Subcellular structures secreting protein hormones

We turn our attention now to the very large class of proteins that are synthesized and sorted in the secretory pathway (Figure-1). Once the ribosomes synthesizing these proteins become bound to the rough endoplasmic reticulum, the proteins enter or cross the endoplasmic reticulum (ER)membrane*cotranslationally* — that is, during their synthesis. Soluble proteins in this class first are localized in the ER lumen and subsequently are sorted to the lumen of other organelles or are secreted from the cell. Likewise, the integral membrane proteins in this class initially are inserted into the rough ER membrane during their synthesis; some remain there, but many eventually become localized to the plasma membrane or membranes of the smooth ER, Golgi complex, lysosomes, or endosomes. The rough ER is an extensive interconnected series of flattened sacs, generally lying in layers. When cells are homogenized, the rough ER breaks up into small closed vesicles, termed *rough microsomes,* with the same orientation (ribosomes on the outside) as that found in the intact cell. The simple experiment shows that immediately after their synthesis secretory proteins are localized in the lumen of ER vesicles, although they have been synthesized on ribosomes bound to the cytosolic face of the ER membrane.

Many important experiments on the secretory pathway take advantage of cells that are specialized for the secretion of specific proteins. These cells contain organelles such as the rough ER and Golgi cisternae in abundance. For example, of the total protein made by hepatocytes (the principal cells of the liver), about 70 percent consists of proteins, such as albumin and transferrin, that are secreted into the blood. Likewise, pancreatic acinar cells synthesize several digestive enzymes that are packaged into zymogen vesicles and secreted into ductules that lead to the intestine. All cells, however, secrete *some* proteins. Extracellular matrix proteins such as collagens, proteoglycans, and fibronectin, for example, constitute about 5 percent of the protein made by most cultured cells. All eukaryotic cells use essentially the same pathway for synthesis and sorting of secretory proteins.

