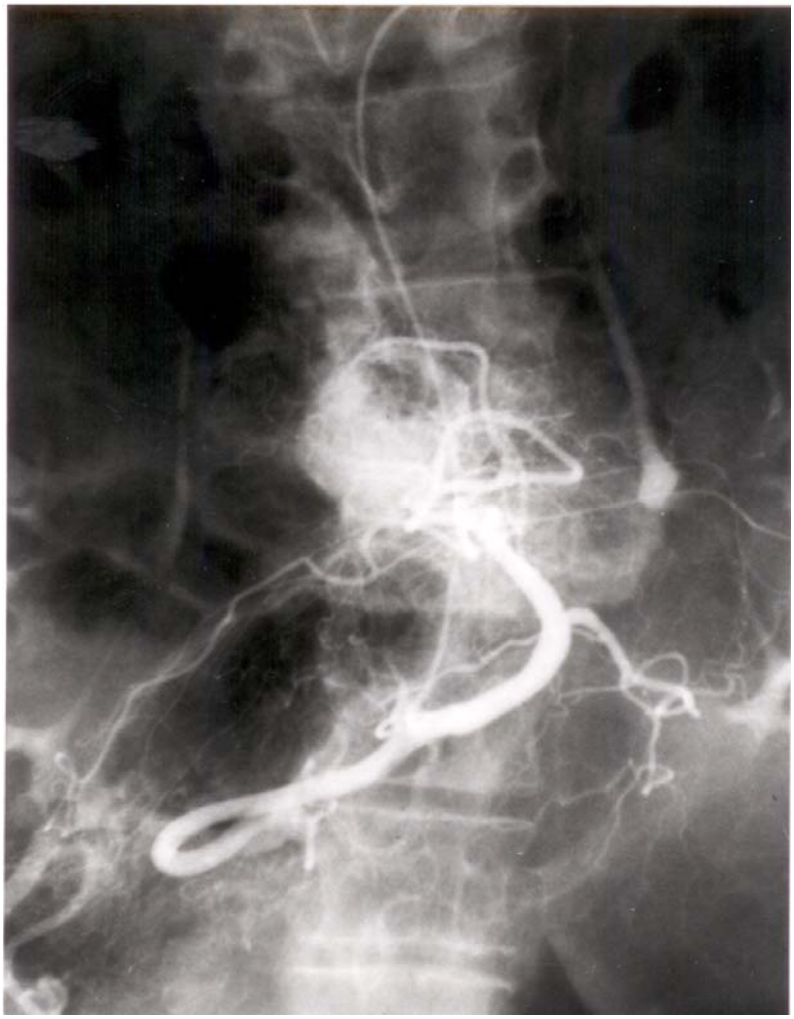
Angular cheilitis (a).
Mildly atrophic and sore tongue of scrotal aspect with preservation of papillae (b).



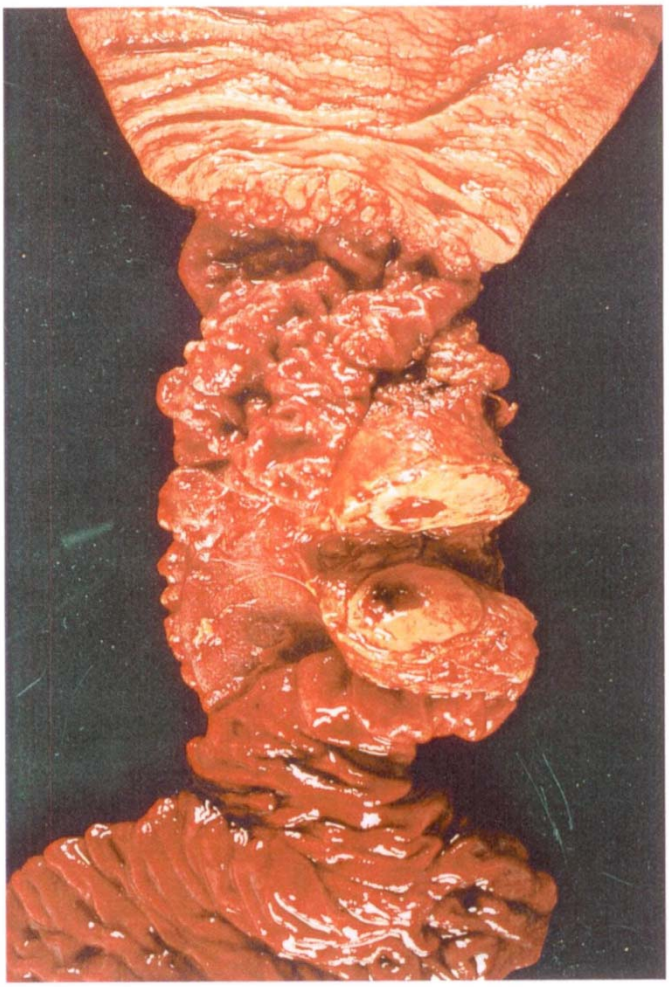
CT scan showing a cystic mass in the inferior part of the head of the pancreas (a).

