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**A Review of "Chromaffin, Enterochromaffin and Related Cells,"
with Some Comments on the APUD
and Paraneuron Concepts***

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R. E. COUPLAND and T. FUJITA (editors):
Chromaffin, Enterochromaffin and Related Cells.

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26 × 19cm, XIV + 400 pages with 248 figures and 31 tables.
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This is a report of a symposium supported by the Naito Foundation and the Eisai Company. It was held in Gifu, Japan, in August, 1975. Participants included contributors from Great Britain, Japan, the United States, Australia, Federal Republic of Germany, Sweden, Denmark and Italy. The chairman of the symposium, Professor R. E. COUPLAND of Nottingham, specifies that chromaffin cells give a positive chromaffin reaction, store and secrete catechol amines, develop from neural crest and are innervated by pre-ganglionic sympathetic nerve fibers. He points out further that groups of chromaffin cells associate with the sympathetic nervous system; a second group is scattered in the epithelial tissue of the gut and respiratory system and a third group consists of amine-storing mast cells found in connective tissues of gut, pancreas and liver, particularly in ungulates. The second and third groups lack some of the features of typical chromaffin cells.

The chromaffin reaction is characterized by the development of a noticeable brown color when cells are exposed to oxidizing agents such as the dichromate or iodate ions. This reaction depends on the presence in the cells of sufficient amounts of easily oxidizable aromatic amines, amongst which the indol amines, histamine and 5-hydroxytryptamine, and the catechol amines, dopamine, adrenaline and noradrenaline are the most important. The book very reasonably includes discussion of cells which contain one or more of these compounds in amounts insufficient to yield a brown color of an intensity which can be clearly perceived in the light microscope. Sensitivity for detecting such compounds can be increased by using their capacity to reduce or to bind silver ions or by fluorescence displays, usually after reactions with formaldehyde vapor or glyoxylic acid.

But the scope of the volume goes further. It presents considerable discussion of scattered endocrine cells which do not display evidence of catechol or indol amines,

* *Editor's Notes:* This book review was originally sent for an ordinary issue of the Arch. histol. jap. However, as the contents of this review concisely summarized the current positions regarding "neuro-endocrine relatives," the editors included it as the concluding article of this supplement with the permission of Professor BENNETT.

which give no chromaffin reaction, and which are found in the intestinal and respiratory tracts and in the pancreas, thyroid, thymus and parathyroids. These cells, together with the cells which contain catechol or indol amines in amounts insufficient to give rise to a visible chromaffin reaction, presumably comprise the "related cells" referred to in this title. Yet the symposium omitted discussion of chromaffin and related cells in the central nervous system, though several catechol amine tracts have been described in the brain and indol amines and catechol amines are present in pineal cells.

What, then, are the criteria which permit some non-chromaffin cells to be regarded as related to chromaffin cells, and which, at the same time, omit very closely related cells in the central nervous system? In general, most of the set of cells chosen for attention at this symposium belong to a group called "paraneurons" by Professor FUJITA. In Chapter 14 FUJITA specifies that a paraneuron is a cell with the following features:

It can produce 1) a neurotransmitter substance and 2) a protein or polypeptide which may possess hormone actions;

It possesses secretory vesicles which may resemble synaptic vesicles or vesicles containing neurosecretory materials;

It has receptor sites which, when subjected to adequate chemical stimuli, lead to release of secretion from another part of the cell;

It is derived from neuroectoderm.