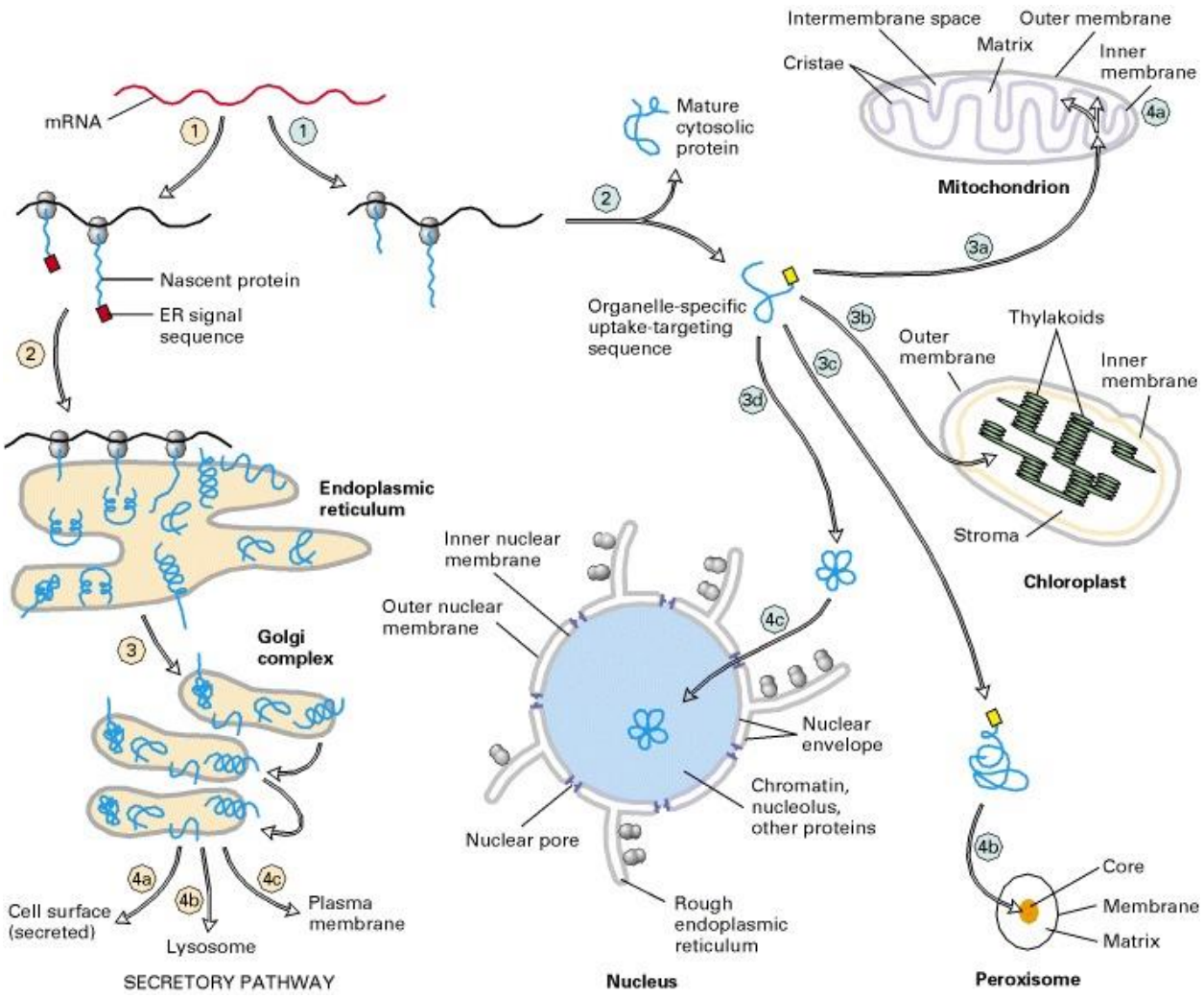

Figure-1Overview of sorting of nuclear-encoded proteins in eukaryotic cells

All nuclear-encoded mRNAs are translated on cytosolic ribosomes. Ribosomes synthesizing nascent proteins in the secretory pathway **1** are directed to the rough endoplasmic reticulum (ER) by an ER signal sequence **2**. After translation is completed in the ER, these proteins move via transport vesicles to the Golgi complex from where they are further sorted to several destinations **4a**, **4b**, **4c** . After synthesis of proteins lacking an ER signal sequence is completed on free ribosomes 1s, the proteins are released into the cytosol **2** . Those with an organelle-specific uptake-targeting sequence are imported into the mitochondrion **3a** , chloroplast **3b** , peroxisome **3c** , or nucleus **3d** . Mitochondrial and chloroplast proteins typically pass through the outer and inner membranes to enter the matrix or stromal space, respectively. Some remain there, and some 4a are sorted to other organellar compartments. Unlike mitochondrial and chloroplast proteins, which are imported in a partially unfolded form, most peroxisomal proteins cross the peroxisome membrane as fully folded proteins 4b.

Secretory Proteins Move from the Rough ER Lumen through the Golgi complex and Then to the Cell Surface.

Most newly made proteins in the ER lumen or membrane are incorporated into small, ≈50-nm-diameter transport vesicles. These either fuse with the *cis*-Golgi or with each other to form the membrane stacks known as the *cis*-Golgi reticulum (network). From the *cis*-Golgi certain proteins, mainly ER-localized proteins, are retrieved to the ER via a different set of *retrograde*transport vesicles. In the process called cisternal migration, or cisternal progression, a new *cis*-Golgi stack with its cargo of luminal protein physically moves from the *cis* position (nearest the ER) to the *trans* position (farthest from the ER), successively becoming first a *medial*-Golgi cisterna and then a *trans*-Golgi cisterna. As this happens, membrane and luminal proteins are constantly being retrieved from later to earlier Golgi cisternae by small retrograde transport vesicles. By this process enzymes and other Golgi resident proteins come to be localized either in the *cis*- or *medial*- or *trans*-Golgi cisternae.Each *cis*-Golgi cisterna, with its protein content, physically moves from the *cis* to the *trans* face of the Golgi stack (red arrows). As this cisternal progression occurs, many luminal and membrane proteins undergo modifications, primarily to attached oligosaccharide chains. Some proteins remain in the *trans*-Golgi cisternae, while others move via small vesicles to the cell surface or to lysosomes. In certain cell types (e.g., nerve cells and pancreatic acinar cells), some soluble proteins are stored in secretory vesicles and are released only after the cell receives an appropriate neural or hormonal signal (regulated secretion). In all cells, certain proteins move to the cell surface in transport vesicles and are secreted continuously (constitutive secretion). Like soluble proteins, integral membrane proteins move via transport vesicles from the rough ER to the *cis*-Golgi and then on to their final destinations. The orientation of a membrane protein, established when it is inserted into the ER membrane, is retained during all the sorting steps: Some segments always face the cytosol; others always face the exoplasmic space (i.e., the lumen of the ER, Golgi cisternae, and vesicles or the cell exterior). Retrograde movement via small transport vesicles retrieves ER proteins that migrate to the *cis*-Golgi and returns them to the ER. Similarly, *cis*- or *medial*-Golgi proteins that migrate to a later compartment are retrieved by small retrograde transport vesicles.