(a)

Selective coeliac and superior mesenteric arteriography showing the presence of a hypervasculated tumor (tumor blush) (b).

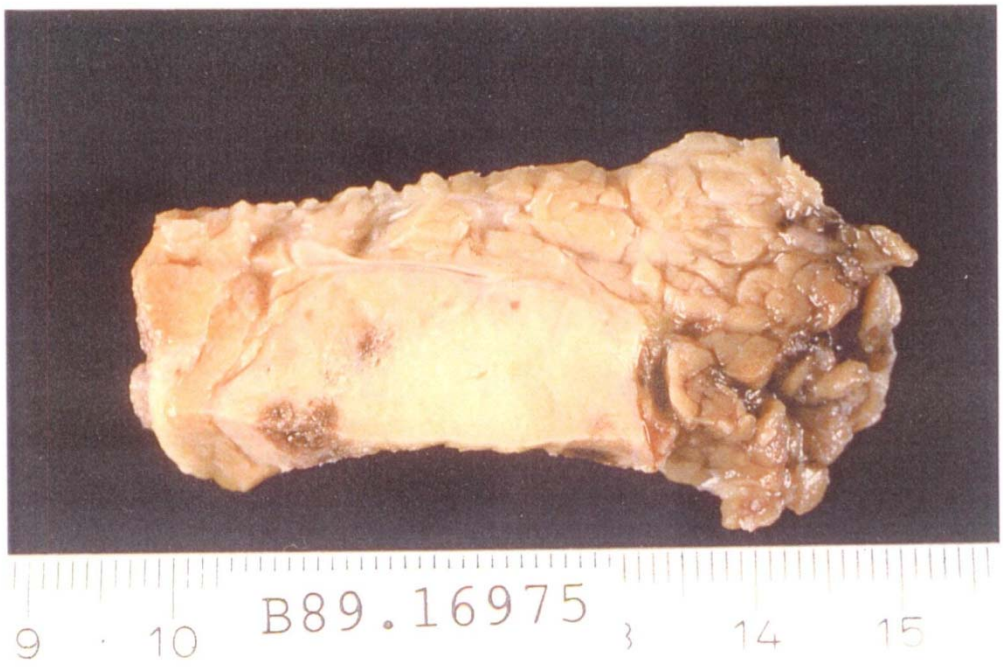


(b)