These are all properties displayed by nerve cells. Probably every nerve cell in the central and sympathetic nervous system displays all of these features. Indeed, chromaffin cells associated with sympathetic ganglia are identified as small interneurons and are discussed in illuminating detail by LEVER, WILLIAMS, YAMAUCHI, MATTHEWS and others in Chapters 7, 8, 9 and 10. Moreover, questionably chromaffin glomus cells in the carotid body, though lacking long processes usually attributed to nerve cells, are described in Chapters 22-24 as regulatory interneurons containing dopamine and other aromatic amines. Yet FUJITA lists the carotid glomus and sympathetic adrenergic interneurons as paraneurons. What is the distinction, then, between neurons and paraneurons, or between nerve and non-nerve? FUJITA, in Chapter 14, lists the following types of cells as examples of paraneurons: adrenal medullary cells, paraganglion cells, adrenergic interneurons, carotid body chief (or glomus) cells, thyroid parafollicular cells, parathyroid chief cells, adenohypophysis cells, mast cells, melanocytes, Merkel cells of skin, pinealocytes, pancreatic islet cells, gastro-enteric, bronchial, urethral and prostatic endocrine cells, gustatory cells in taste buds, hair cells of acoustic, vestibular and lateral line systems, and retinal photoreceptors. He does not list olfactory receptor cells, which recently have been shown to be renewed constantly, in contrast to typical neurons, which are not replaced, once formed. He does not mention candidate types of cells in invertebrates. Moreover, neuroectodermal origins of several of the cell types listed,—for example, endocrine cells of pancreas and of the alimentary, respiratory, and urinary tracts,—have not been clearly established and have even been denied by careful workers. Yet these cells, except for those in the urinary tract, receive considerable notice in eight chapters (Chapters 14-21). Chapter 13 discusses paraneuronic cells in mammalian skin, yet sensory cells of lateral line, acoustic and visual systems are mentioned only in the list of paraneuron types. One notes that all the cell types selected

for extensive consideration in the symposium are listed as paraneurons, yet some correspond to rather typical neurons, some are included as paraneurons though an embryological basis for inclusion has not been established and some members of the paraneuron list are excluded from special attention. Thus the central theme of the volume does not include all the cell types listed as paraneurons by FUJITA in Chapter 14, but is encompassed entirely by the paraneuron concept.

Chapter 11, by A. G. E. PEARSE, is entitled, "Neurotransmission and the APUD concept." One can ask, how does the APUD concept relate to the central theme of the volume? PEARSE has evolved and amplified this concept over the past decade so that the definition he now prefers does not correspond to the concepts which engendered the APUD acronym. APUD stands for amine precursor uptake and decarboxylation and refers to the observation that various types of cells found in many parts of the body have the capacity to take up the aromatic amino acids, tryptophan or tyrosine, or their hydroxylated derivatives, dihydroxyphenylalanine (DOPA) or 5-hydroxytryptophan (5-HTP), and then decarboxylate the precursor as an intermediary step on the way to synthesis of melanin or one of the active aromatic amines such as the catechol amines (adrenaline, noradrenaline or dopamine) or the indol amines (serotonin [5-hydroxytryptamine], histamine or melatonin). The chromaffin cells discussed in this book have the capabilities reflected by the APUD acronym. PEARSE states that APUD cells are derivatives of neuroectoderm, though mast cells, blood basophiles and intestinal endocrine cells producing 5-hydroxytryptamine or catechol amines have not been traced to such an origin, even though they meet the concept reflected in the APUD acronym. From time to time PEARSE has modified his definition of APUD cells so that, at present, the concept does not correspond closely to the ideas symbolized in the acronym. For example, in Chapter 11, PEARSE states, "... the APUD series ... has come to encompass virtually all the cells responsible for peptide hormone production," though he adds, "... in many instances, the relevant cells have lost not only their in vivo capacity to synthesize and store amines, but also the specific decarboxylating enzyme responsible for the APUD process itself."

These quotations from PEARSE reflect one of the serious difficulties of the APUD concept. The difficulty resides in the fact that the functions reflected in the acronym are not central to the functions of peptide hormone production, which forms the basis of PEARSE's current definition. Peptide hormones are made by methods used for the synthesis of all peptides and proteins. These reactions do not involve decarboxylation of amine precursors nor the uptake of hydroxylated aromatic amino acids. Cells producing catechol and indol amines are included in the APUD series and have characteristics which conform to the APUD acronym. Yet, though they synthesize proteins which bind the aromatic amines they secrete, no hormonal action has been demonstrated for these proteins. To make matters worse, neuroectodermal origins of many peptide hormone producing cells have not been demonstrated and have even been denied. Some of these cells are listed by PEARSE in Chapter 11 as "APUD cells of unproven origin."