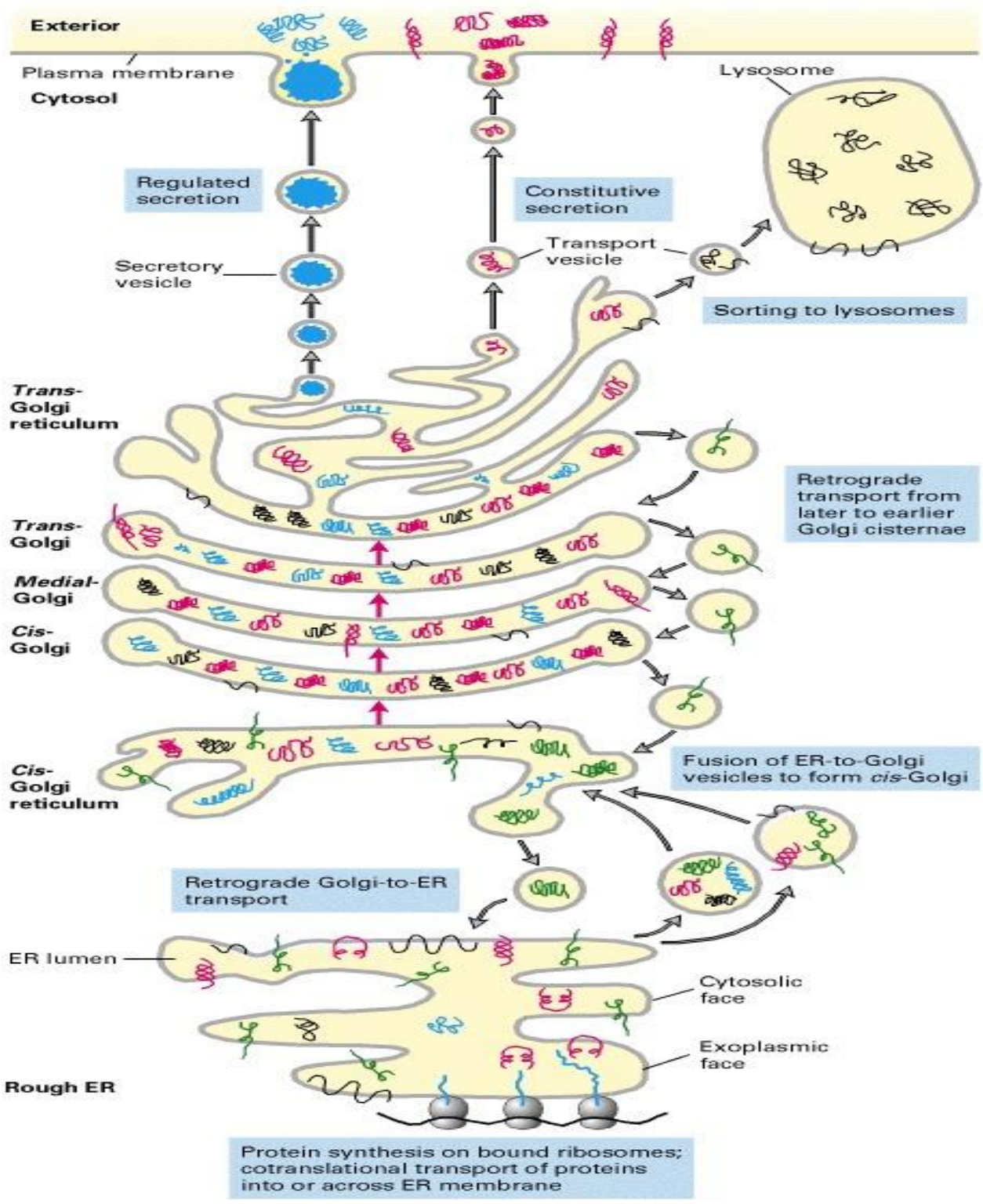

Figure-2.The secretory pathway of protein synthesis and sorting

Process of Hormones Secretion

In earlier studies it was suggested that the mechanisms of hormone release from glands containing secretory granules encompassed holocrine and apocrine secretion (1, 2) as well as other forms of release, for example:

(a) extrusion of intact secretory granules into the cell exterior (3);

(b) release from a "free hormone pool" (3,4);

(c) release by diffusion of hormones out of the granules into the cytoplasm (4, 5); or

(d) release by dissolution of the granule membrane in the cytoplasm (6). The final common path proposed for the last three mechanisms of release was the diffusion across the plasma membrane of the hormones from the cytoplasmic sap into the cell exterior. However, in 1957 another mechanism of secretion was proposed by De Robertis & Vaz Ferreira, which suggested that secretion was by "reverse pinocytosis" (exocytosis), a mechanism whereby the membrane of the secretory granule fuses with the plasmalemma allowing the escape of the content of the granules to the cell exterior (7).Exocytosis seems to be the most logical process of hormone release. If the disadvantages of the other forms of release are considered, it is immediately obvious that exocytosis is the simplest, the most economical, and the most efficient mechanism for releasing not only hormones but also enzymes and transmitter substances from the exocrine glands and nerve terminals respectively. If hormones were released from granules into the cytosol they would diffuse in all directions; large quantities of the hormones would be destroyed by enzymes present in the cytosol and only a fraction of the hormones would reach the cell exterior. Furthermore, the hormones in question would have to cross at least two membranes, those of the granules and that of the cell. Finally, if release were not by exocytosis, since the molecular size of the hormones which are stored in granules varies from very simple molecules like adrenaline to more complex ones like somatotropin or adrenocorticotropin, special transport mechanisms through these membranes, namely, the granule and the plasmalemma, would have to exist.

