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Professional Biographical Sketch

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After graduating from City College of New York (CCNY) in 1953, I entered the army. By the next year, I was in the 219th Preventive Medicine Unit in Korea. Many soldiers in the field were dying from hemorrhagic fever. My duty was to roam the Korean countryside and set mousetraps to see if we could find clues that would give us an understanding of what was causing their deaths. We brought the captured mice back to the laboratory to remove mites from their skin. We evaluated these mites and sent this information to the Armed Forces Institute of Pathology (AFIP). The cause of this was determined to be due to a virus that was carried by these mites, and the mice were their hosts. Little did I know that almost 50 years later I would develop an atlas of the mouse brain.

In 1955, the Army transferred me to Tokyo, Japan to the 406th Medical General Laboratory Pathology Department. There my duties were to assist the pathologist at autopsies and in the laboratory. I was in charge of special stains on pathological tissues. I also learned a variety of histological procedures such as embedding tissue and preparing paraffin blocks to cut tissue sections for staining and evaluation by the pathologists. I also learned how to use the freezing microtome to prepare tissue sections at surgery for biopsies to determine subsequent surgical manipulation such as removal of cancer tissue. From this experience, I became very interested in histology to the point where I read the entire textbook written by Maximow and Bloom.

Following my army experience, I obtained a job at the Brooklyn College of Pharmacy where I assisted in the Department of Pharmacology student laboratory course and was in charge of the care and handling of laboratory animals. There I learned animal handling, drug injections, and surgery.

In December 1957, I married Ilene Hammer and we began our life together, which has been ongoing for 47 years.

With this background and an interest in histology, I was accepted to the Department of Anatomy at Ohio State University (OSU) College of Medicine with Dr. Morton Alpert as my advisor. My thesis work was the study of a pigment (lipofuscin, ceroid) in a variety of tissues such as the mouse adrenal cortex and in atherosclerotic plaques in human post-mortem aortas. My experience with post-mortem dissections was important for this work. In addition, my work with special stains gave me the experience and insight to develop a histochemical stain for lipofuscin pigment (Alpert *et al.*, 1960). It was during this time I met Dr. Bernard Marks of the Department of Pharmacology who served as a co-advisor and collaborator on this project.

In 1958, I received a Master's Degree in Anatomy. My advisor, Dr. Alpert, accepted a position in the medical school at the University of Indiana in Indianapolis. It was only natural, in view of my experience in pharmacology that I transferred to the Department of Pharmacology with Drs. Bernard Marks and Chauncey Leake who was also Assistant Dean of the medical school. It was a great honor to be exposed to the teachings of this famous scientific statesman who created *Current Contents*©, the weekly digest of Life Sciences Journals. From this arose the Institute for Scientific Information that was eventually responsible for Pub Med, the Internet's contribution to the public dissemination of scientific information.

In this Department, I was fortunate to meet Dr. Joan Vernikos, a postdoctoral fellow from England. She was working in the field of adrenocorticotropic hormone (ACTH) and stress. I was interested in this area of research. She became my co-advisor and guided me, along with Dr. Marks, through my thesis work on the effect of stress on the synthesis and release of ACTH from the pituitary gland. This study showed that the anterior pituitary of intact rats can take up a labeled amino acid and incorporate it into ACTH and that stress can increase this rate of incorporation. This was one of the first studies to use 2-dimensional electrophoresis (high voltage electrophoresis and chromatographic separation) of radioactive extracts on Whatman paper sheets.

Every extract revealed the presence of the same

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radioactive spot (spot 1). Stress increased the amount of radioactivity incorporation into this spot. Using a biological assay method, spot 1 was identified as ACTH. It was concluded that the effect of the acute stress was to cause an increased synthesis of ACTH in the adenohypophysis of rats.

What remained with me was the abiding interest in 2-dimensional electrophoresis. Twenty-one years later (1983), I set up the newly developed method (O'Farrell, 1975) of 2-dimensional gel electrophoresis for the isolation and some identification of proteins.

In April 1962, I attended the FASEB meeting (Federation of American Scientists and Experimental Biologists) in Atlantic City. There I went to a symposium talk given by Dr. George Koelle, the Chairman of Pharmacology at the University of Pennsylvania in Philadelphia. I was intensely interested in his work. Here was a pharmacologist that used a histochemical procedure in his studies - the first marriage of pharmacology and neuroanatomy that I had encountered. This, as it turned out, was the professional path that I was destined to take. Drs. Koelle and Friedenwald developed the histochemical stain for acetylcholinesterase (AChE) a very important marker for the localization of the cholinergic nerves anywhere in the body. This procedure was to be partially responsible for inspiring me to study the peripheral and central nervous system. I met with Dr. Koelle in Atlantic City. He became a very important link in the chain of my life.

It was in mid-1962 while perusing the literature (in *Current Contents*©) that I discovered a series of publications by Swedish groups in Lund and Stockholm. This series was soon to change my scientific life forever. These papers describe a procedure developed by Falck (in Lund) and Hillarp (in Stockholm) (Falck, 1962; Falck *et al.*, 1962). The histochemical procedure was a fluorescence method for the histochemical demonstration of catecholamines and serotonin, which revealed for the first time that these amines accumulated in synaptic nerve terminals of the brain and peripheral organs.

I received my doctoral degree in August 1962. I then moved with Ilene and my first child to the University of Pennsylvania Medical School Pharmacology Department as a Postdoctoral Fellow. I revealed my great interest in setting up this fluorescence microscopic procedure. I already had experience with fluorescence microscopy while working on lipofuscin, which was an autofluorescent pigment. I also had experience with paraffin blocks of human tissue and the use of the autotechnicon while working in the Army Medical Corps. All that remained was to develop a procedure for freeze-drying tissue that was frozen in isopentane surrounded by liquid nitrogen. The catecholamine procedure was difficult and laborious. I obtained a newly developed freeze-dryer. The frozen tissue was exposed to vacuum drying for several days. The dry tissue was exposed to heated paraformaldehyde powder. The formaldehyde fumes reacted with the catechol and indoleamines in the tissue, which resulted in fluorescent aminergic nerves that were visualized with the fluorescence microscope. This dried tissue was embedded in paraffin, placed in a vacuum oven, and then cut on a microtome. The paraffin sections were processed and viewed in the fluorescence microscope.

This was a period of pure magic. Everyday in every tissue that we looked at there were discoveries. We studied the eyes (Laties and Jacobowitz, 1964; 1966), the gut (Jacobowitz, 1965b; Jacobowitz and Nemir, 1969; Nemir *et al.*, 1971), ganglia (Jacobowitz, 1967; 1970; Jacobowitz and Woodward, 1968), heart (Jacobowitz *et al.*, 1967; Friedman *et al.*, 1968; Vogel *et al.*, 1969; Brus and Jacobowitz, 1970), ovary (Jacobowitz and Wallach, 1967), urinary system (Wein *et al.*, 1972a,b; 1973; 1974; 1975a,b) and skin (Jacobowitz and Laties, 1968).

In Dr. Koelle's lab, I learned the histochemical procedure for AChE. Using this procedure I completed the first coexistence studies between the catecholaminergic and the cholinergic nerves (Jacobowitz, 1965; 1974; Jacobowitz and Koelle, 1965a,b; Raezer *et al.*, 1973). The catecholamine histofluoresence procedure was also very useful in studying the effects of drugs on the aminergic nerves (Patil *et al.*, 1968; 1972; Goldman and Jacobowitz, 1971). Early studies were done on the recently developed drug, 6-hydroxydopamine (6-OHDA), the first drug developed that resulted in the reversible destruction of the noradrenergic varicose nerve terminals in peripheral organs. At higher doses, intraventricular administration also destroyed nerve fibers in the brain.

Up to this time (1969), I had carefully stayed away from studying the brain primarily because I felt intimidated by the enormous monumental work on the brain being published by the Swedish workers (e.g., Dahlström and Fuxe, 1964; 1965). However, a recent rat brain map atlas by König and Klippel (1963) made it possible for me to initiate studies on the brain. With Richard Kostrzewa, my first graduate student, we studied the influence of 6-hydroxydopa (6-OHDOPA) on noradrenergic terminals and axons on the rat brain (Jacobowitz and Kostrzewa, 1971). He directed his thesis work to the study of 6-OHDOPA on peripheral organs and the brain (Kostrzewa and Jacobowitz, 1972;

1973). This landmark work resulted in a Pharmacological Review of 6-OHDA, which later turned out to be a citation classic in *Current Contents*© (Kostrzewa and Jacobowitz, 1974).

By 1970, I began to know my way around in the brain. It was at this time that I met with Dr. Irv Kopin, Chief of the Laboratory of Clinical Science at the National Institute of Mental Health. He offered me a position of Section Chief in his laboratory. Dr. Kopin was indeed one of the big links in my life's chain. I was also very pleased to be in the same laboratory with Dr. Julius Axelrod, a Nobel laureate. In September 1971, my family - now Ilene and our two sons - arrived in Bethesda, Maryland, where we have lived for 33 years.

At NIH, I set up a lab known as the Section on Histopharmacology, a term used to indicate the marriage of histochemical and pharmacological/biochemical disciplines, a reflection of my past work experience. Here I had the opportunity to interact with many people with a variety of interests. There was much interest by research collaborators in pursuing studies of catecholaminergic/serotonergic and cholinergic nerves.

My section on Histopharmacology has been a unique laboratory dedicated to the belief that the challenge of uncovering the secrets to brain function lies in the unraveling of neuronal connectivity. To this end, we dedicate a portion of our work to mapping the brain's neuronal systems. The guiding principle is that knowledge of the building blocks gives us clues about how the nervous systems operate and how they might fail due to disease and injury.

The focus in the laboratory was on multi-disciplinary studies. We directed much of our work towards brain aminergic/cholinergic nerve localization, mapping and behavioral alterations (Richardson and Jacobowitz, 1973; Richardson *et al.*, 1974; Miliaressis *et al.*, 1975; 1976; St. Laurent *et al.*, 1975; 1976; Cruce *et al.*, 1976; 1978; Lewis *et al.*, 1976). The study of the effects of 6-OHDA was continued (Jacobowitz, 1975; Jacobowitz *et al.*, 1975; Liuzzi *et al.*, 1977; Massari *et al.*, 1978), in addition to other drugs (Hanbauer *et al.*, 1974; Roizen *et al.*, 1975; Grobecker *et al.*, 1977). Chromaffin cells and organ and cell cultures (Jacobowitz and Greene, 1974; Webb *et al.*, 1975; Jacobowitz *et al.*, 1976) were studied.

In 1972, Dr. Mickey Palkovits, a Neuroanatomist from Hungary, visited the laboratory of Clinical Science. We undertook the first effort of mapping the whole brain localization of both catecholaminergic and cholinergic (AChE) nerves in the rat brain (Jacobowitz and Palkovits, 1974; Palkovits and Jacobowitz, 1974). We both sat at one microscope "cheek-to-cheek" for about one year carefully mapping terminal fields and cell bodies -- a very tedious endeavor. Some years later this work became a scientific classic in *Current Contents*©. Soon after, Dr. Paul MacLean and I did a "cheek-to-cheek" mapping of catecholaminergic neurons and serotonergic perikarya in a pygmy monkey brain (Jacobowitz and MacLean, 1978).