PEARSE cites a 1975 paper by TAKOR-TAKOR and PEARSE which assigns the origin of Rathke's pouch to "specialized neuroectoderm of the ventral neural ridge" rather than to stomadeal ectoderm. PEARSE does not assign thyrotrophs and gonadotrophs to the APUD series, though these cells do produce peptide hormones and are assigned to derivation from Rathke's pouch. PEARSE lists as APUD cells those in the hypothal-

amus which secrete oxytocin, vasopressin, somatostatin and the hypothalamic releasing hormones. He also lists the pineal cells which manufacture melatonin. However, he does not list cells elsewhere in the central nervous system which use as transmitters peptide hormones such as somatostatin, substance P, luteinizing hormone or thyrotropin releasing factors or melanocyte stimulating hormone, nor does he cite any of the neurons in the central nervous system which use catechol or indol amines. Yet all are derived from neural ectoderm. One thinks further of angiotensin, chorionic gonadotrophin, somatomedin and erythropoietin as peptide hormones produced outside the nervous system, the source cells of which are not assigned to the APUD class by PEARSE, which are not derived from neural ectoderm, and which have not been associated with uptake of aromatic amine precursor or with decarboxylation. Moreover, he omits from his list all cells of the central nervous system except those which make oxytocin and vasopressin and the hypothalamic cells mentioned above, though many other central nervous system cells make peptide hormones and others possess the capabilities reflected in the APUD acronym. These inconsistencies suffice to make the APUD concept of little value, whether one considers the concept as defined by the capabilities reflected in the APUD acronym or as defined by the capability to produce peptide hormones.

In a recent paper in *Nature* (262, pp. 92-94, 1976) PEARSE calls attention to the fact that substance P, somatostatin, gastrins and vasoactive intestinal peptide are all present in the brain and in the intestinal tract alike. Moreover, he seeks to extend the boundaries of neuroectoderm to include not only the cells of origin of Rathke's pouch but also the otic placodes and the endocrine placenta. He then states, "... the cells of the APUD series constitute a third division of the nervous system," the other two being the somatic and the autonomic divisions, respectively. Thus this recent characterization of the APUD concept brings it close to FUJITA's idea of paraneurons. Yet PEARSE, who attended the conference at which FUJITA presented the paraneuron idea as set forth in Chapter 14 of the volume under review, makes no reference to FUJITA's prior formulation.

The paraneuron and APUD concepts have some similarities and some differences. The paraneuron concept is more sharply defined. Both postulate origins of relevant cells from neuroectoderm and both seek to accommodate cell members which do not appear to come from neuroectoderm. There is overlap of membership of several cell types which are listed by FUJITA and by PEARSE alike as members of their series but PEARSE includes some cells in APUD which FUJITA does not list as paraneurons and FUJITA includes some cells in his series which PEARSE omits. This reviewer judges the paraneuron idea to be sounder and more powerful than the APUD concept and anticipates that it will have a longer period of usefulness. The APUD concept corresponds less well to the central theme of the symposium.

PEARSE (p. 153), FUJITA (p. 191) and other authors contributing to the book use the term, "biogenic amine." One can ask, is the symposium concerned primarily with cells which produce biogenic amines? Before answering this question, one must ask, what is meant by this term? The term "biogenic amines" suggests the concept of "amines which engender life," or alternatively, "amines engendered by life." The first of these concepts is invalid. The second is insufficiently restricted, as there is a vast number of amines engendered by living cells, of which those to which the term, "biogenic" are usually applied are but a very small fraction. This