Ultrastructural observations have suggested that secretory granules originate from the Golgi apparatus (15, 17, 36-43). The Golgi complex can be viewed as the membrane-bound compartment of the cell which is believed to be involved in the following functions (44):

(a). The synthesis of certain polysaccharides;

(b). The synthesis or attachment of carbohydrates side chains of glycoproteins (especially the addition of terminal ·sugars such as galactose, fucose, and sialic acid).

(c). The assembling of secretory materials and formation of membrane-bound granules containing these materials.

(d). The assembling of Iysosomes.

According to some investigators (45) the Golgi apparatus is part of the "endomembrane system" of the cell. This system is formed by the following components:

Rough endoplasmic reticulum → Smooth endoplasmic reticulum → Golgi apparatus → secretory granules. The arrows here indicate the direction of the "membrane flow," a term which has been introduced to describe the process of physical transfer of membranes from one cell compartment to another (45). This process of membrane transfer may or may not be accompanied by the concomitant transfer through this endomembrane system of secretory products, in this particular case, prohormones and hormones. Morphological evidence showing the packing of secretory products into granules in the stacked Golgi cisternae, especially in the maturing phase of the Golgi, has been published. This ultra structural evidence has been collected from studies on all types of secretory cells of the adenohypophysis (15, 17, 36, 37); and 8 cells of the pancreas (37-40); the adrenal medulla (11, 12); the neurosecretory cells of the hypothalamus-neurohypophysial system 43); and in many other secretory systems in which secretory products are packed in vesicles (46).

Mechanisms of release (Energy Requirement and the Possible Role of ATP)

It has been shown that in many secretory tissues the release reaction is an energydependent process (68-71). Because secretion is blocked by inhibitors of oxidative phosphorylation and glycolysis (68-71) and because these two processes are important in the generation of ATP, it is possible that the energy requirement for the secretory process is in the form of ATP. However, it is not known whether the energy requirement is necessary to maintain a general cell function or whether it is needed in some of the steps involved in stimulus-secretion coupling. Although the molecular events involved in the secretory process have not yet been completely elucidated, much information has been obtained by studying the effects of A TP on secretory granules isolated from the adrenal medulla. In the presence of MgH, ATP produces structural changes in the chromaffin granules (72) and there is a simultaneous release of catecholamines, endogenous ATP, and soluble proteins (73). When ATP acts on chromaffin granules it is hydrolyzed by enzymes present in granule membranes, and part of the Pi so liberated is transphosphorylated to granule membranes (74). The effects of ATP on chromaffin

granules can be blocked at either an early (ATP hydrolysis, transphosphorylation) or at a subsequent (conformational changes) step (75). On the basis of the above and other observations, Poisner & Trifar6 proposed a hypothetical model for the molecular events involved in exocytosis (73). In this model the stimulation of the chromaffin cells induces an increase in intracellular Ca^{2+} (this being due either to increased Ca^{2+} entry or to liberation from intracellular sources). Then, CaH may form a link between anionic groups (possibly phospholipids) of both granule and plasma membranes (73). It is known that CaH can cause the aggregation of chromaffin and other secretory granules (76, 77). Moreover, it has been shown that CaH causes the attachment of secretory granules of leucocytes to their membranes (78). ATP, which is released from the plasma membrane upon stimulation (79) and is perhaps also freed from some other places within the cell, acts on chromaffin granules. During this interaction ATP is hydrolyzed by granule membrane enzymes; membrane protein and lipid are phosphorylated; and this is followed by the production of some conformational change (contractile event?) in the granule membrane leading to the release of soluble granule components. This hypothesis involves Ca2+ as one of the principal elements in membrane fusion. In connection with this it has been shown that ATP induces the synthesis of diphosphatidyl inositol in granule membranes (25, 80, 81). This lipid has great affinity for Ca2+ and, in some membranes, there is a direct correlation between Ca2+ binding and diphosphatidyl inositol content (82). It should be remembered that this hypothesis has been formulated on the basis of in vitro observations and although it accommodates all that is known about the release reaction, further work is necessary to see whether this mechanism will operate during release in vivo.

Recombinant DNATechnology in the Treatment of Diabetes: Insulin Analogs

After more than half a century of treating diabetics with animal insulins, recombinant DNA technologies and advanced protein chemistry made human insulin preparations available in the early 1980s.As the next step, over the last decade, insulinanalogswereconstructedbychangingthestructureof the native protein with the goal of improving the therapeutic properties of it, because the pharmacokinetic characteristics of rapid-, intermediate-, and long-acting preparations of human insulin make it almost impossible to achieve sustained normoglycemia. The first clinically available insulin analog, lispro, confirmed the hopes by showing that improved glycemic control can be achieved without an increase in hypoglycemic events. Two new insulin analogs, insulin glargine and

insulinaspart,haverecentlybeenapprovedforclinicaluseinthe United States, and several other analogs are being intensively tested. Thus, it appears that a rapid acceleration of basic and clinical research in this arena will be seen, which will have direct significance to both patients and their physicians.Theintroductionofnewshort-actinganalogsandthe development of the first truly long-acting analogs and the developmentofanalogswithincreasedstability,lessvariability, and perhaps selective action, will help to develop more individualized treatment strategies targeted to specific patient characteristics and to achieve further improvements in glycemic control. Data on the currently available and tested analogs, as well as data on those currently being developed, are reviewed.