This was a time when there was a surge of interest in brain function and disease. Researchers discovered that the loss of dopaminergic nerves in the brain caused human Parkinson's Disease. In addition, the importance of norepinephrine and serotonin in the action of antidepressant drugs resulted in brain anatomical studies to visualize the influence of drugs on the uptake and release of these neurotransmitters. The availability of maps of the localization of neurochemicals was of great interest.

Furthermore, Dr. Palkovits developed a new technique in our labortory known as the "micropunch" method (Palkovits, 1973). This landmark procedure allowed us to microdissect discrete nuclei and other regions of the brain, which provided tiny amounts of brain tissue (e.g., 5-80 µg protein). This tissue was assayed by a new microradioenzymatic procedure developed by Dr. David Henry in Dr. Kopin's section (Coyle and Henry, 1973). This combination of micropunching discrete nuclei and quantitating amine (norepinephrine, dopamine, serotonin) levels in the brain was an explosion that was of considerable importance in brain physiology, pharmacology, and neuroanatomy. The era of both histochemical and biochemical mapping was launched.

Thus, in the 1970's, our Section was involved with studies concerning major neurotransmitters and neuronal enzymes. We were able to study the influence of a variety of behaviors, stressors and genetic mutants on specific neurochemicals (Richardson and Jacobowitz, 1973; Richardson *et al.*, 1974; Miliaressis *et al.*, 1975; St. Laurent *et al.*, 1975; 1976; Cruce *et al.*, 1976; 1978; Lewis *et al.*, 1976; Miliaressis and Jacobowitz, 1976). Much work was done on the effect of stress (Palkovits *et al.*, 1975; Thoa *et al.*, 1975; 1977; Kobayashi *et al.*, 1975; Moyer *et al.*, 1977; 1978), reproductive neuroendocrine processes (Crowley *et al.*, 1978a,b,c; 1979a,b), and brain pathways (Palkovits *et al.*, 1974; Jacobowitz, 1978). We also studied the localization of glutamic acid decarboxylase (Massari *et al.*, 1976; Hoover *et al.*, 1977; 1978). Thus, it was the mapping of potential brain neuroregulators that served as a springboard of ideas from which behavioral studies emanate.

Scientists used the catecholamine histofluoresce technique until about the mid 1970s. I believe that this powerful procedure was responsible for the modern day rapid growth of neuroscience. But a new scientific landmark procedure came on the scene in the early 1970s. It was the immunofluorescent procedure (Geffen *et al.*, 1969; Hartman, 1973). Antibodies to tyrosine hyroxylase became the marker for identification of catecholamine-containing neurons. This simple antibody technique quickly replaced the immunofluoresent procedure for identification of adrenergic nerves and chromaffin cells in the periphery and central nervous system. This exciting new development began a new era of the study of peptides as potential neurotransmitters. This was a time when antibodies were generated against known peptides (oxytocin, vasopressin) and new peptides were being isolated, purified, and antiserum was being made available. I recognized that my professional interests had come full circle starting from my thesis work with peptides in the pituitary back to peptides in both the brain and periphery. From that point on, my interest in peptides had returned and I was eager to study the localization of peptides within the neurons in the brain and ganglia.

With Tom O'Donahue, a graduate student in the Department of Pharmacology at Howard University working on his thesis under my guidance at NIH, we embarked on a study of alpha-melanocyte stimulating hormone (alpha-MSH) utilizing immunohistochemical, biochemical, and behavioral methods (Jacobowitz and O'Donohue, 1978; O'Donohue *et al.*, 1979a,b,c,d; 1980a,b; 1981b,c; 1982; Tizabi *et al.*, 1982). This was an exciting time of constant discovery.

With Dr. Gerhard Skofitsch, a postdoctoral fellow from Austria, we initiated a program of immunohistochemical mapping of the localization of peptides in cells and fibers of the brain. We prepared whole-brain maps of corticotrophin releasing factor (CRF), galanin, calcitonin gene-related peptide (CGRP), melanin concentrating hormone (MCH), and atrial natriuretic factor (ANF).

Our laboratory has now produced complete maps of 13 whole brain neuronal systems - more than have come out of any other laboratory in the world: catecholamines/serotonin (Jacobowitz and Palkovits, 1974; Palkovits and Jacobowitz, 1974; Jacobowitz and MacLean, 1978); alpha-MSH (Jacobowitz *et al.*, 1978); bovine pancreatic polypeptide (Olschowka *et al.*, 1981); motilin (Jacobowitz *et al.*, 1981); gonadotropin releasing factor (Jacobowitz *et al.*, 1983); ANF (Thoa *et al.*, 1972); CRF (Skofitsch and Jacobowitz, 1985b); galanin (Skofitsch and Jacobowitz, 1985c; Jacobowitz *et al.*, 2004); CGRP (Skofitsch *et al.*, 1985a); MCH (Skofitsch *et al.*, 1985c); ANF (Skofitsch *et al.*, 1985b); calretinin (Jacobowitz and Winsky, 1991); in addition to an atlas of the chemoarchitectonics of the developing mouse brain (Jacobowitz and Abbott, 1998). The major "depository" of information concerning sites of localization for potent brain neurochemicals has given birth to a field of neuroscience that I have described as "Brain Cartography".

Knowledge of the localization of peptides in the brain enabled Drs. Giora Feuerstein, Debra Diz, and Matthew Sills, Postdoctoral Fellows in the lab, to use a microinjection technique to examine the effects on blood pressure and heart rate following injections of a variety of neuropeptides (alpha-MSH, TRH, bradykinin, dermorphin, CGRP, ANF) into discrete nuclei of the hypothalamus and preoptic area [see Diz and Jacobowitz, 1983; 1984a,b,c; Diz *et al.*, 1984; Sills *et al.*, 1985; Nguyen *et al.*, 1986; Sills and Jacobowitz, 1988)], in addition to prostaglandin and prostacyclin (Feuerstein *et al.*, 1981a,b; 1982a,b). We also studied CGRP injection in the amygdala (Nguyen *et al.*, 1986). The data demonstrated the diversity of central cardiovascular actions of these peptides and emphasized the advantage of microinjection methods in defining central cardiovascular effects. Unlike either intracerebroventricular injections or electrical stimulations, the results of which represent a more widespread involvement of neuronal structures, intraparenchymal injections allowed us to localize the discrete anatomical sites for cardiovascular actions and to provide preliminary information on the peripheral mechanism(s) responsible for the effects elicited at each site by each peptide.

In the early 1980s, localization and physiological changes in the concentration of peptides were studied by the use of radioimmunoassay (RIA) (O'Donohue *et al.*, 1980a,b; 1981a,b; Charlton *et al.*, 1981; 1982; Helke *et al.*, 1981a,b; Moody *et al.*, 1981a,b; Tizabi *et al.*, 1982; 1985; Skofitsch and Jacobowitz, 1985b,d; 1986; Zamir *et al.*, 1986a,b; Rodriguez-Sierra *et al.*, 1987b). Technical advances allowed us to study the autoradiographic distribution of peptide binding sites and mRNA localization (Kamiya *et al.*, 1981; Rotter and Jacobowitz, 1984; Skofitsch *et al.*, 1985a,c; 1986; Clarke *et al.*, 1986; Hamill *et al.*, 1986; Jacobowitz and Skofitsch, 1986; 1991; Millan *et al.*, 1986; Millan *et al.*, 1990; Ramaswamy and Jacobowitz, 1990; Sutin and Jacobowitz, 1990a,b; Skofitsch and Jacobowitz, 1991; 1992a,b; Kresse *et al.*, 1992; 1995).

In 1983, a syndrome similar to idiopathic parkinsonism developed in humans after intravenous self-administration of an illicit drug preparation in which *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was responsible for the toxicity. At that time, Dr. Kopin and his group initiated a study of intravenous injection of MPTP into rhesus monkeys. I joined this group to carry out the catecholamine histofluorescent procedure in order to study the influence of this drug on the dopamine in substantia nigra/ventral tegmental area and the striatum in the monkey brain. We soon discovered that MPTP produced a disorder like parkinsonism (akinesia, rigidity, postural tremor, flexed posture, eyelid closure, drooling) that was reversed by the administration of L-dopa. MPTP treatment decreased the release of dopamine and dopamine accumulation in swollen, distorted axons in the nigrostriatal pathway just above the substantia nigra, followed by severe nerve loss in the pars compacta of the substantia nigra and a marked reduction in the dopamine content of the striatum. The pathological and biochemical changes produced by MPTP were similar to the well-established changes in patients with parkinsonism.

This landmark work provided a model that is used today to examine mechanisms and explore therapies of parkinsonism (Burns *et al.*, 1983). Interestingly, MPTP did not destroy the ventral tegmental area dopamine cell bodies (A10) in contrast to the A9 cells in the substantia nigra compacta (Jacobowitz *et al.*, 1984). This observation allowed us to explain why there was a transient behavioral recovery following tissue implants in hemiparkinsonian primates following intracarotid artery injection of MPTP (Bankiewicz *et al.*, 1986; 1988; 1990; 1991; Kopin *et al.*, 1993). The dopamine cells projected fibers to the medial aspect of the caudate nucleus which sprouted new fibers following surgical implantation of a variety of tissues.

In the early 1980s NIH had a policy whereby senior scientists were urged to undertake high-risk long-term research projects. The hope was that such undertakings would yield groundbreaking results (perhaps with clinical implications). It was in the midst of this highly productive peptide era that I made the judgment that there was a need for basic research on proteins. It seemed to me that as the peptide era stabilized, research of proteins would take off. At that time, the word "proteomics" was unknown. In 1975, a landmark publication by Patrick O'Farrell (1975) described a new procedure that was known as 2-dimensional gel electrophoresis (2DE). Over time, 2DE has proven to be a useful and powerful research technique, capable of resolving large numbers of proteins based upon their differences in both isoelectric points and molecular weight. This intrigued me since I had worked with 2-dimensional "filter paper" electrophoresis while working on my doctoral thesis (Jacobowitz *et al.*, 1963). Polyacrylamide gel replaced the filter paper.

This breakthrough launched the "protein era" for me. I established a new research program whose purpose was "protein identification". With Dr. Bill Heydorn, a postdoctoral fellow, and with the technical assistance of Joe Creed, we began our work on protein discovery utilizing 2DE. The long-term goal was to discover a unique protein that deserved our undivided attention.

At that time, few investigators had attempted to apply the technique of 2DE to the separation of proteins from nervous tissue. We initiated a study with the goal of surveying the major proteins visible by silver stain on two-dimensional polyacrylamide gels from a variety of discrete neuroanatomical regions of the brain. Here, my experience with brain mapping and the micropunch procedure proved invaluable. We studied 25 different regions of the rat brain, including a number of cortical areas as well as nuclei from the hypothalamus, amygdala, thalamus, forebrain, and hindbrain. After 2DE separation of the protein, we stained them using the procedure described by Merril *et al.* (Merrill *et al.*, 1981; 1982). We accomplished quantitative analysis of individual protein spots by computer-assisted densitometry based upon the procedure of Goldman *et al.* (1982). This was in the early days of the large computer with an image processor that required an operator to measure each gel successively for a particular protein spot. Patterns of proteins common to all gels were used for orientation, and a measurement window was then positioned over the protein of interest. Differences in the intensity of each protein, and hence the relative quantity of each protein, could be demonstrated among the different regions. We then assigned a permanent indexing number to each protein that we measured. We used this numbering system when identifying specific proteins for more detailed study. In each gel, the ordinate and abscissa were marked for molecular weight and isoelectric range, respectively.