reviewer knows of no generally agreed upon definition of this term though it is most frequently applied to physiologically active catechol and indol amines such as serve as neurotransmitters. This reviewer takes the view that the ideas represented in the roots of a term should reflect the ideas embodied in the term itself. By this standard, the term has little merit. One can ask, does the term, "biogenic," have a relation to the word "biogenesis," used by HUXLEY a century ago to refer to the concept that all life derives from previous life? Does "biogenic" mean the same thing as "biogenetic," an adjective which reflects a property embodied in "biogenesis"? Or one can inquire if the word, "biogenic," has a relation to the term "biogen," introduced early in the twentieth century to refer to hypothetical and vaguely defined units supposed to exist in protoplasm, and in 1915 to 1918 equated by some biochemists and physiologists to large molecules, such as proteins, thought to be present in cells and embodying some important living principle? It is clear that "biogenic," when combined with "amines," has implications unrelated to the concepts implicit in the roots used in constructing the word, nor is it related to the intended meanings of other words derived from the same roots during the past century, nor does it refer to a specific, well-defined class of amines. Clearly the main theme of the symposium volume under review is not embodied in the concepts suggested by the term "biogenic amine," a term unworthy of such a meritorious volume.

One cannot evaluate the topics considered in this symposium without considering the effects of several powerful currents which are changing some basic concepts in biology. One such current is evident in embryological studies and involves a serious erosion of the status of the concept that the three germ layers are constituted of three classes of cells, each fundamentally determined to be different from the other two and each destined to give rise only to certain restricted classes of mature cells or tissues, to which the other germ layers do not contribute.

For example, it used to be thought that only mesoderm gives rise to connective tissue. Now we know that ectodermal neural crest cells—from neuroectoderm at that—give rise to odontoblasts, to dental pulp connective tissue and to much of the cartilage and bone of the developing face. Collagenous connective tissue fibers were once regarded as characteristic products of mesodermal cells called fibroblasts, or, in less differentiated form, mesenchymal cells. Now we know that collagen is synthesized in substantial amounts by cells representing all three germ layers: by muscle, epidermal epithelial cells, odontoblasts from neural crest, kidney epithelial cells, endodermal intestinal epithelial cells, liver hepatocytes, and many other cell types. What about muscle, whose apparently secure position as a mesodermal derivative was challenged when the iris muscles were shown to be muscular portions of ectodermal epithelial cells? Myoepithelial cells were also traced to ectoderm and endoderm but this was insufficient to engender serious questions about the germ layer concept. Now we recognize actin and myosin as characteristic proteins of muscle cells and almost daily have opportunity to read of discovery of one or both of these muscle proteins in cells not regarded as muscle, and representing all three germ layers. Examples of cells in which one or both of these proteins are found are endodermal intestinal epithelial cells, mesodermal leukocytes, platelets and megakaryocytes, ectodermal glial cells and neurons. Actin and myosin have been found in ameba and even outside the animal kingdom, in slime moulds and in *Nitella*. In the face of such findings as these and with the recognition that all cells in a metazoon

organism are equipped with all the genes assigned to that individual, we find ourselves abandoning the view that, as a cell takes this or that special position in an embryo, it somehow becomes committed, along with all its fellows in the same germ layer to activate only a set of genes characteristic of that germ layer, a set different from the sets activated in cells of the other two germ layers. The three germ layers remain as convenient topographic features which can be used to trace the morphological development of many cells, organs and tissues. But topography does not accurately or consistently reflect biochemical capability. Thus this reviewer is not troubled if all cells of a certain class, such as, for example, paraneurons, or cells capable of making myosin, or capable of contraction, do not come from a single class of topographically localizable embryonic cells such as neuroectoderm or mesoderm. Thus, in the view of this reviewer, FUJITA and PEARSE overemphasize the importance of the claim that all paraneurons, or APUD cells, or cells producing peptide hormones, must come from neuroectoderm. It now appears that some of the cells these authors wish to assign to their series may not turn out to be of neuroectodermal origin. Should this prove to be the case, the values of the concepts of paraneuron and APUD would be little changed.