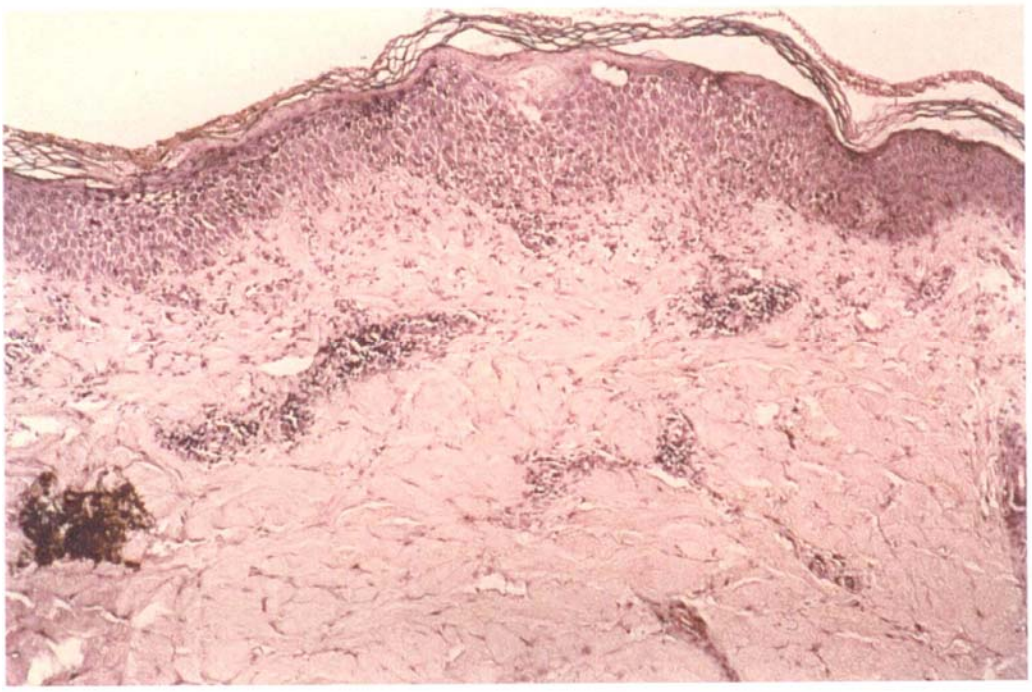


(a)

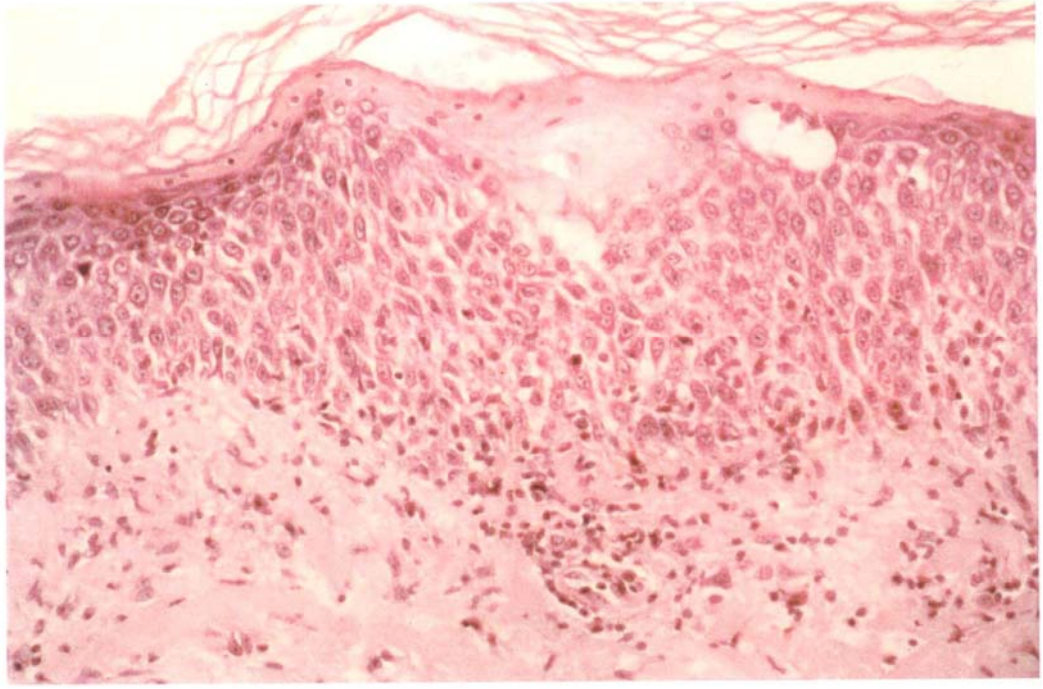
Cut-surface of the tumor (a) (b).



(b)



(a)



(b)

Biopsy specimen of same lesion (a) (b), showing mild epidermal hyperplasia and hyperkeratosis accompanied by irregular parakeratosis. Moreover diffuse spongiosis along with mild epidermal perivascural infiltrate of lymphocytes and histiocytes. Hypochromatic areas replacing parts of the granular layer.