The results demonstrated that all proteins examined varied somewhat in concentration among the different brain regions. The majority (53%) of proteins selected for quantitation were found to vary less than four-fold in concentration between the neuroanatomical areas with the lowest and highest detected amounts. In contrast, approximately 10% of the proteins examined varied widely in the quantity measured in each brain region, with concentration values ranging more than ten-fold between the regions with the lowest and highest detected amounts. The result of this study was once again an "atlas" containing the charge, mass, and when possible, the relative quantity of each of the proteins chosen for analysis. This atlas of brain proteins served as a basis for future studies of proteins from the central nervous system (Heydorn *et al.*, 1983; Jacobowitz and Heydorn, 1984).

At this time, (mid 1980s), mass spectrometry was not yet used to identify proteins. We used Western Blots for protein identification (Heydorn *et al.*, 1985a,b; 1986a,c; 1988; Narayan *et al.*, 1985; Santer *et al.*, 1988b). We simply pointed out protein spots on our 2DE gels that changed in density following experimental manipulations. For example, the influence of a variety of drugs (Heydorn *et al.*, 1984; 1986b; Narayan *et al.*, 1985; Rodriguez-Sierra *et al.*, 1986; Sills *et al.*, 1986), brain cancer (Narayan *et al.*, 1984; 1985; 1986a,b), and gonadal steroids (Scouten *et al.*, 1985b) were studied. Also, the uptake of ^{35}S-methionine into proteins (Gold *et al.*, 1984; Santer *et al.*, 1986; Rodriguez-Sierra *et al.*, 1987a), and the influence of stereotactic brain lesions on proteins in cholinergic and adrenergic neurons (Heydorn *et al.*, 1985c,d), in addition to sex differences (Gold *et al.*, 1983), genetic polymorphisms (Scouten *et al.*, 1985a), and iron deficiency (Youdim *et al.*, 1986) on protein density were studied.

A major breakthrough came when Dr. Lois Winsky, a post doctoral fellow in our laboratory, was studying proteins localized to the cochlear nucleus and other major auditory brain regions in the rabbit, guinea pig, and rats (Winsky *et al.*, 1989a,b). Using 2DE she revealed a protein designated as protein 10. The appearance of protein 10 on gels was similar to that of a calcium binding protein previously identified in our laboratory in ammonium sulfate fractions of rat brain (Santer *et al.*, 1988a). Incubation of nitrocellulose blots in $^{45}Ca^{2+}$ revealed that protein 10 was a calcium binding protein. We then went on to isolate and purify this protein from guinea pig brain using column chromatography. Then we generated rabbit antiserum to protein 10. Using a gas-phase sequencer, Dr. Brian Martin (Winsky *et al.*, 1989c) revealed that the amino acid sequence of fragments from proteolytic digestion of protein 10 had an 86% sequence identity with a calcium-binding protein that had been recently reported by Rogers (Rogers, 1987) who was studying calbindin in the chicken retina and isolated a cDNA clone that was approximately 50% homologous to calbindin. He named this protein "calretinin" which we thought a misnomer, because it was localized in many regions of the brain. At first, we thought it was a brain specific protein, but we later found it in sensory nerves in the periphery. However, we chose to stay with "calretinin" rather than confuse the literature.

At this point, the era of 2DE was over. In 1992, Dr. Ken Strauss, a postdoctoral fellow, joined us and initiated the era of molecular biology in the lab and soon published the nucleotide sequence of rat calretinin cDNA (Strauss and Jacobowitz, 1993a). He also developed a technique for the measurement of mRNA in rat brain micropunches without prior isolation of RNA (Strauss and Jacobowitz, 1993b; Strauss *et al.*, 1994a,b; 1995; Marini *et al.*, 1997).

Dr. Krystyna Isaacs set up cell culture in the laboratory and studied calretinin in cells of the substantia nigra compacta (Isaacs *et al.*, 1996; 1997 1998; 2000; Iwasaki *et al.*, 1998). She also mapped the colocalization of calretinin and tyrosine hydroxylase (dopamine-containing cells) in the rat substantia nigra (Isaacs and Jacobowitz, 1994), in addition to colocalization of calretinin with CGRP, VIP, and substance P in sensory nerves within villi of the rat intestine (Isaacs *et al.*, 1995).

With the antiserum that we generated, we did much work using immunofluroescence microscopy that revealed a wide distribution of calretinin throughout the brain (Winsky *et al.*, 1996; 1989c; 1992; Dechesne *et al.*, 1991; Jacobowitz and Winsky, 1991; Guylas *et al.*, 1992; Arai *et al.*, 1992a,b; 1993a,b,c; 1994; 1995; 1996; Ren *et al.*, 1993; Ichikawa *et al.*, 1993a,b; 1994a,b; 1995; 1997; Floris *et al.*, 1994; Abbott and Jacobowitz, 1995; 1999; Krzywkowski *et al.*, 1995; Montpied *et al.*, 1995; Winsky and Jacobowitz, 1995; Mineta *et al.*, 1996; Cimini *et al.*, 1997; Jacobowitz *et al.*, 1997; Parks *et al.*, 1997).

Dr. Jacek Kúznicki, a senior scientist from Poland, joined us and carried out biochemical studies (Strauss *et al.*, 1994c; Kuznicki *et al.*, 1994a,b; 1995a,b; 1996). He studied changes in hydrophobicity induced by Ca^{2+} binding which may be relevant for calretinin functions. He also showed that calretinin changes in conformation on calcium binding that involve the interaction of different parts of the molecule.

The work on calretinin was the culmination of a high-risk, long-term study using 2DE in search of an interesting protein. We spent approximately 10 years working on calretinin. Although the exact mechanism of action of the calcium binding protein is not known, it has become an important marker in a variety of cell types in the nervous system. It has also become an important protein and diagnostic marker in the pathophysiology of malignant mesothelioma, a cancer of the mesothelial cells lining the lung (Gotzos *et al.*, 1992; Schwaller *et al.*, 1995; 1997; Gander *et al.*, 1996). In our laboratory, Dr. Abraham Kallarakal discovered a point mutation in the coding region of the calretinin gene in mesothelioma cell lines that have resulted in an amino acid change of alanine 110 to threonine (CR-A110T) (Kallarakal *et al.*, 1999).

I am pleased that there are approximately 1,200 Pub Med citations identifying "calretinin." This somehow justifies the high-risk endeavor taken some 20 years ago. I credit our ability to thrive in this field to being a senior NIH scientist whose hands are not tied to a granting agency, but was allowed the freedom to explore and change direction freely. But of course, in the end, research productivity is anticipated.

One final freedom granted to me was an undertaking in the early 1990s. The question of the embryonic development of the brain arose. The mouse appeared to be the animal of choice because of all the work going on with "knockout," mutant and transgenic mice. I, therefore, initiated an immunohistochemical study on calretinin in the whole embryo brain at a variety of time studies (E11/12 to day of birth). When I entered these brains, I was shocked to learn that I knew nothing about the anatomical location of discrete regions in the developing brain. It soon became clear that this was yet another mountain to climb.

I began studying the enormous anatomical literature of the development of brain areas at different time periods. It was an eye-opener to realize how quickly things change in such short periods of time. After cutting and staining many embryonic brains, the light began to shine through and things began to fall into place. I then began to reach into my armamentarium of past experience with special stain, i.e., Nissl stain, AChE histochemical stain, and immunohistochemical stains for catecholamines, serotonin, calretinin and calbindin. I began accumulating sets of slides covering five different time periods. At about this time, Dr. Louise Abbott, a young scientist from the University of Illinois, visited my lab for 6 months and we undertook a study of calretinin development in the hindbrain (Abbott and Jacobowitz, 1995). Dr. Abbott became an expert in cutting these tiny brains in the cryostat. My technician (Quy Ha) helped with all the staining. I spent much of my day time at the microscope taking photos.

I soon realized that I had sets of slides of mice brains with six different stains. It became clear that each stain brought out different regions of the brain with the discrete localization of cell bodies, axons, and terminal fields. It was knowledge of these stains in the adult rat brain that helped the "light shine in" and made identification of brain regions and specific neuronal systems (e.g., dopaminergic, noradrenergic, and serotonergic pathways). At this time, I realized that I had the makings of an atlas of the developing mouse brain. The chemicals focused attention on the identification of brain regions and neuronal systems. This gave birth to the term "chemoarchitectonics" for the use in a brain atlas. It took two more years to complete the "Chemoarchitectonic Atlas of the Developing Mouse Brain." It was the first all-color atlas showing adjacent sections utilizing six different regions. It is currently the state-of-the-art atlas of the developing mouse brain. This allows research scientists to identify more accurately major anatomical structures in the developing brain not easily delineated by conventional Nissl and Myelin stains. The use of distinct precise chemical landmarks to derive chemoarchitectonic maps will help to reduce the subjectivity that can occur when deriving maps based solely on cytoarchitectonic criteria. The combination of chemical neuroanatomy with classical cytoarchitectonic criteria yields a powerful source of information, providing state-of-the-art brain cartography.

In a sense, it is the study of the neurotoxicity of the brain that focuses attention on "**Neurotoxicity Research**".

I am currently an Adjunct Professor at the Uniformed Services University across the street from NIH. I continue to do research in the Department of Anatomy, Physiology, and Genetics. I very much enjoy working with graduate students, postdoctoral fellows, and senior scientists. I am working with diseases of the brain (Parkinson's Disease, Canavan disease, schizophrenia, bipolar disease, the Alzheimer mouse, and the Down Syndrome mouse-trisomy). I am also collaborating on studies in the periphery, particularly cystic fibrosis (with Dr. Harvey Pollard's group), thyroid cancer, and pig ischemic hearts. I am learning much and I find that I can apply my 45 years of experience in a variety of projects.

And finally, to complete the cycle, I have set up once again 2-dimensional gel electrophoresis. It is comparatively easy to do because now we can purchase the first- and second-dimension gels. The major difference now is that with mass spectrometry, we can punch out the stained spots and identify the proteins within days. Can the life of a scientist be more wonderful than that?

References

Abbott LC and DM Jacobowitz (1995) Development of calretinin-immunoreactive unipolar brush-like cells and an afferent pathway to the embryonic and early postnatal mouse cerebellum. *Anat. Embryol. (Berl.)* **191**, 541-559.

Abbott LC and DM Jacobowitz (1999) Developmental expression of calretinin-immunoreactivity in the thalamic eminence of the fetal mouse. *Int. J. Dev. Neurosci.* **17**, 331-345.

Alpert M, DM Jacobowitz and BH Marks (1960) A simple method for the demonstration of lipofuscin pigment. *J. Histochem. Cytochem.* **8**, 153-158.