Molecular Genetics and Diagnosis of Thyroid Cancer

Thyroid cancer is a common type of endocrine malignancy, and its incidence has been steadily increasing in many regions of the world. Initiation and progression of thyroid cancer involves multiple genetic and epigenetic alterations, of which mutations leading to the activation of the MAPK and PI3K–AKT signaling pathways are crucial. Common mutations found in thyroid cancer are point mutation of the *BRAF* and *RAS* genes as well as *RET/PTC* and *PAX8/PPARγ* chromosomal rearrangements. The mutational mechanisms seem to be linked to specific etiologic factors. Chromosomal rearrangements have a strong association with exposure to ionizing radiation and possibly with DNA fragility, whereas point mutations probably arise as a result of chemical mutagenesis. A potential role of dietary iodine excess in the generation of *BRAF* point mutations has also been proposed. Somatic mutations and other molecular alterations have been recognized as helpful diagnostic and prognostic markers for thyroid cancer and are beginning to be introduced into clinical practice, to offer a valuable tool for the management of patients with thyroid nodules.

Molecular Genetics of Neuroendocrine Tumors

Through insights into the molecular genetics of neuroendocrine tumors (NETs), the genes predisposing to multiple endocrine neoplasia (MEN) syndromes were identified. In MEN1, tumors occur in the parathyroids, endocrine pancreas, anterior pituitary, adrenal glands and thymic neuroendocrine tissues. The *MEN1* gene encodes a putative growth-suppressor protein, *menin*, binding JunD, a transcriptional factor belonging to the AP-1 complex. However, new partners binding menin remain to be found. The *MEN1* gene might be involved in 1–50% of sporadic NETs. Another critical mechanism involved in NETs is the deregulation of the RET-

signalling pathways by oncogenic point mutations responsible for MEN2 syndromes. MEN2 refers to the inherited forms of medullary thyroid carcinoma. The *RET* proto-oncogene, a tyrosine-kinase receptor, is activated by missense mutations occurring either in the extracellular dimerization domain or intracellular tyrosine kinase catalytic regions. In both cases the receptor is constitutionally activated in the absence of natural ligands. Endocrine tumors also belong to the clinical pattern of Recklinghausen (NF1) and von Hippel-Lindau (VHL) diseases. The genes for both syndromes have been characterized and provide new pathways for endocrine tumorigenesis related to G-protein physiology *(NF1)* and transcriptional regulation and/or endothelial cell proliferation *(VHL)*, respectively. Here, we propose a basic overview of recent data on genetic events leading a normal endocrine cell towards a fully malignant phenotype.

Diagnosis of Endocrine Disease: Diagnostic approach to TSH-producing Pituitary Adenoma

Thyrotropin (TSH)-secreting adenomas (TSHomas) are the rarest form of pituitary adenomas, and most endocrinologists will see few cases in a lifetime, if any. In most cases, the diagnostic approach is complicated and cases may be referred after being presented as a syndrome of inappropriate TSH secretion or as a pituitary mass. This review aims to cover the past, present and possible future diagnostic approaches to TSHomas, including different clinical presentations, laboratory assessment and imaging advances. The differential diagnoses will be discussed, as well as possible coexisting disorders. By evaluating the existing reports and reviews describing this rare condition, this review aims to present a clinically practical suggestion on the diagnosic workup for TSHomas, Major advances and scientific breakthroughs in the imaging area in recent years, facilitating diagnosis of TSHomas, support the belief that future progress within the imaging field will play an important role in providing methods for a more efficient diagnosis of this rare condition.

References:

1. Graumann, W. 1956. Beobachtungen uber Bildung and Sekretion perjodatreaktiver Staffe im Nebennierenmark des Gold-hamsters. Z. Anat. Entwicklungsgesch. 119:415-30
2. Franzen, D. 1964. Beitrage zur morphologie und chemohistologie des Nebennierenmarks des Goldhamsters (Mesocricetus auratus). Anat. Anz. 115:35-38
3. Cramer, W. 1918. Further observations on the thyroid-adrenal apparatus. A histochemical method for the demonstration of the adrenalin granules in the suprarenal gland. J. Physiol. London 52:viii-x 0
4. Hillarp, N. A. 1960. Different pools of catecholamines stored in the adrenal medulla. Acta Physiol. Scand. 50:8-22
5. Thorn, N. A. 1965. Role of calcium in the release of vasopressin and oxytocin from posterior pituitary protein. Acta Endocrinol. 50:357-64
6. Blaschko, H., Welch, A. D. 1953. Localization of adrenaline in cytoplasmic particles of the bovine adrenal medulla. Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. 219: 17-22
7. Lever, J. D., Findlay, J. A. 1966. Similar structural basis for the storage and release of secretory material in adrenomedullary and ,g-pancreatic cells. Z. ZellJorsch. Mikrosk. Anat. 74:317-24
8. De Robertis, E., Vaz Ferreira, A. 1957. Electron microscope study of the secretion of catechol-containing droplets in the adrenal medulla. Exp. Cell Res. 12:568-74
9. Diner, O. 1967. L'expulsion des granules de la medulla-surrenale chez Ie hamster. CR. Acad. Sci. Paris 265: 616-19
10. Nagasawa, J., Douglas, W. W., Schulz, R. A. 1970. Ultrastructural evidence of secretion by exocytosis and of "synaptic vesicle" formation in posterior pituitary glands. Nature 227:407-9
11. Gomez-Acebo, J., Parrilla, R., Candela, L. R. 1968. Fine structure of the A and D cells of the rabbit endocrine pancreas in vivo and incubated in vitro. I. Mechanism of secretion of the A cells. J. Ce ll BioI. 36:33-44
12. Lacy, P. E. 1961. Electron microscopy of the beta cell of the pancreas. Am. J. Med. 31:851-59
13. Farquhar, M. G. 1969. In Lysosomes in Biology and Pathology, ed. J. T. Dingle, H. B. Fell, 2:462-82. Amsterdam: North-Holland. 668 pp.
14. Rennels, E. G., Shiino, M. 1968. Ultrastructural manifestations of pituitary release of ACTH in the rat. Arch. Anat. Histol. Embryol. 51 :575-90
15. Farquhar, M. G. 1961. Origin and fate of secretory granules in cells of the anterior pituitary gland. Trans. NY Acad. Sci. 23:346-51
16. Smith, R. E., Farquhar, M. G. 1966. Lysosome function in the regulation of the secretory process in cells of the anterior pi tuitary gland. J. Cell Bioi . 31:319-47
17. Farquhar, M. G., Skutelsky, E. H., Hopkins, E. R. 1975. See Ref. 10, pp. 83-135
18. Farquhar, M. G. 1971. Processing of secretory products by cells of the anterior pituitary gland. Mem. Soc. Endocrinol. 19:79-122
19. Pelletier, G., Peillon, F., Vila-Porcile, E. 1971. An ultrastructural study of sites of granule extrusion in the anterior pituitary of the rat. Z. ZellJorsch. Mikrask. Anat. 115:501-7
20. Palade, G. E. 1959. In Subcellular Particles. ed. T. Hayashi, pp. 64-83. New York: Ronald. 213 pp.
21. Banks, P., Helle, K. 1965. The release of protein from the stimulated adrenal medulla. Biachem. J. 97:40C-4IC
22. Douglas, W. W., Poisner, A. M., Rubin, R. P. 1965. Efflux of adenine nucleotides from perfused adrenal glands exposed to nicotine and other chromaffin cell stimulants. J. Physiol. London 179:130-37