7. Discussion and conclusion

A. Discussion

The glucagonoma syndrome is a striking manifestation of a rare neoplastic disease. The typical glucagonoma patient is a middle-aged person with mild diabetes, who complains above all of a pruritic and recurrent skin rash.

Our patient presented with a fairly long 4-year history of severe dermatitis, which had the following particularities. The rash would always start at the lower limbs, only to be followed by the involvement of most of his body, first sparing his face, 3 years later also severely attacking his face and tongue. The lesions in general had the tendency to become *more and more severe* in the course of time. First his feet would only have lichenified lesions accompanied by diffuse desquamation, but later in the course of his dermatitis he would suffer from terrible pain due to fissurated soles, which proved to be so intense and incapacitating as to have him consider suicide. Edema of lower limbs wasn't present from the beginning, then gradually appeared and became more and more important. First lesions were erythematous and scaly, only to become oozing.
The dermatitis evolved in a rather classical fashion: the rash would evolve over 2-3 weeks with some of the characteristic changes, interrupted by phases of remission lasting between 2-3 weeks. But on two occasions he experienced unexplained and unusual remission, lasting respectively 8 months and over a year. The multiple biopsies performed were unfortunately never taken from the edge of fresh lesions, showing a not helpful uncharacteristic histopathologic picture, which confirms the reports that stress the importance of taking biopsies from fresh lesions.
The diagnosis most often advanced was eczema, the proposed differential diagnosis including psoriasis, Sneddon-Wilkinson disease, parapsoriasis en plaques and some kind of deficiency disease. As Stacpoole pointed out, the rash may be classic for NME or may appear similar to any one of a number of more common dermatoses (86). The latter seems to have been partly the case for our patient, delaying the diagnosis. But still, the typical cycle of occurrence and remission, non-responsive to any treatment, seen in the context of the other symptoms, should have allowed a quicker diagnosis, which demonstrates the importance of detailed considerations of all complaints.

Our patient did not suffer from diabetes mellitus. But he presented a pathologic glucose tolerance test with a peak value of 11.7 mmol/l; other than that fasting glucose levels were always within normal range.
The important weight loss occurring in most cases was present too (13 kg), which helped to motivate further investigations.
The patient presented also the typical normochromic, normocytic anemia, with low plasma iron

concentrations. Iron administrations did not improve the anemic state.
Moreover he suffered from the condition which might be responsible for up to 50% of death causes due to glucagonomas, namely thromboembolic complications. He had extensive bilateral deep vein thrombosis, motivating the introduction of a filter of Guenther in VCI and quick surgery of the tumor, for fear of deadly embols.
Due to his tumor, he experienced what has been called a “neurologic paraneoplastic syndrome” (82, 83,85,133): decrease in cognitive functions (diminished faculty to understand things, lack of intellectual mobility, reduced short-term memory), impaired handwriting, lower limb weakness and myotatic hyperreflexia of extremities. All of these disorders proved to be reversible.
Some of the other features encountered by our case were fungal skin superinfections, diarrhea, hypertrophic folds in duodenal mucosa and generalized amyotrophy.

Now let us turn to the biological findings. Hyperglucagonemia must be present to confirm the diagnosis. Our case had a relatively low plasma glucagon level of 800 pg/ml, but still presented almost all of the clinical and metabolic abnormalities, confirming the absence of correlation between high glucagon concentration and severity of symptoms.
Due to the absence of diabetes, plasma insulin levels were not analyzed.
He presented a very characteristic and almost complete panhypoaminoacidemia, with some amino acids less than 25% of normal, particularly the glycogenic ones (alanine, glutamine, glycine, serine). Our case further confirmed the following laboratory findings as part of the syndrome: hypoalbuminemia, hyposiderinemia, hypoproteinemia, discrete hypozincemia and elevated ESR.

Localisation methods in search of the tumor confirmed computed tomography scanning as the initial procedure of choice. An abdominal US which had been performed prior to the scanner had failed to show any unusual mass, because the pancreas could not be visualized. Selective coeliac and superior mesenteric arteriography revealed the typical tumor “blush”.