Arai M, R Arai, K Kani and DM Jacobowitz (1992a) Immunohistochemical localization of calretinin in the rat lateral geniculate nucleus and its retino-geniculate projection. *Brain*

Res. **596**, 215-222.

Arai R, DM Jacobowitz and T Hida (1992b) Calbindin D28k and calretinin in oxytocin and vasopressin neurons of the rat supraoptic nucleus. A triple-labeling immunofluorescence study. *Cell Tissue Res.* **298**, 11-19.

Arai M, R Arai, K Sasamoto, K Kani, T Maeda, S Deura and DM Jacobowitz (1993a) Appearance of calretinin-immunoreactive neurons in the upper layers of the rat superior colliculus after eye enucleation. *Brain Res.* **613**, 341-346.

Arai R, DM Jacobowitz and S Deura (1993b) Immuno-histochemical localization of calretinin-, calbindin-D28k- and parvalbumin-containing cells in the hypothalamic paraventricular and supraoptic nuclei of the rat. *Brain Res.* **618**, 323-327.

Arai R, DM Jacobowitz and S Deura (1993c) Ultrastructural localization of calretinin immunoreactivity in lobule V of the rat cerebellum. *Brain Res.* **613**, 300-304.

Arai R, DM Jacobowitz and S Deura (1994) Distribution of calretinin, calbindin-D28k, and parvalbumin in the rat thalamus. *Brain Res. Bull.* **33**, 595-614.

Arai R, DM Jacobowitz and I Nagatsu (1995) Up-regulation of calretinin in the supraoptic nucleus of the rat after chronic salt loading. *Brain Res.* **673**, 339-343.

Arai R, DM Jacobowitz and I Nagatsu (1996) Calretinin is differentially localized in magnocellular oxytocin neurons of the rat hypothalamus. A double-labeling immunofluorescence study. *Brain Res.* **735**, 154-158.

Bankiewicz KS, EH Oldfield, CC Chiueh, JL Doppman, DM Jacobowitz and IJ Kopin (1986) Hemiparkinsonism in monkeys after unilateral internal carotid artery infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Life Sci.* **39**, 7-16.

Bankiewicz KS, RJ Plunkett, IJ Kophin, DM Jacobowitz, WT London and EH Oldfield (1988) Transient behavioral recovery in hemiparkinsonian primates after adrenal medullary allografts. *Prog. Brain Res.* **78**, 543-549.

Bankiewicz KS, RJ Plunkett, IJ Kopin, DM Jacobowitz, U di Porzio, WT London and EH Oldfield (1990) Recovery from MPTP-induced parkinsonism in primates after fetal dopaminergic brain implants, histochemical and behavioral studies. *J. Neurosurgery* **72**, 231-244.

Bankiewicz KS, RJ Plunkett, DM Jacobowitz, IJ Kopin and EH Oldfield (1991) Fetal nondopaminergic neural implants in parkinsonian primates. Histochemical and behavioral studies. *J. Neurosurg*. **74**, 97-104.

Brus R and DM Jacobowitz (1970) The effects of DMPP on adrenergic nerves in perfused hearts. *Eur. J. Pharmacol.* **11**, 1-12.

Burns RS, CC Chiueh, SP Markey, MH Ebert, DM Jacobowitz and IJ Kopin (1983) A primate model of parkinsonism, selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci. USA* **80**, 4546-4550.

Charlton CG, TL O'Donohue, RL Miller and DM Jacobowitz (1981) Secretin immunoreactivity in rat and pig brain. *Peptides* **2** Suppl. 1, 45-49.

Charlton CG, TL O'Donohue, RL Miller and DM Jacobowitz (1982) Secretin in the rat hypothalamo-pituitary system, localization, identification and characterization. *Peptides* **3**, 565-567.

Cimini V, KR Isaacs and DM Jacobowitz (1997) Calretinin in the rat pituitary, colocalization with thyroid-stimulating hormone. *Neuroendocrinology* **65**, 179-188.

Clarke PB, GS Hamill, NS Nadi, DM Jacobowitz and A Pert (1986) ^{3}H-nicotine- and ^{125}I-alpha-bungarotoxin-labeled nicotinic receptors in the interpeduncular nucleus of rats. II. Effects of habenular deafferentation. *J. Comp. Neurol.* **251**, 407-413.

Coyle JT and D Henry (1973) Catecholamines in fetal and newborn rat brain. *J. Neurochem.* **21**, 61-67.

Crowley WR, TL O'Donohue, JM George and DM Jacobowitz (1978a) Changes in pituitary oxytocin and vasopressin during the estrous cycle and after ovarian hormones, evidence for mediation by norepinephrine. *Life Sci.* **23**, 2579-2585.

Crowley WR, TL O'Donohue and DM Jacobowitz (1978b) Sex differences in catecholamine content in discrete brain nuclei of the rat, effects of neonatal castration or testosterone treatment. *Acta Endocrinol. (Copenh.)* **89**, 20-28.

Crowley WR, TL O'Donohue, H Wachslicht and DM Jacobowitz (1978c) Effects of estrogen and progesterone on plasma gonadotropins and on catecholamine levels and turnover in discrete brain regions of ovariectomized rats. *Brain Res.* **154**, 345-357.

Crowley WR, TL O'Donohue and DM Jacobowitz (1979a) Involvement of central catecholamines in reproductive neuroendocrine processes, In *Catecholamines, Basic and Clinical Frontiers. Proceedings of 4th Intl. Catecholamine Symposium* (Usdin E, IJ Kopin and J Barchas, Eds.), (Pergamon Press, Oxford, England), pp 1272-1274.

Crowley WR, TL O'Donohue, EA Muth and DM Jacobowitz (1979b) Effects of ovarian hormones on levels of luteinizing hormone in plasma and on serotonin concentrations in discrete brain nuclei. *Brain Res. Bull.* **4**, 571-574.

Cruce JA, NB Thoa and DM Jacobowitz (1976) Catecholamines in the brains of genetically obese rats. *Brain Res.* **101**, 165-170.

Cruce JA, NB Thoa and DM Jacobowitz (1978) Catecholamines in discrete areas of the hypothalamus of obese and castrated male rats. *Pharmacol. Biochem. Behav.* **8**, 287-289.

Dahlström A and K Fuxe (1964) Evidence fo the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand.* **62**, 1-55.

Dahlström A and K Fuxe (1965) Evidence for the existence for monoamine neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neuron systems. *Acta Physiol. Scand.* **64**, 1-36.

Dechesne CJ, L Winsky, HN Kim, G Goping, TD Vu, RJ Wenthold and DM Jacobowitz (1991) Identification and ultrastructural localization of a calretinin-like calcium-binding protein (protein 10) in the guinea pig and rat inner ear. *Brain Res.* **560**, 139-148.

Diz DI and DM Jacobowitz (1983) Cardiovascular effects of intrahypothalamic injections of alpha-melanocyte stimulating hormone. *Brain Res.* **270**, 265-272.

Diz DI and DM Jacobowitz (1984a) Cardiovascular actions of four neuropeptides in the rat hypothalamus. *Clin. Exp. Hypertens. A* **6**, 2085-2090.

Diz DI and DM Jacobowitz (1984b) Cardiovascular effects of discrete intrahypothalamic and preoptic injections of bradykinin. *Brain Res. Bull.* **12**, 409-417.

Diz DI and DM Jacobowitz (1984c) Cardiovascular effects produced by injections of thyrotropin-releasing hormone in specific preoptic and hypothalamic nuclei in the rat. *Peptides* **5**, 801-808.

Diz DI, JA Vitale and DM Jacobowitz (1984) Increases in heart rate and blood pressure produced by injections of dermorphin into discrete hypothalamic sites. *Brain Res.* **294**, 47-57.

Falck B (1962) Observations on the possibilities for the cellular localization of monoamines with a fluroescence method. *Acta Physiol. Scand.* **56**, Suppl. 197, 1.

Falck B, NÅ Hillarp, G Thieme and A Torp (1962) Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J. Histochem. Cytochem.* **10**, 348-354.