A second important current which is changing our viewpoint relates to an expansion of our concept of hormones and the recognition that all nerve cells are, at the same moment, capable of secretion and are hence gland cells. The brain is a gland. Along with this is the recognition that the electrical properties characteristic of nerve cells are now recognized in cells of many types. Thus the distinction between nerve and non-nerve is not sharp. Let us look at each of these general features in turn.

Turning first to the secretory capacity of nerve cells, we note that on page 153 of Chapter 11, PEARSE reminds us that the great neurophysiologist, G. H. BISHOP, recognized that "the prime function of a neuron is to produce and apply to other tissues a chemical activator." This secretory product is a hormone and its delivery is an endocrine function. We now recognize a versatile array of specific secretions of nerve cells. All of them are amines. They can be classified in accordance with chemical composition:

Table 1. A classification of transmitter amines

Aromatic amines			Aliphatic amines		
Indol amines	Catechol amines	Purine amines	Quaternary amines	Amino acids	Peptides
Melatonin	Adrenaline	Adenosine	Acetylcholine	γ -Aminobutyric acid	Thyrotropin releasing factor
5-Hydroxytryptamine	Noradrenaline			Glycine	Vasopressin
Histamine ?	Dopamine			Arginine	Oxytocin
				Aspartic acid	Somatostatin
				Lysine etc.	Gastrin
					Substance P etc.

In the face of such an array of amines, one sees no merit in selecting an arbitrary

fraction and designating them as "biogenic." Some of the peptides, of course, contain aromatic amino acids and hence could be classified as either aromatic or aliphatic. The list grows year by year. Recent additions to the peptide category are the endorphins and enkephalins. Collectively, the amine hormones of nerve cells are often designated as "neurotransmitters." The presence of neurotransmitters in cells outside the nervous system comprises one of the criteria for assignment to the category FUJITA presents as "paraneurons." However, FUJITA includes melanocytes in his list of paraneurons, even though they do not produce any neurotransmitter substances. Yet in producing melanin, melanocytes use several of the metabolic steps employed to produce catechol amines. The presence of many neurotransmitter substances in numerous categories of cells outside the nervous systems has served to blur the boundary between nerve and non-nerve and to inspire attempts to formulate helpful generalizing concepts such as those embodied in the paraneuron idea.

New findings have forced us to revise even further our concepts of nerve cells. For decades teachers have presented the anterior horn cell, or cell of Deiters, as a typical nerve cell. It was said that a typical nerve cell has one axon, one soma or perikaryon and many branching dendrites. The dendrites were envisioned as receptor areas to perceive signals from axons of other cells terminating on the dendrites. The soma embodied the genetic and synthetic machinery of the cell. The axon carried a "typical," rapid nerve impulse characterized by depolarization and a spike action potential and ended in one or more synaptic terminals or "boutons." Little of this remains intact. In 1956, BISHOP pointed out that the majority of neurons do not give rise to spike potentials, but use graded potentials. Such graded potentials may be either hyperpolarizing or depolarizing. Morphologically as well, the Deiters type cell cannot be regarded as typical. The work of GOLGI, CAJAL and others has shown that the majority of neurons are cells with elaborate but rather short branches and that in many instances processes cannot be easily assigned to either axon or dendrite category. Horizontal and amacrine cells in the retina are good examples. The glomus cells of the carotid body are recognized as interneurons, yet their processes are few, very short and almost unbranched. To confuse the classic model of a neuron further, the electron microscope has shown us that any portion of a nerve cell can have presynaptic features and any portion can be postsynaptic in character. Thus some regions of dendrites or of the cell somata of Deiters-like cells are presynaptic and can secrete neurotransmitters to activate postsynaptic regions of other cells. Moreover, it has been discovered that well-defined axons often have receptor areas, some very near the terminal boutons, which can perceive neurotransmitter release from axon terminals of other cells ending presynaptically on their axonic receptor surfaces. Thus the old concepts of dendrites as characteristically receptor areas and of axons being exclusively conducting and transmitting in function have been irretrievably compromised. Many "reciprocal synapses" or "glomerular synaptic complexes" have been discovered, wherein much signal processing occurs. Release of synaptic vesicles gives rise to micropotentials, which, along with graded responses, reflect the electrical features of most of the signal processing in the nervous system. Spike potentials are used only when a signal must be transmitted rapidly over a comparatively long distance. Thus an effort to trace a pathway in the central nervous system by following spike potentials will probably miss much of the sequence.