As soon as the diagnosis was established, subcutanous injections of octreotide were started, which resulted in a spectacular and very rapid disappearence of the patient’s entire rash. Continuation of this antisecretory therapy on a long term basis was discussed, if confronted with the impossibility of surgery. Also improved diet and hypervitaminic supplements resulted in some weight gain and improved mental state. The apparent absence of metastasis made surgery a very promising option. The result was a definitive cure. Microscopically the resected tissue ressembled descriptions of glucagonoma tumor histopathology. lmmunohistochemistry of the resected tissue showed what seems to be most often the case: a pluri-hormonal-secreting endocrine tumor.

The post-operative evolution was very favourable, without any complications. He has never experienced a recurrence of NME, has reached his ideal weight again, has not had any thromboembolic complication since and dos not suffer anymore of any of the many neuropsychiatric

disorders he had had.
The time course of his fasting plasma glucagon level was surprising and cannot be explained. After an initial drop of glucagonemia in the immediate post-operative period within normal range, he all of a sudden had again plasma glucagon values which were too high. No tumor recidive could be discovered nor were there any symptoms associated. Within a few months his glucagon plasma levels normalized again.
All the laboratory values normalized quickly, with notably a gradual normalization of all amino acids.

The patient lived again a disease-free life. He died of causes incident to old age in 2008, nineteen years after his surgery.

B. Conclusion

Cancer of the exocrine pancreas constitutes the most prevalent tumor of the pancreas, responsible for 95 % of all tumors (exocrine and endocrine). The remaining 5 % are represented by neuroendocrine tumors, which considered together are less malignant than the tumors arising from the exocrine pancreas, but are accompanied by “unusual” clinical features associated to hormonal secretion, helping in establishing diagnosis. Insulinomas are the most common pancreatic endocrine tumors, followed in order of decreasing frequency by nonfunctioning endocrine tumors, gastrinomas and VIPomas. Then follow glucagonomas, with an estimated annual incidence of 1 in 20 million per year. Glucagonoma syndrome is a rare disorder, but very characteristic and thus recognizable by its typical clinical presentation. You should suspect the diagnosis in patients with unexplained recurrent and therapy-resistant dermatitis, particularly if associated with mild diabetes (or glucose intolerance) and/or thromboembolism, along with important weight loss. Neuropsychiatric disorders may be present too. Your laboratory findings will include a normochronic, normocytic anemia- with low plasma iron concentrations- which does not improve on oral iron. You may also encounter hypoalbuminemia, hypocholesterolemia, hypoproteinemia and an elevated ESR. Diagnosis rests on the demonstration of elevated plasma glucagon levels, decreased plasma amino acid levels and a histopathological study of tumor tissue.
Because of the slow growth of these tumors and the clinical symptoms above all associated to hormonal secretion and not to the tumor bulk, you can, if you make the diagnosis early and in the absence of metastasis, completely heal your patient. If surgery is not possible, palliative methods have proven to be quite satisfactorily in reducing effects of hormone secretion.

Our experience confirms other reports: a patient with a hormone - secreting glucagonoma tumor will most probably present the striking manifestations of its syndrome. Our severely afflicted patient had

all of the major features of the glucagonoma syndrome. Four years after the initial symptoms he finally underwent surgery, and thus was completely cured in the absence of metastasis. Our experience also confirms the often spectacular effect of subcutaneous octreotide injections in reducing NME.

Future research and applications centre around the somatostatin analog octreotide: a new method, already applied in specialized centres, uses radiolabeled somatostatin analog scintiscans with a high detection sensitivity.
A potential future radiation therapy uses specially prepared octreotide molecules which are bound and even internalized by glucagonoma cells. In order for radiolabeled somatostatin receptor-targeting peptides to be efficacious, the tumor needs to have a high immunopositivity for somatostatin receptors. A very recent paper talks of the profit to be gained in improving peptide analogs targeting other tumor-related receptors, such as glucagon-like peptide-1 (GLP-1) receptor. Thus peptide-receptor radionuclide therapy (PRRT) could be a promising new treatment of glucagonomas (131).

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