Feuerstein G, SA Adelberg, IJ Kopin and DM Jacobowitz (1981a) Cardiovascular and sympathetic responses to PGF2 alpha injection into hypothalamic nuclei. *Brain Res. Bull.* **6**, 203-207.
Feuerstein G, S Adelberg, IJ Kopin and DM Jacobowitz (1981b) Central cardiovascular effects of prostacyclin. *Neuropharmacology* **20**, 1085-1090.
Feuerstein G, SA Adelberg, IJ Kopin and DM Jacobowitz (1982a) Hypothalamic sites for cardiovascular and sympathetic modulation by prostaglandin E2. *Brain Res.* **231**, 335-342.
Feuerstein G, CJ Helke, RL Zerbe, DM Jacobowitz and IJ Kopin (1982b) Mechanisms involved in central cardiovascular effects of prostaglandin F2 alpha. *Am. J. Physiol.* **242**, R545-R551.
Floris A, M Dino, DM Jacobowitz and E Mugnaini (1994) The unipolar brush cells of the rat cerebellar cortex and cochlear nucleus are calretinin-positive, a study by light and electron microscopic immunocytochemistry. *Anat. Embryol. (Berl.)* **189**, 495-520.
Friedman WF, PE Pool, DM Jacobowitz, SC Seagren and E Braunwald (1968) Sympathetic innervation of the developing rabbit heart. Biochemical and histochemical comparisons of fetal, neonatal, and adult myocardium. *Circ. Res.* **23**, 25-32.
Gander JC, V Gotzos, B Fellay and B Schwaller (1996) Inhibition of the proliferative cycle and apoptotic events in WiDr cells after down-regulation of the calcium-binding protein calretinin using antisense oligodeoxynucleotides. *Exp. Cell. Res.* **225**, 399-410.
Geffen LB, BG Livett and RA Rush (1969) Immunological localization of chromogranins in sheep sympathetic neurones, and their release by nerve impulses. *J. Physiol.* **204**, 58P-59P.
Gold MA, WE Heydorn, GJ Creed and DM Jacobowitz (1983) Sex differences in specific proteins in the preoptic medial nucleus of the rat hypothalamus. *Neuroendocrinology* **37**, 470-472.
Gold MA, WE Heydorn, GJ Creed, JL Weller, DC Klein and DM Jacobowitz (1984) *In vitro* [^{35}S]-methionine labeled protein synthesis in microdissected discrete brain areas, marked regional differences revealed by 2-dimensional gel electrophoresis. *Electrophoresis* **5**, 116-121.
Goldman D, CR Merril, RJ Polinsky and MH Ebert (1982) Lymphocyte proteins in Huntington's disease, quantitative analysis by use of two-dimensional electrophoresis and computerized densitometry. *Clin. Chem.* **28**, 1021-1025.
Goldman H and DM Jacobowitz (1971) Correlation of norepinephrine content with observations of adrenergic nerves after a single dose of 6-hydroxydopamine in the rat. *J. Pharmacol. Exp. Ther.* **176**, 119-133.
Gotzos V, B Schwaller, N Hetzel, M Bustos-Castillo and MR Celio (1992) Expression of the calcium binding protein calretinin in WiDr cells and its correlation to their cell cycle. *Exp. Cell. Res.* **202**, 292-302.
Grobecker H, MF Roizen, DM Jacobowitz and IJ Kopin (1977) Effect of prolonged treatment with adrenergic neuron blocking drugs on sympathoadrenal reactivity in rats. *Eur. J. Pharmacol.* **46**, 125-133.
Gulyas AI, R Miettinen, DM Jacobowitz and TF Freund (1992) Calretinin is present in non-pyramidal cells of the rat hippocampus--I. A new type of neuron specifically associated with the mossy fibre system. *Neuroscience* **48**, 1-27.
Hamill GS, PB Clarke, A Pert and DM Jacobowitz (1986) ^{3}H-nicotine and ^{125}I-alpha-bungarotoxin-labeled nicotinic receptors in the interpeduncular nucleus of rats. I. Subnuclear distribution. *J. Comp. Neurol.* **251**, 398-406.
Hanbauer I, DM Jacobowitz and IJ Kopin (1974) Effects of vinblastine on noradrenergic axons. *Br. J. Pharmacol.* **50**, 219-225.
Hartman BK (1973) Immunofluorescence of dopamine-ß-hydroxylase. Application of improved methodology to the localization of the peripheral and central noradrenergic nervous system. *J. Histochem. Cytochem.* **21**, 312-332.
Helke CJ, JA DiMicco, DM Jacobowitz and IJ Kopin (1981a) Effect of capsaicin administration to neonatal rats on the substance P content of discrete CNS regions. *Brain Res.* **222**, 428-431.
Helke CJ, DM Jacobowitz and NB Thoa (1981b) Capsaicin and potassium evoked substance P release from the nucleus tractus solitarius and spinal trigeminal nucleus *in vitro*. *Life Sci.* **29**, 1779-1785.
Heydorn WE, GJ Creed, D Goldman, D Kanter, CR Merril and DM Jacobowitz (1983) Mapping and quantitation of proteins from discrete nuclei and other areas of the rat brain by two-dimensional gel electrophoresis. *J. Neurosci.* **3**, 2597-2606.
Heydorn WE, GJ Creed and DM Jacobowitz (1984) Effect of desmethylimipramine and reserpine on the concentration of specific proteins in the parietal cortex and the hippocampus of rats as analyzed by two-dimensional gel electrophoresis. *J. Pharmacol. Exp. Ther.* **229**, 622-628.
Heydorn WE, GJ Creed, PJ Marangos and DM Jacobowitz (1985a) Identification of neuron-specific enolase and nonneuronal enolase in human and rat brain on two-dimensional polyacrylamide gels. *J. Neurochem.* **44**, 201-209.
Heydorn WE, GJ Creed, H Wada and DM Jacobowitz (1985b) Immunological evidence for the existence of two subforms of soluble glutamic oxaloacetic transaminase (sGOT) in human and rat brain. *Neurochem. Int.* **7**, R33-R41.
Heydorn WE, KQ Nguyen, GJ Creed, and DM Jacobowitz (1985c) Effect of reduction of cholinergic input on the concentration of specific proteins in different cortical regions of the rat brain. *Brain Res.* **339**, 209-218.
Heydorn WE, GJ Creed, CR Creveling and DM Jacobowitz (1986a) Studies on catechol-*O*-methyltransferase in rat brain using two dimensional gel electrophoresis. *Neurochem. Int.* **8**, 581-588.
Heydorn WE, GJ Creed, KQ Nguyen and DM Jacobowitz (1986b) Effect of 5,7-dihydroxytryptamine on the concentration of individual proteins in different areas of the rat brain. *Brain Res.* **368**, 193-196.
Heydorn WE, P Gierschik, GJ Creed, G Milligan, A Spiegel and DM Jacobowitz (1986c) The beta subunit of the guanine nucleotide regulatory proteins, identification of its location on two-dimensional gels of brain tissue and its regional and subcellular distribution in brain. *J. Neurosci. Res.* **16**, 541-552.
Heydorn WE, KQ Nguyen, GJ Creed, RM Kostrzewa and DM Jacobowitz (1986d) Effects of bilateral lesion of the locus coeruleus and of neonatal administration of 6-hydroxydopamine on the concentration of individual proteins in rat brain. *Brain Res.* **367**, 31-38.
Heydorn WE and DM Jacobowitz (1988) The use of two-dimensional gel electrophoresis to study proteins in the central nervous system, In *Neuronal and Glial Proteins* (Marangos PJ, Campbell and RM Cohen, Eds.), (Academic Press: New York, NY), pp 25-68.
Hoover DB, EA Muth and DM Jacobowitz (1977) A method for sectioning microwave-fixed brain prior to microdissection and acetylcholine analysis. *Neurosci. Lett.* **5**, 247-251.
Hoover DB, EA Muth and DM Jacobowitz (1978) A mapping of the distribution of acetycholine, choline acetyltransferase and acetylcholinesterase in discrete areas of rat brain. *Brain Res.* **153**, 295-306.
Ichikawa H, DM Jacobowitz and T Sugimoto (1992) Calretinin-immunoreactivity in the oro-facial and pharyngeal regions of the

rat. *Neurosci. Lett.* **146**, 155-158.

Ichikawa H, DM Jacobowitz and T Sugimoto (1993a) Calretinin-immunoreactive neurons in the trigeminal and dorsal root ganglia of the rat. *Brain Res.* **617**, 96-102.

Ichikawa H, S Mitani, H Hijiya, T Nakago, DM Jacobowitz and T Sugimoto (1993b) Calretinin-immunoreactivity in trigeminal neurons innervating the nasal mucosa of the rat. *Brain Res.* **629**, 231-238.

Ichikawa H, T Deguchi, S Mitani, T Nakago, DM Jacobowitz, T Yamaai and T Sugimoto (1994a) Neural parvalbumin and calretinin in the tooth pulp. *Brain Res.* **647**, 124-130.

Ichikawa H, T Deguchi, T Nakago, DM Jacobowitz and T Sugimoto (1994b) Parvalbumin, calretinin and carbonic anhydrase in the trigeminal and spinal primary neurons of the rat. *Brain Res.* **655**, 241-245.

Ichikawa H, T Deguchi, T Nakago, DM Jacobowitz and T Sugimoto (1995) Parvalbumin- and calretinin-immunoreactive trigeminal neurons innervating the rat molar tooth pulp. *Brain Res.* **679**, 205-211.

Ichikawa H, DM Jacobowitz and T Sugimoto (1997) Coexpression of calretinin and parvalbumin in Ruffini-like endings in the rat incisor periodontal ligament. *Brain Res.* **770**, 294-297.

Isaacs KR and DM Jacobowitz (1994) Mapping of the colocalization of calretinin and tyrosine hydroxylase in the rat substantia nigra and ventral tegmental area. *Exp. Brain Res.* **99**, 34-42.

Isaacs KR, L Winsky, KI Strauss and DM Jacobowitz (1995) Quadruple colocalization of calretinin, calcitonin gene-related peptide, vasoactive intestinal peptide, and substance P in fibers within the villi of the rat intestine. *Cell. Tissue Res.* **280**, 639-651.

Isaacs KR, G de Erausquin, KI Strauss, DM Jacobowitz and I Hanbauer (1996) Differential effects of excitatory amino acids on mesencephalic neurons expressing either calretinin or tyrosine hydroxylase in primary cultures. *Brain Res. Mol. Brain Res.* **36**, 114-126.

Isaacs KR, ME Wolpoe and DM Jacobowitz (1997) Calretinin-immunoreactive dopaminergic neurons from embryonic rat mesencephalon are resistant to levodopa-induced neurotoxicity. *Exp. Neurol.* **146**, 25-32.

Isaacs KR, I Hanbauer and DM Jacobowitz (1998) A method for the rapid analysis of neuronal proportions and neurite morphology in primary cultures. *Exp. Neurol.* **149**, 464-467.

Isaacs KR, ME Wolpoe and DM Jacobowitz (2000) Vulnerability to calcium-induced neurotoxicity in cultured neurons expressing calretinin. *Exp. Neurol.* **163**, 311-323.

Iwasaki K, KR Isaacs and DM Jacobowitz (1998) Brain-derived neurotrophic factor stimulates neurite outgrowth in a calretinin-enriched neuronal culture system. *Int. J. Dev. Neurosci.* **16**, 135-145.

Jacobowitz DM (1965a) A method for the demonstration of both acetycholinesterase and catecholamine in the same nerve trunk. *Life Sci.* **12**, 297-303.

Jacobowitz DM (1965b) Histochemical studies of the autonomic innervation of the gut. *J. Pharmacol. Exp. Ther.* **149**, 358-364.

Jacobowitz DM (1967) Histochemical studies of the relationship of chromaffin cells and adrenergic nerve fibers to the cardiac ganglia of several species. *J. Pharmacol. Exp. Ther.* **158**, 227-240.

Jacobowitz DM (1970) Catecholamine fluorescence studies of adrenergic neurons and chromaffin cells in sympathetic ganglia. *Fed. Proc.* **29**, 1929-1944.

Jacobowitz DM (1974) The Peripheral Autonomic System, In *The Peripheral Autonomic Nervous System* (Hubbard JI, Ed.) (Plenum Press, New York), pp 87-110.

Jacobowitz DM (1975) Long-term effects on periheral adrenergic nerves of 6-hydroxydopamine injected into newborn rats, In *Chemical Tools in Catecholamine Research. 6-Hydroxydopamine as a Ddenervation Tool in Catecholamine Research* (Jonsson G, T Malmfors and C Sachs, Eds.) (North-Holland Publishing Co.: Amsterdam, The Netherlands), pp 153-162.

Jacobowitz DM (1978) Monaminergic pathways in the central nervous system, In *Psychopharmacology, A Generation of Progress* (Lipton MA, A Di Mascio and K Killiam, Eds.) (Raven Press, New York), pp. 119-130.

Jacobowitz DM and LC Abbott (1998) *Chemoarchitectonic Atlas of the Developing Mouse Brain* (CRC Press: Boca Raton, FL).

Jacobowitz DM and LA Greene (1974) Histofluorescence study of chromaffin cells in dissociated cell cultures of chick embryo sympathetic ganglia. *J. Neurobiol.* **5**, 65-83.

Jacobowitz DM and WE Heydorn (1984) Two-dimensional gel electrophoresis used in neurobiological studies of proteins in discrete areas of the rat brain. *Clin. Chem.* **30**, 1996-2002.

Jacobowitz DM and GB Koelle (1965) Histochemical correlations of acetylcholinesterase and catecholamines in postganglionic autonomic nerves of the cat, rabbit, and guinea pig. *J. Pharmacol. Exp. Ther.* **148**, 225-237.

Jacobowitz DM and R Kostrzewa (1971) Selective action of 6-hydroxydopa on noradrenergic terminals, mapping of preterminal axons of the brain. *Life Sci.* **10**, 1329-1342.

Jacobowitz DM and AM Laties (1968) Direct adrenergic innervation of a teleost melanophore. *Anat. Rec.* **162**, 501-504.