But are the principles reflected in the electrical and secretory activity of nerve cells found only in the nervous system, or in cells from only a single germ layer? The answer is, of course, no. Spike potentials are engendered by several varieties of striated muscle cells and by electroplax cells of electric organs, some of which are derived from striated muscle cells and some from gland cells. Graded potentials have been recorded from smooth muscle cells, many gland cells, and even from many plant cells. KANNO, in Chapter 5, describes nerve-like electrical behavior of adrenal medullary cells when secreting in response to acetylcholine application. In previous papers he has described similar behavior of pancreatic acinar cells. The process of eccytosis, using the gate mechanism of synhymenosis, characterizes alike the release of synaptic transmitters, of pancreatic acinar secretions, of insulin, or of any other secretory product involving peptides or proteins. Thus there is no feature of the electrical or the secretory behavior of nerve which is exclusively found in nerve cells, again making difficulty in distinguishing between nerve and non-nerve.

In 1959 FURSHPAN and POTTER discovered "electrical synapses" between nerve cells. This discovery revealed that, in some cases, one nerve cell could communicate with its immediate neighbor by direct passage of ions from the cytoplasm of one cell to that of its neighbor through low-resistance pathways traversing the membranes of the two adjacent cells. Later, the sites of these pathways were recognized morphologically in the electron microscope and came to be called "communicating junctions" or more commonly by the less appropriate term, "gap junctions." Communicating junctions, or electrical synapses, have been found abundantly in many parts of the nervous systems of vertebrates and invertebrates and clearly have an important role in processing neural signals. But they are also found abundantly outside the nervous system. For example, the vertebrate heart is not a syncytium, as was taught for many years, but is composed of individual cells, each containing one or a few nuclei. The cells are connected with each other by myo-myotic electrical synapses or communicating junctions permitting excitatory signals to spread ionically from cell to cell without the synaptic delay characterizing chemical synapses. Smooth muscle cells are joined by similar communicating junctions, serving analogous functions. So are hosts of epithelial and connective tissue cells, as are many cells in developing embryos. Thus the presence or absence of electrical synapses or communicating junctions cannot be used to distinguish nerve from non-nerve, nor are these features exclusively characteristic of the products of any one germ layer.

From this brief review of recent important currents, it emerges that one searches in vain for clear-cut distinctions between nerve cells and non-nerve cells. The theme of the symposium reported in the volume under review deals mainly with cells outside the nervous system proper which share many of their characteristics with nerve cells. Like nerve cells, they secrete specific amines and are hence gland cells. As in the case of nerve cells, their secretory processes are characterized by electrical events. Like nerve cells, they possess receptor areas sensitive to specific chemicals which can either excite or inhibit the release of neurotransmitter hormones. As for embryonic origin, all nerve cells come from neural crest and neural plates. Some of the cells discussed in the volume under review have been traced to origins in neural ectoderm, some are said to originate from other embryonic regions, and the origins of others are unknown. It is useful to explore the properties of endocrine cells outside the proper nervous system in order to determine which resemble nerve cells most

closely and hence qualify for a designation reflecting that affinity. The paraneuron concept reflects such an effort.

This volume under review serves as a vehicle to explore the merits of this effort against a background of rapidly changing concepts relating to the nervous system and to embryologic development. As such, it merits the thoughtful attention of scholars interested in the principles of organization and control in metazoons.

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