23. Viveros, O. H., Arqueros, L., Kirshner, N. 1968. Release of catecholamines and dopamine ,g-oxidase from the adrenal medulla. Life SCI: 7:609-18
24. Poisner, A. M., Trifaro, J. M., Douglas, W. W. 1967. The fate of the chromaffin granule during catecholamine release from the adrenal medulla. II. Loss of protein and retention of lipid in subcellular fractions. Biochem. Pharmacol. 16;2101-8
25. Viveros, O. H., Arqueros, L., Connett, R. J., Kirshner, N. 1969. Mechanism of secretion from the adrenal medulla. 4. The fate of the storage vesicles following insulin and reserpine administration. Mol. Pharmacol. 5:69-82
26. Trifar6, J. M. 1973. See Ref. 58, pp. 501-3
27. Viveros, O. H., Arqueros, L., Kirshner, N. 1969. Quantal secretion from adrenal medulla; all or none release of storage vesicle content. Science 165;911-\3
28. Uttentha1, L. 0., Livett, B. G., Hope, D. B. 1971. Release of neurophysin together with vasopressin by a Ca2+dependent mechanism. Phi/os. Tr ans. R. Soc. London Ser. B 261:379-80 29. Nordmann, J. J., Dreifuss, 1. J., Legros, 1. J. 1971. A correlation of release of "polypeptide hormones" and of immunoreactive neurophysin from isolated rat neurohypophyses. Experientia 27;\344-45
30. Rubenstein, A. H., Clark, J. L., Malani, F., Steiner, D. F. 1969. Secretion of proinsulin C-peptide by pancreatic f3 cells and its circulation in blood. Nature 224:697-99
31. Fillion, G. M. B., Siorach, S. A., Uvnas, B. 1970. The release of histamine, heparin, and granule protein from rat mast cells treated with compound 48/80 in vitro. Acta Physiol. Scand. 78;547-60 32. Trifar6, J. M., Poisner, A. M., Douglas, W. W. 1967. The fate of the chromaffin granule during catecholamine release from the adrenal medulla. I. Unchanged effiux of phospholipid and cholesterol. Biochem. Pharmacol. 16:20952100
33. Schneider, F. H., Smith, A. D., Winkler, H. 1967. Secretion from the adrenal medulla: biochemical evidence for exocytosis. Br. 1. Pharmacol. 31;94-104
34. Edwards, B. A., Edwards, M. E., Thorn, N. A. 1973. The release in vitro of vasopressin unaccompanied by the axoplasmic enzymes; lactic acid dehydrogenase and adenylate kinase. Acta Endocrinol. 72:417-24
35. Matthews, E. K., Legros, J. J., Grau, J. D. Nordmann, J. J., Dreifuss, J. J. 1973. Release of neurohypophysial hormones by exocytosis. Nature 241;86-88
36. Kirshner, N., Sage, H. J., Smith, W. J., Kirshner, A. G. 1966. Release of catecholamines and specific protein from adrenal glands. Science 154:529-31
37. Tixier-Vidal, A., Picart, R. 1971. Electron microscopic localization of glycoproteins in pituitary cells of duck and quail. 1. Histochem. Cytochem. 19; 775-97
38. Fawcett, D. W., Long, 1. A., Jones, A. L. 1969. The ultrastructure of endocrine glands. Recent Prog. Horm. Res. 25:315-68
39. Lazarus, S. S., Shapiro, S., Volk, B. W. 1968. Secretory granule formation and release in rabbit pancreatic A-cells. Diabetes 17 :152-60
40. Howell, S. L., Kostianovsky, M., Lacy, P. E. 1969. Beta granule formation in isolated islets of Langerhans. A study by electron microscopic radioautography. J. Cell Bioi. 42;695-705
41. Kobayashi, S., Fujita, T. 1969. Fine structure of mammalian and avian pancreatic islets with special reference to D cells and nervous elements. Z. Zellfo rsch. Mikrosk. Anat. 100;340-63
42. De Robertis. E., Sabatini. D. D. 1960. Submicroscopic analysis of the secretory process in the adrenal medulla. Fed. Proc. 19;5iJppl. 5, pp. 70-78
43. Coupland, R. E. 1972. See Ref. 6, pp. 16-45
44. Sachs, H., Fawcett, P., Takabatake, Y., Portanova, R. 1969. Biosynthesis and release of vasopressin and neurophysin. Recent Prog. Horm. Res. 25;447-84
45. Whaley, W. G., Dauwalder, M., Kephart, J. E. 1972. Golgi apparatus: Influence on cell surfaces. Science 175:596-99

46. Morrc, D. J., Keenan, T. W., Huang, C. M. 1974. See Ref. 4, pp. \07-25
47. Jamieson, J. D., Palade, G. E. 1967. Intracellular transport of secretory proteins in the pancreatic exocrine cell. I. Role oft he peripheral elements of Golgi complex. J. Cell BioI. 34:577-96
48. Hopkins, C. R. 1972. The biosynthesis, intracellular transport, and packaging of melanocyte-stimulating peptides in the amphibian pars intermedia. J. Cell BioI. 53:642-53
49. Caro, L. G., Palade, G. E. 1964. Protein synthesis, storage and discharge in the pancreatic exocrine cell. An autoradiography study. J. Cell BioI. 20:473-95
50. Castle, J. D., Jamieson, J. D., Palade, G. E. 1972. Radioautographic analysis of the secretory process in the parotid acinar cell of the rabbit. J. Ce ll Bioi. 53:290-311
51. Howell, S. L. 1972. Role of A TP in the intracellular translocation of proinsulin and insulin in the rat pancreatic /3 cell. Nature 235:85-86
52. Sachs, H., Takabatake, Y. 1964. Evidence for a precursor in vasopressin biosynthesis. Endocrinology 75:943-48
53. Burford, G. D., Jones, C. W., Pickering, B. T. 1971. Tentative identification of a vasopressin-neurophysin and an oxytocin-neurophysin in the rat. Biochem. J. 124:809-13
54. Winkler, H., Hortnagl, H., Schopf, J. A. L., Zur Nedden, G. 1971. Bovine adrenal medulla: Synthesis and secretion of radioactively labelled catecholamines and chromogranins. NaunynSchmiedebergs Arch. Pharmakol. 271: 193-203
55. Winkler, H., Schopf, J. A. L., Hortnagl, H., Hortnagl, H. 1972. Bovine adrenal medulla: Subcellular distribution of newly synthesized catecholamines, nuc1eotides, and chromogranins. NaunynSchmiedebergs Arch. Pharmacal. 273: 43-61
56. Malamed, S., Poisner, A. M., Trifar6, J. M., Douglas, W. W. 1968. The fate of the chromaffin granule during catecholamine release from the adrenal medulla. III. Recovery of a purified fraction of electron-translucent structures. Biochem. Pharmacol. 17 :241-46
57. Viveros, O. H., Arqueros, L., Kirshner, N. 1969. Mechanism of secretion from the adrenal medulla. 5. Retention of storage vesicle membranes following release of adrenaline. Mol. Pharmacol. 5:342-49
58. Trifar6, J. M., Duerr, A. C. 1976. Isolation and characterization of a Golgirich fraction from the adrenal medulla. Biochim. Biophys. Acta 421:153-67
59. Trifar6, J. M., Duerr, A. c., Pinto, J. E. B. 1976. Membranes of the adrenal medulla: A comparison between the membranes of the Golgi apparatus and chromaffin granules. Mol. Pharmacal. 12:536-45
60. Benedeczky, I., Smith, A. D. 1972. Ultrastructural studies on the adrenal medulla of golden hamster: origin and fate of secretory granules. Z. Zel/forsch. Mikroskok. Anal. 124:367-86
61. Baker, B. L. 1974. See Ref. 35, pp. 191224
62. Pictet, R. , Rutter, W. J. 1972. In Handb. Physiology, Endocrinology I: 25-66
63. Douglas, W. W., Nagasawa, J., Schulz, R. A. 1971. Coated microvesicles in neurosecretory terminals of posterior pituitary glands shed their coats to become smooth "synaptic" vesicles. Nature 232:340-41
64. Nagasawa, J., Douglas, W. W. 1972. Thorium dioxide uptake into adrenal medullary cells and the problem of recapture of granule membrane following exocytosis. Brain Res. 37:141-45
65. Orci, L., Junod, A., Pictet, R., Renold, A. E., Rouiller, C. 1968. Granuloysis in A cells of endocrine pancreas in spontaneous and experimental diabetes in animals. J. Cell Bioi. 38:462-66 66. Rufener, C. 1973. Autophagy of secretory granules in the rat neurohypophysis. Neuroendocrinology 13:314--20
67. Holtzman, E., Dominitz, R. 1968. Cytochemical studies of Iysosomes, Golgi apparatus and endoplasmic reticulum in secretion and protein uptake by adrenal medulla cells of the rat. J. Histochem. Cytochem. 16:320-36