Jacobowitz DM and PD MacLean (1978) A brainstem atlas of catecholaminergic neurons and serotonergic perikarya in a pygmy primate (*Cebuella pygmaea*). *J. Comp. Neurol.* **177**, 397-416.

Jacobowitz DM and P Nemir Jr (1969) The autonomic innervation of the esophagus of the dog. *J. Thorac. Cardiovasc. Surg.* **58**, 678-684.

Jacobowitz DM and TL O'Donohue (1978) alpha-Melanocyte stimulating hormone, immunohistochemical identification and mapping in neurons of rat brain. *Proc. Natl. Acad. Sci. USA* **75**, 6300-6304.

Jacobowitz DM and M Palkovits (1974) Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. I. Forebrain (telencephalon, diencephalon). *J. Comp. Neurol.* **157**, 13-28.

Jacobowitz DM and G Skofitsch (1986) Calcitonin gene-related peptide in the central nervous system, neuronal and receptor localization, biochemical characterization and functional studies, In *Neural and Endocrine Peptides and Receptors* (Moody TW, Ed.) (Plenum Publishing Co.: New York), pp 247-288.

Jacobowitz DM and G Skofitsch (1991) Localization of galanin cell bodies in the brain by immunocytochemistry and in situ hybridization histochemistry, In *International Symposium on Galanin, a new Multifunctional Peptide in the Neuro-endocrine System* (Hökfelt T, T Bartfai, DM Jacobowitz and D Ottoson, Eds.) (Wenner-Gren Intl. Symposium Series, London), pp 69-92.

Jacobowitz DM and EE Wallach (1967) Histochemical and chemical studies of the autonomic innervation of the ovary. *Endocrinology* **81**, 1132-1139.

Jacobowitz DM and L Winsky (1991) Immunocytochemical localization of calretinin in the forebrain of the rat. *J. Comp. Neurol.* 304, 198-218.

Jacobowitz DM and JK Woodward (1968) Adrenergic neurons in the cat superior cervical ganglion and cervical sympathetic nerve trunk. A histochemical study. *J. Pharmacol. Exp. Ther.* **162**, 213-226.

Jacobowitz DM, BH Marks and J Vernikos-Danellis (1963) The effect of acute stress on the pituitary uptake on serine -1-C^{14} into ACTH. *Endocrinology* **72**, 592-597.

Jacobowitz DM, T Cooper and HB Barner (1967) Histochemical and chemical studies of the localization of adrenergic and cholinergic nerves in normal and denervated cat hearts. *Circ. Res.* **20**, 289-298.

Jacobowitz DM, AH Katcher, LH Turner and SN Wampler (1975) Acute and chronic effects of 6-hydroxydopamine in dogs, In, *Chemical Tools in Catecholamine Research. 6-Hydroxydopamine as a Denervation Tool in Catecholamine Research* (Jonsson G, T Malmfors and C Sachs, Eds.) (North-Holland Publishing Co.: Amsterdam, The Netherlands), pp 239-245.

Jacobowitz DM, LA Greene and NB Thoa (1976) Chromaffin cells in culture. *SHEW Publ No (NIH) 76-942, Fogarty Center Conference*, 215-222.

Jacobowitz DM, TL O'Donohue, WY Chey and TM Chang (1981) Mapping of motilin-immunoreactive neurons of the rat brain. *Peptides* **2**, 479-487.

Jacobowitz DM, H Schulte, GP Chrousos and DL Loriaux (1983) Localization of GRF-like immunoreactive neurons in the rat brain. *Peptides* **4**, 521-524.

Jacobowitz DM, RS Burns, CC Chiueh and IJ Kopin (1984) *N*-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) causes destruction of the nigrostriatal but not the mesolimbic dopamine system in the monkey. *Psychopharmacol. Bull.* **20**, 416-422.

Jacobowitz DM, KR Isaacs and V Cimini (1997) Triple colocalization of tyrosine hydroxylase, calretinin, and calbindin D-28k in the periventricular hypophyseal dopaminergic neuronal system. *Adv. Pharmacology* **42**, 37-40.

Jacobowitz DM, A Kresse and G Skofitsch (2004) Galanin in the brain, chemoarchitectonics and brain cartography - a historical review. *Peptides* **25**, 433-464.

Kallarakal AT, J Kuznicki, L Winsky, K Amin and DM Jacobowitz (1999) Identification and characterization of a point mutation in the coding region of calretinin in mesothelioma. *Proc. Am. Assn. Cancer Res.*, Philadelphia, PA, pp 279-280.

Kamiya HO, A Rotter and DM Jacobowitz (1981) Muscarinic receptor binding following cholinergic nerve lesions of the cingulate cortex and hippocampus of the rat. *Brain Res.* **209**, 432-439.

Kobayashi RM, M Palkovits, JS Kizer, DM Jacobowitz and IJ Kopin (1975) Selective alterations of catecholamines and tyrosine hydroxylase activity in the hypothalamus following acute and chronic stress, In *Catecholamines and Stress* (Usdin E, R Kvetnansky and IJ Kopin, Eds.) (Pergamon Press: Oxford, England), pp 29-38.

König JFR and RA Klippel (1963) *The Rat Brain. A Sterotaxic Atlas* (Krieger Publishing: New York).

Kopin IJ, KS Bankiewicz, RJ Plunkett, DM Jacobowitz and EH Oldfield (1993) Tissue implants in treatment of parkinsonian syndromes in animals and implications for use of tissue implants in humans. *Adv. Neurol.* **60**, 707-714.

Kostrzewa R and DM Jacobowitz (1972) The effect of 6-hydroxydopa on peripheral adrenergic neurons. *J. Pharmacol. Exp. Ther.* **183**, 284-297.

Kostrzewa R and DM Jacobwitz (1973) Acute effects of 6-hydroxydopa on central monoaminergic neurons. *Eur. J. Pharmacol.* **21**, 70-80.

Kostrzewa RM and DM Jacobowitz (1974) Pharmacological actions of 6-hydroxydopamine. *Pharmacol. Rev.* **26**, 199-288.

Kresse A, DM Jacobowitz and G Skofitsch (1992) Distribution of calcitonin gene-related peptide in the central nervous system of the rat by immunocytochemistry and *in situ* hybridization histochemistry. *Ann. NY Acad. Sci.* **657**, 455-457.

Kresse A, DM Jacobowitz and G Skofitsch (1995) Detailed mapping of CGRP mRNA expression in the rat central nervous system, comparison with previous immunocytochemical findings. *Brain Res. Bull.* **36**, 261-274.

Krzywkowski P, DM Jacobowitz and Y Lamour (1995) Calretinin-containing pathways in the rat forebrain. *Brain Res.* **705**, 273-294.

Kuznicki J, L Winsky and DM Jacobowitz (1994a) Ca(2+)-dependent and independent interactions of calretinin with hydrophobic resins. *Biochem. Mol. Biol. Int.* **33**, 713-721.

Kuznicki J, L Winsky and DM Jacobowitz (1994b) Hydrophobicity of calretinin, Hetergeneity and partial calcium dependence. *Biochem. Mol. Biol.* **33**, 712-720.

Kuznicki J, KI Strauss and DM Jacobowitz (1995a) Conformational changes and calcium binding by calretinin and its recombinant fragments containing different sets of EF hand motifs. *Biochemistry* **34**, 15389-15394.

Kuznicki J, TL Wang, BM Martin, L Winsky and DM Jacobowitz (1995b) Localization of Ca(2+)-dependent conformational changes of calretinin by limited tryptic proteolysis. *Biochem. J.* **308**, 607-612.

Kuznicki J, KR Isaacs and DM Jacobowitz (1996) The expression of calretinin in transfected PC12 cells provides no protection against Ca(2+)-overload or trophic factor deprivation. *Biochim. Biophys. Acta* **1313**, 194-200.

Laties A and DM Jacobowitz (1964) A histochemical study of the adrenergic and cholinergic innervation of the anterior segment of the rabbit eye. *Invest. Ophthalmol.* **47**, 592-600.

Laties AM and DM Jacobowitz (1966) A comparative study of the autonomic innervation of the eye in monkey, cat, and rabbit. *Anat. Rec.* **156**, 383-395.

Lewis MJ, JL Costa, DM Jacobowitz and DL Margules (1976) Tolerance, physical dependence and opiod-seeking behavior, dependence of diencephalic norepinephrine. *Brain Res.* **107**, 156-165.

Liuzzi A, FH Foppen, JM Saavedra, DM Jacobowitz and IJ Kopin (1977) Effect of NGF and dexamethasone on phenylethanolamine-*N*-methyl transferase (PNMT) activity in neonatal rat superior cervical ganglia. *J. Neurochem.* **28**, 1215-1220.

Marini AM, KI Strauss and DM Jacobowitz (1997) Calretinin-containing neurons in cultured rat cerebellar cultures. *Brain Res. Bull.* **42**, 279-288.

Massari VJ, Z Gottesfeld and DM Jacobowitz (1976) Distribution of glutamic acid decarboxylase in certain rhombenecephalic and thalamic nuclei of the rat. *Brain Res.* **118**, 147-151.

Massari VJ, Y Tizabi, Z Gottesfeld and DM Jacobowitz (1978) A fluorescence histochemical and biochemical evaluation of the effect of p-chloroamphetamine on individual serotonergic nuclei in the rat brain. *Neuroscience* **3**, 339-344.

Merril CR, D Goldman, SA Sedman and MH Ebert (1981) Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. *Science* **211**, 1437-1438.

Merril CR, D Goldman and ML VanKeuren (1982) Simplified silver protein detection and image enhancement in polyacrylamide gels. *Electrophoresis* **3**, 17-23.

Miliaressis E, A Bouchard and DM Jacobowitz (1975) Strong positive reward in median raphe, specific inhibition by parachlorophenylalanine. *Brain Res.* **98**, 194-201.

Miliaressis E and DM Jacobowitz (1976) Hyperthermia following self-stimulation of the median raphe in the rat. *Pharmacol. Biochem. Behav.* **4**, 477-479.

Millan MA, DM Jacobowitz, RL Hauger, KJ Catt and G Aguilera (1986) Distribution of corticotropin-releasing factor receptors in primate brain. *Proc. Natl. Acad. Sci. USA* **83**, 1921-1925.

Millan MA, DM Jacobowitz, KJ Catt and G Aguilera (1990) Distribution of angiotensin II receptors in the brain of nonhuman primates. *Peptides* **11**, 243-253.

Mineta Y, H Koyanagi, M Morimoto, K Harano, T Totoki and DM Jacobowitz (1996) Immunocytochemical study of parvalbumin, calbindin D-28k, and calretinin in the superficial dorsal horn of the rat spinal cord following unilateral hindpaw inflammation. *J. Anesth.* **10**, 211-217.

Montpied P, L Winsky, JW Dailey, PC Jobe and DM Jacobowitz (1995) Alteration in levels of expression of brain calbindin D-28k and calretinin mRNA in genetically epilepsy-prone rats. *Epilepsia* **36**, 911-921.

Moody TW, TL O'Donohue and DM Jacobowitz (1981a) Biochemical localization and characterization of bombesin-like peptides in discrete regions of rat brain. *Peptides* **2**, 75-79.