68. Blaschko, H., Jerrome, D. W., RobbSmith, A. H. T., Smith, A. D., Winkler, H. 1968. Biochemical and morphological studies on catecholamine storage in human phaeochromocytoma. Clin. Sc i. 34:453-65
69. Hokin, L. E. 1951. The synthesis and secretion of amylase by pigeon pancreas in vitro. Biochemistry 48:320-26
70. Coore, H. G., Randle, P. J. 1964. Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem J. 93:66--78
71. Douglas, W. W., Ishida, A., Poisner, A. M. 1965. The effect of metabolic inhibitors on the release of vasopressin from the isolated neurohypophysis. J. Physiol. London 181:753-59
72. Kirshner, N., Smith, W. J. 1969. Metabolic requirements for secretion from the adrenal medulla. Life Sci. 8(part 1): 799-803
73. Trifar6, J. M., Poisner, A. M. 1967. The role of A TP and ATPase in the release of catecholamines from the adrenal medulla. II. A TP-evoked fall in optical density of isolated chromaffin granules. MoL Pharmacol. 3:572-80
74. Poisner, A. M., Trifar6, J. M. 1967. The role of A TP and ATPase in the release of catecholamines from the adrenal medulla. I. A TP-evoked release of catecholamines, ATP, and protein from isolated chromaffin granules. Mol. Pharmacol. 3:561-71
75. Trifaro, J. M., Dworkind, J. 1971. Phosphorylation of membrane components of adrenal chromaffin granules by adenosine triphosphate. Mol. Pharmacal. 7:52-65
76. Dworkind, J., Trifaro, J. M. 1971. Chromaffin granules: Effects of ions and ATP on 'catecholamine content, ATPase activity, and membrane phosphorylation. Experientia 27:1277-79
77. Banks, P. 1966. An interaction between chromaffin granules and calcium ions. Biochem. J JOI:18C-20C
78. Vilhardt, H., Jrgensen, T. 1972. Free Row electrophoresis of isolated seen:tory granules from bovine neurohypophyses. Experientia 28:852-53
79. Woodin, A. M., French, J. E., Marchesi, V. T. 1963. Morphological changes associated with the extrusion of protein induced in the polymorphonuclear leucocyte by staphy lococca1 leucocidin. Biachem. J 87:567-71
80. Abood, L. G. 1966. Interrelationships between phosphates and calcium in bioelectric phenomena. In t. Rev. Neurobiol. 9:223-61
81. Trifaro, J. M. 1972. Solubilization and partial characterization of the phosphorylated component(s) of the membrane of adrenal chromaffin granules. FEBS Lett. 23:237-40
82. Trifaro, J. M., Dworkind, J. 1975. Phosphorylation oft he membrane components of chromaffin granules: Synthesis of diphosphatidylinositol and presence of phosphatidylinositol kinase in granule membranes. Can. J. Physiol. Pharmacol. 53:479-92