Moody TW, NB Thoa, TL O'Donohue and DM Jacobowitz (1981b) Bombesin-like peptides in rat spinal cord, biochemical characterization, localization and mechanism of release. *Life Sci.* **29**, 2273-2279.

Moyer JA, LR Herrenkohl and DM Jacobowitz (1977) Effects of stress during pregnancy on catecholamines in discrete brain regions. *Brain Res.* **121**, 385-393.

Moyer JA, LR Herrenkohl and DM Jacobowitz (1978) Stress during pregnancy, effect on catecholamines in discrete brain regions of offspring as adults. *Brain Res.* **144**, 173-178.

Narayan RK, WE Heydorn, GJ Creed, PL Kornblith and DM Jacobowitz (1984) Proteins in normal, irradiated, and postmortem human brain quantitatively compared by using two-dimensional gel electrophoresis. *Clin. Chem.* **30**, 1989-1995.

Narayan RK, WE Heydorn, GJ Creed and DM Jacobowitz (1985) Identification of major proteins in human cerebral cortex and brain tumors. *J. Protein Chem.* **4**, 375-389.

Narayan RK, WE Heydorn, GJ Creed and DM Jacobowitz (1986a) Protein patterns in various malignant human brain tumors by two-dimensional gel electrophoresis. *Cancer Res.* **46**, 4685-4694.

Narayan RK, WE Heydorn, GJ Creed, PL Kornblith and DM Jacobowitz (1986b) Two-dimensional gel electrophoretic protein patterns in high-grade human astrocytomas, In *Biology of Brain Tumour* (Walker MD and DGT Thomas, Eds.) (Martinus Nijhoff Publishers), pp 7-14.

Nemir P Jr, M Fallahnejad, B Bose, DM Jacobowitz, AS Frobese and HR Hawthorne (1971) A study of the causes of failure of esophagocardiomyotomy for achalasia. *Am. J. Surg.* **121**, 143-149.

Nguyen KQ, MA Sills and DM Jacobowitz (1986) Cardiovascular effects produced by microinjection of calcitonin gene-related peptide into the rat central amygdaloid nucleus. *Peptides* **7**, 337-339.

O'Donohue TL and DM Jacobowitz (1980a) Studies of alpha-melanotropin in the central nervous system, In *Polypeptide Hormones* (Beers RF and EG Bassett, Eds.) (Raven Press: New York), pp 203-222.

O'Donohue TL and DM Jacobowitz (1980b) Studies of alpha-MSH-containing nerves in the brain, In *Progress in Biochemical Pharmacology. Endogenous Peptides and Centrally Active Drugs* (Paoletti R, Ed.) (Karger Press: Basel), pp 69-83.

O'Donohue TL, CG Charlton, CJ Helke, DM Jacobowitz and RL Miller (1979a) Identification of alpha-melanocyte stimulating hormone immunoreactivity in rat and human brain and cerebrospinal fluid, In *Peptides, Structure and Biological Function* (Gross E and J Meienhofer, Eds.) (Pierce Chemical Co.: Rockford, IL), pp 897-900.

O'Donohue TL, GE Holmquist and DM Jacobowitz (1979b) Effect of hypophysectomy on alpha-melanotropin in discrete regions of the rat brain. *Neurosci. Lett.* **14**, 271-274.

O'Donohue TL, RL Miller and DM Jacobowitz (1979c) Identification, characterization and stereotaxic mapping of intraneuronal alpha-melanocyte stimulating hormone-like immunoreactive peptides in discrete regions of the rat brain. *Brain Res.* **176**, 101-123.

O'Donohue TL, RL Miller, RC Pendleton and DM Jacobowitz (1979d) A diurnal rhythm of immunoreactive alpha-melanocyte-stimulating hormone in discrete regions of the rat brain. *Neuroendocrinology* **29**, 281-287.

O'Donohue TL, VJ Massari, Y Tizabi and DM Jacobowitz (1980a) Identification and distribution of alpha-melanotropin in discrete regions of the cat brain. *Brain Res. Bull.* **4**, 829-832.

O'Donohue TL, RL Miller, RC Pendleton and DM Jacobowitz (1980b) Demonstration of an endogenous circadian rhythm of alpha-melanocyte stimulating hormone in the rat pineal gland. *Brain Res.* **186**, 145-155.

O'Donohue TL, MC Beinfeld, WY Chey, TM Chang, G Nilaver, EA Zimmerman, H Yajima, H Adachi, M Poth, RP McDevitt and DM Jacobowitz (1981a) Identification, characterization and distribution of motilin immunoreactivity in the rat central nervous system. *Peptides* **2**, 467-477.

O'Donohue TL, CG Charlton, NB Thoa, CJ Helke, TW Moody, A Pert, A Williams, RL Miller and DM Jacobowitz (1981b) Release of alpha-melanocyte stimulating hormone into rat and human cerebrospinal fluid *in vivo* and from rat hypothalamus slices *in vitro*. *Peptides* **2**, 93-100.

O'Donohue TL, GE Handelmann, T Chaconas, RL Miller and DM Jacobowitz (1981c) Evidence that *N*-acetylation regulates the behavioral activity of alpha-MSH in the rat and human central nervous system. *Peptides* **2**, 333-344.

O'Donohue TL, GE Handelmann, RL Miller and DM Jacobowitz (1982) N-acetylation regulates the behavioral activity of alpha-melanotropin in a multineurotransmitter neuron. *Science* **215**, 1125-1127.

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**, 4007-4021.

Olschowka JA, TL O'Donohue and DM Jacobowitz (1981) The distribution of bovine pancreatic polypeptide-like immunoreactive neurons in rat brain. *Peptides* **2**, 309-331.

Palkovits M (1973) Isolated removal of hypothalamic or other brain nuclei of the rat. *Brain Res.* **59**, 449-450.

Palkovits M and DM Jacobowitz (1974) Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. II. Hindbrain (mesencephalon, rhombencephalon). *J. Comp. Neurol.* **157**, 29-42.

Palkovits M, JS Richardson and DM Jacobowitz (1974) A histochemical study of ventral tegmental acetylcholinesterase-containing pathway following destructive lesions. *Brain Res.* **81**, 183-188.

Palkovits M, RM Kobayashi, JS Kizer, DM Jacobowitz and IJ Kopin (1975) Effects of stress on catecholamines and tyrosine hydroxylase activity of individual hypothalamic nuclei. *Neuroendocrinology* **18**, 144-153.

Parks TN, RA Code, DA Taylor, DA Solum, KI Strauss, DM Jacobowitz and L Winsky (1997) Calretinin expression in the chick brain stem auditory nuclei develops and is maintained independently of cochlear nerve input. *J. Comp. Neurol.* **383**, 112-121.

Patil PN and DM Jacobowitz (1968) Steric aspects of adrenergic drugs. IX. Pharmacologic and histochemical studies on isomers of cobefrin (alpha-methylnorepinephrine). *J. Pharmacol. Exp. Ther.* **161**, 279-295.

Patil PN, K Fudge and DM Jacobowitz (1972) Steric aspects of adrenergic drugs. 18. Alpha-Adrenergic receptors of mammalian aorta. *Eur. J. Pharmacol.* **19**, 79-87.

Raezer DM, AJ Wein, DM Jacobowitz and JN Corriere Jr (1973) Autonomic innervation of canine urinary bladder. Cholinergic and adrenergic contributions and interaction of sympathetic and parasympathetic nervous systems in bladder function. *Urology* **2**, 211-221.

Ramaswamy SG and DM Jacobowitz (1990) A novel autoantibody from a rabbit preimmune serum that immunostains myelinated nerves of the brain. *Brain Res. Bull.* **25**, 193-197.

Ren K, MA Ruda and DM Jacobowitz (1993) Immunohistochemical localization of calretinin in the dorsal root ganglion and spinal cord of the rat. *Brain Res. Bull.* **31**, 13-22.

Richardson JS and DM Jacobowitz (1973) Depletion of brain norepinephrine by intraventricular injection of 6-hydroxydopa, a biochemical, histochemical and behavioral study in rats. *Brain Res.* **58**, 117-133.

Richardson JS, N Cowan, R Hartman and DM Jacobowitz (1974) On the behavioral and neurochemical actions of 6-hydroxydopa and 5,6-dihydroxytryptamine in rats. *Res. Commun. Chem. Pathol. Pharmacol.* **8**, 29-44.

Rodriguez-Sierra JF, WE Heydorn, GJ Creed and DM Jacobowitz (1986) Isolation of specific proteins affected by estradiol in the arcuate-median eminence of prepuberal female rats. *Brain Res.* **399**, 379-382.

Rodriguez-Sierra JF, WE Heydorn, GJ Creed and DM Jacobowitz (1987a) Incorporation of amino acids into proteins of the hypothalamus of prepuberal female rats after estradiol treatment. *Neuroendocrinology* **45**, 459-464.

Rodriguez-Sierra JF, DM Jacobowitz and CA Blake (1987b) Effects of neuropeptide Y on LH, FSH and TSH release in male rats. *Peptides* **8**, 539-542.

Rogers JH (1987) Calretinin, a gene for a novel calcium-binding protein expressed principally in neurons. *J. Cell. Biol.* **105**, 1343-1353.

Roizen MF, IJ Kopin, M Palkovits, M Brownstein, JS Kizer and DM Jacobowitz (1975) The effect of two diverse inhalation anesthetic agents on serotonin in discrete regions of the rat brain. *Exp. Brain Res.* **24**, 203-207.

Rotter A and DM Jacobowitz (1984) Localization of substance P, acetylcholinesterase, muscarinic receptors and alpha-bungarotoxin binding sites in the rat interpeduncular nucleus. *Brain Res. Bull.* **12**, 83-94.

Santer DM, WE Heydorn, GJ Creed, DC Klein and DM Jacobowitz (1986) Subfornical organ, effects of salt loading and water deprivation on *in vitro* radioamino acid incorporation into individual proteins. *Brain Res.* **372**, 107-114.

Santer DM, WE Heydorn, GJ Creed, T Fukuda and DM Jacobowitz (1988a) Localization of calcium binding proteins of rat cortex on 2-dimensional gels II. Analysis of calcium binding proteins in ammonium sulfate fractions of rat brain. *Neurochem. Int.* **12**, 225-236.

Santer DM, WE Heydorn, GJ Creed and DM Jacobowitz (1988b) Localization of calcium binding proteins in rat cortex on 2-dimensional gels I. Identification of calmodulin and the beta subunit of calcineurin. *Neurochem. Int.* **12**, 215-223.

Schwaller B, MR Celio and W Hunziker (1995) Alternative splicing of calretinin mRNA leads to different forms of calretinin. *Eur. J. Biochem.* **230**, 424-430.

Schwaller B, I Durussel, D Jermann, B Herrmann and JA Cox (1997) Comparison of the Ca^{2+}-binding properties of human recombinant calretinin-22k and calretinin. *J. Biol. Chem.* **272**, 29663-29671.

Scouten CW, WE Heydorn, GJ Creed, CW Malsbury and DM Jacobowitz (1985a) An apparent genetic polymorphism for a protein present in the hypothalamus of Sprague-Dawley rats. *Brain Res.* **330**, 170-173.

Scouten CW, WE Heydorn, GJ Creed, CW Malsbury and DM Jacobowitz (1985b) Proteins regulated by gonadal steroids in the medial preoptic and ventromedial hypothalamic nucleic of male and female rats. *Neuroendocrinology* **41**, 237-245.

Sills MA, WE Heydorn, RM Cohen, GJ Creed and DM Jacobowitz (1986) Effect of chronic treatment with clorgyline on the relative concentration of specific proteins in the hippocampus and parietal cortex of the rat. *Neuropharmacology* **25**, 143-150.

Sills MA and DM Jacobowitz (1988) Propranolol and methylatropine antagonize the cardiovascular effects produced by microinjection of the TRH analog MK-771 into the preoptic suprachiasmatic nucleus. *Peptides* **9**, 893-898.

Sills MA, KQ Nguyen and DM Jacobowitz (1985) Increases in heart rate and blood pressure produced by microinjections of atrial natriuretic factor into the AV3V region of rat brain. *Peptides* **6**, 1037-1042.

Skofitsch G and DM Jacobowitz (1985a) Calcitonin gene-related peptide coexists with substance P in capsaicin sensitive neurons and sensory ganglia of the rat. *Peptides* **6**, 747-754.

Skofitsch G and DM Jacobowitz (1985b) Distribution of corticotropin releasing factor-like immunoreactivity in the rat brain by immunohistochemistry and radioimmunoassay, comparison and characterization of ovine and rat/human CRF antisera. *Peptides* **6**, 319-336.

Skofitsch G and DM Jacobowitz (1985c) Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. *Peptides* **6**, 509-546.

Skofitsch G and DM Jacobowitz (1985d) Quantitative distribution of calcitonin gene-related peptide in the rat central nervous system. *Peptides* **6**, 1069-1073.

Skofitsch G and DM Jacobowitz (1986) Quantitative distribution of galanin-like immunoreactivity in the rat central nervous system. *Peptides* **7**, 609-613.

Skofitsch G and DM Jacobowitz (1991) Distribution of galanin binding sites in the central nervous system, In *International Symposium on Galanin, a new Multifunctional Peptide in the Neuro-endocrine System* (Hokfelt T, T Bartfai, DM Jacobowitz and D Ottoson, Eds.) (Wenner-Gren International Symposium Series), pp 93-106.

Skofitsch G and DM Jacobowitz (1992a) Calcitonin and calcitonin gene-related peptide receptor binding sites in the rat central nervous system. *Ann. NY Acad. Sci.* **657**, 420-422.

Skofitsch G and DM Jacobowitz (1992b) Calcitonin and calcitonin gene-related peptide, receptor binding sites in the central nervous system, In *Handbook of Chemical Neuroanatomy, Vol XI, Neuropeptide Receptors in the CNS* (Björklund A, T Hökfelt and M Kuhar, Eds.) (Elsevier Science Publishers: Amsterdam), pp 92-144.

Skofitsch G, TR Insel and DM Jacobowitz (1985a) Binding sites for corticotropin releasing factor in sensory areas of the rat hindbrain and spinal cord. *Brain Res. Bull.* **15**, 519-522.

Skofitsch G, DM Jacobowitz, RL Eskay and N Zamir (1985b) Distribution of atrial natriuretic factor-like immunoreactive neurons in the rat brain. *Neuroscience* **16**, 917-948.

Skofitsch G, DM Jacobowitz and N Zamir (1985c) Immunohistochemical localization of a melanin concentrating hormone-like peptide in the rat brain. *Brain Res. Bull.* **15**, 635-649.

Skofitsch G, MA Sills and DM Jacobowitz (1986) Autoradiographic distribution of ^{125}I-galanin binding sites in the rat central nervous system. *Peptides* **7**, 1029-1042.

St Laurent J, MF Roizen, E Miliaressis and DM Jacobowitz (1975) The effects of self-stimulation on the catecholamine concentration or discrete areas of the rat brain. *Brain Res* **99**, 194-200.

St Laurent J, MF Roizen, H Beckman, E Miliaressis, FK Goodwin and DM Jacobowitz (1976) Neurochemical changes in discrete areas of the rat brain after self-stimulation from the area ventralis tegmenti, In *Brain-Stimulation Reward* (Wanquier A and ET Rolls, Eds.) (North-Holland Publishing Co.: Amsterdam, The Netherlands), pp 283-289.

Strauss KI and DM Jacobowitz (1993a) Nucleotide sequence of rat calretinin cDNA. *Neurochem. Int.* **22**, 541-546.

Strauss KI and DM Jacobowitz (1993b) Quantitative measurement of calretinin and beta-actin mRNA in rat brain micropunches without prior isolation of RNA. *Brain Res. Mol. Brain Res.* **20**, 229-239.

Strauss KI, KR Isaacs, QN Ha and DM Jacobowitz (1994a) Calretinin is expressed in the Leydig cells of rat testes. *Biochim. Biophys. Acta* **121**, 435-440.

Strauss KI, DM Jacobowitz and J Schulkin (1994b) Dietary calcium deficiency causes a reduction in calretinin mRNA in the substantia nigra compacta-ventral tegmental area of rat brain. *Brain Res. Mol. Brain Res.* **25**, 140-142.

Strauss KI, J Kuznicki, L Winsky and DM Jacobowitz (1994c) Recombinant and native rat calretinin share physical-chemical properties. *Prot. Expres. Purif.* **5**, 187-191.

Strauss KI, J Schulkin and DM Jacobowitz (1995) Corticosterone effects on rat calretinin mRNA in discrete brain nuclei and the testes. *Brain Res. Mol. Brain Res.* **28**, 81-86.

Sutin EL and DM Jacobowitz (1990a) Detection of CCK mRNA in the motor nucleus of the rat trigeminal nerve with in situ hybridization histochemistry. *Brain Res. Mol. Brain Res.* **8**, 63-68.

Sutin EL and DM Jacobowitz (1990b) Localization of substance P mRNA in cholinergic cells of the rat laterodorsal tegmental nucleus, in situ hybridization histochemistry and immunocytochemistry. *Cell. Mol. Neurobiol.* **10**, 19-31.

Thoa NB, B Eichelman, JS Richardson and DM Jacobowitz (1972) 6-Hydroxydopa depletion of brain norepinephrine and the function of aggressive behavior. *Science* **178**, 75-77.

Thoa NB, Y Tizabi and DM Jacobowitz (1975) The effect of prolonged isolation on the catecholamine and serotonin concentration of discrete areas of the rat brain, In *Catecholamines and Stress* (Usdin E, R Kvetnansky and IJ Kopin, Eds.) (Pergamon Press: Oxford, England), pp 61-68.

Thoa NB, Y Tizabi and DM Jacobowitz (1977) The effect of isolation on catecholamine concentration and turnover in discrete areas of the rat brain. *Brain Res.* **131**, 259-269.

Tizabi Y, TL O'Donohue and DM Jacobowitz (1982) Changes in alpha-melanotropin in discrete brain areas of isolated aggressive mice. *Peptides* **3**, 429-431.

Tizabi Y, G Skofitsch and DM Jacobowitz (1985) Effect of chronic reserpine and desmethylimipramine treatment on CRF-like immunoreactivity of discrete brain areas of rat. *Brain Res.* **335**, 389-391.

Vogel JH, DM Jacobowitz and CA Chidsey (1969) Distrubution of norepinephrine in the failing bovine heart. Correlation of chemical analysis and fluorescene microscopy. *Circ. Res.* **24**, 71-84.

Webb JG, J Moss, IJ Kopin and DM Jacobowitz (1975) Biochemical and histofluorescence studies of catecholamines in superior cervical ganglia in organ culture. *J. Pharmacol. Exp. Ther.* **193**, 489-502.

Wein AJ, JV Leoni, JG Gregory, HW Schoenberg and DM Jacobowitz (1972a) The effect of 6-hydroxydopamine on canine ureteral contractility, further evidence in favor of a non-neurogenic theory of ureteral function. *J. Urol.* **108**, 402-405.

Wein AJ, JV Leoni, HW Schoenberg and DM Jacobowitz (1972b) A study of the adrenergic nerves in the dog ureter. *J. Urol.* **108**, 232-233.

Wein AJ, DM Jacobowitz, JN Corriere Jr., and JJ Murphy (1973) Chemical sympathectomy with 6-hydroxydopamine, a useful research tool. *Surg. Forum* **24**, 560-562.

Wein AJ, JG Goregory, WJ Cromie, JN Corriere Jr. and DM Jacobowitz (1974) Sympathetic innervation and chemical sympathectomy of canine urinary bladder. *Urology* **4**, 27-32.

Wein AJ, JV Leoni, DM Raezer, DM Jacobowitz and JN Corriere Jr. (1975a) The effect of acute chemical sympathectomy on the competence of the canine ureterovesical junction. *Urol. Res.* **3**, 95-97.

Wein AJ, DM Raezer, DM Jacobowitz, WF Gadbois, GS Benson and JN Corriere (1975b) Effect of long-term chemical sympathectomy on function of canine urinary tract. *Urology* **6**, 200-204.

Winsky L and DM Jacobowitz (1995) Effects of unilateral cochlea ablation on the distribution of calretinin mRNA and immunoreactivity in the guinea pig ventral cochlear nucleus. *J. Comp. Neurol.* **354**, 564-582.

Winsky L, JA Harvey, SE McMaster and DM Jacobowitz (1989a) A study of proteins in the auditory system of rabbits using two-dimensional gels, identification of glial fibrillary acidic protein and vitamin D-dependent calcium binding protein. *Brain Res.* **493**, 136-146.

Winsky L, H Nakata and DM Jacobowitz (1989b) Identification of calcium binding protein primarily localized to the cochlear nucleus. *Neurochem. Int.* **15**, 381-389.

Winsky L, H Nakata, BM Martin and DM Jacobowitz (1989c) Isolation, partial amino acid sequence, and immunohistochemical localization of a brain-specific calcium-binding protein. *Proc. Natl. Acad. Sci. USA* .**86**, 10139-10143.

Winsky L, P Montpied, R Arai, BM Martin, and DM Jacobowitz (1992) Calretinin distribution in the thalamus of the rat, immunohistochemical and in situ hybridization histochemical analyses. *Neuroscience* **50**, 181-196.

Winsky L, KR Isaacs and DM Jacobowitz (1996) Calretinin mRNA and immunoreactivity in the medullary reticular formation of the rat, colocalization with glutamate receptors. *Brain Res.* **741**, 123-133.

Youdim MB, MA Sills, WE Heydorn, GJ Creed and DM Jacobowitz (1986) Iron deficiency alters discrete proteins in rat caudate nucleus and nucleus accumbens. *J. Neurochem.* **47**, 794-799.

Zamir N, G Skofitsch, MJ Bannon and DM Jacobowitz (1986a) Melanin-concentrating hormone, unique peptide neuronal system in the rat brain and pituitary gland. *Proc. Natl. Acad. Sci. USA* **3**, 1528-1531.

Zamir N, G Skofitsch and DM Jacobowitz (1986b) Distribution of immunoreactive melanin-concentrating hormone in the central nervous system of the rat. *Brain Res.* **373**, 240-245.