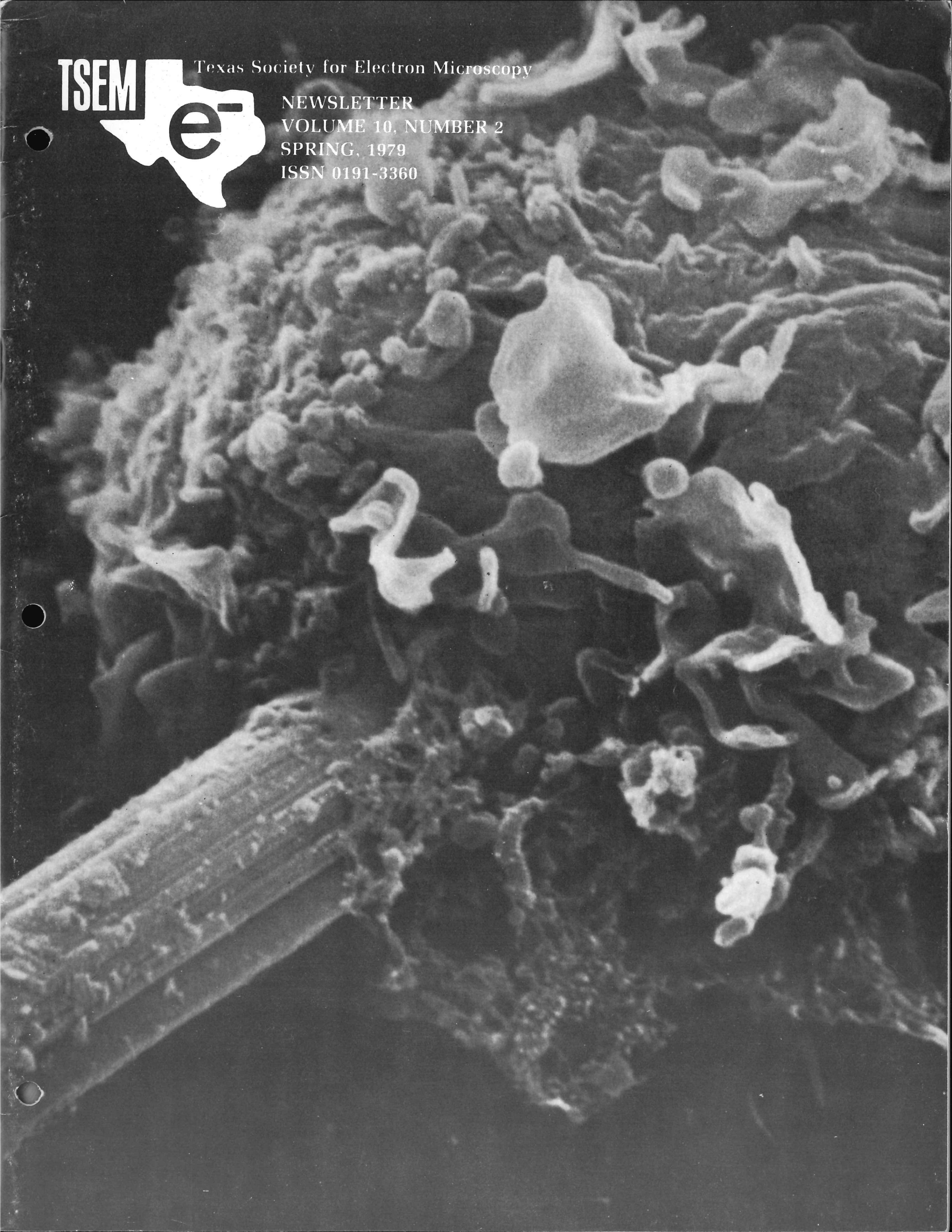

TSEM

Texas Society for Electron Microscopy

NEWSLETTER
VOLUME 10, NUMBER 2
SPRING, 1979
ISSN 0191-3360

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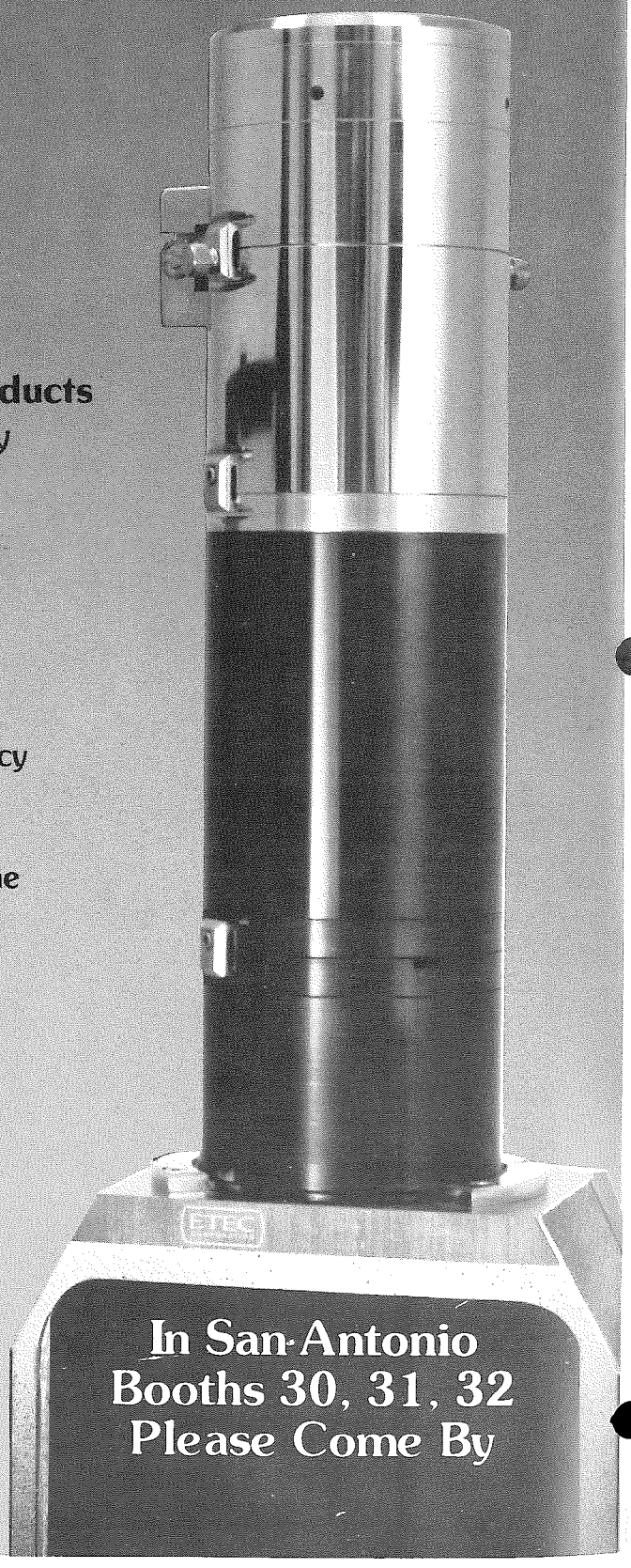

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Contents

Volume 10, Number 2

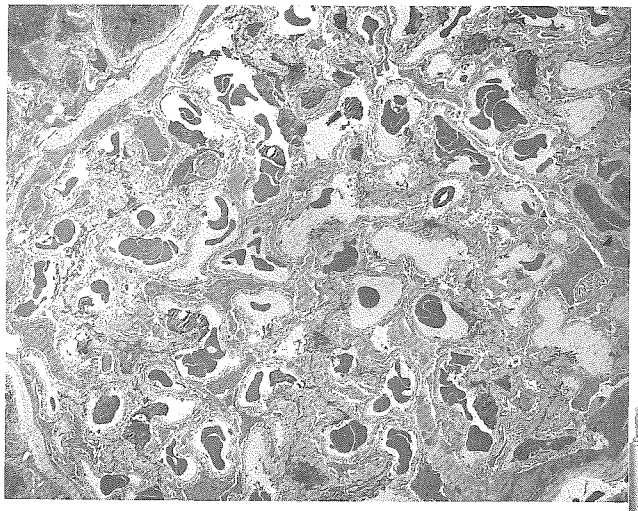
Spring, 1979

ISSN 0191-3360

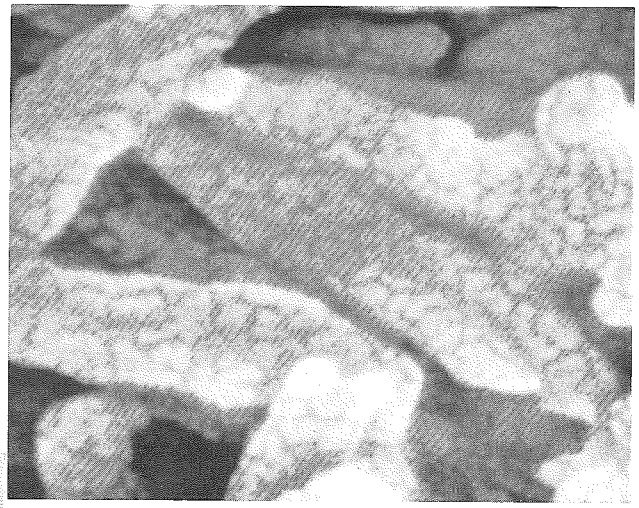
Texas Society for Electron Microscopy
"For the purpose of dissemination of research with the electron microscope"

ON THE COVER
Photo courtesy of: Myles L. Mace, Jr., Ted McLemore, Russell Martin, and B. R. Brinkley, Baylor College of Medicine. Scanning electron micrograph of human Pulmonary Alveolar Macrophage (PAM) phagocytising amosite asbestos fiber. Freshly lavaged PAMs incubated with asbestos fibers begin phagocytic activity within five minutes, frequently totally engulfing smaller fibers within 60 minutes. In this example, the macrophage has engulfed one end of an asbestos fiber.

Hitachi/Perkin-Elmer introduces H-300 the world's first Universal Electron Microscope (UEM)



Mouse glomerules at 700X, full frame; wider field of view, with full frame, obtainable at 250X.



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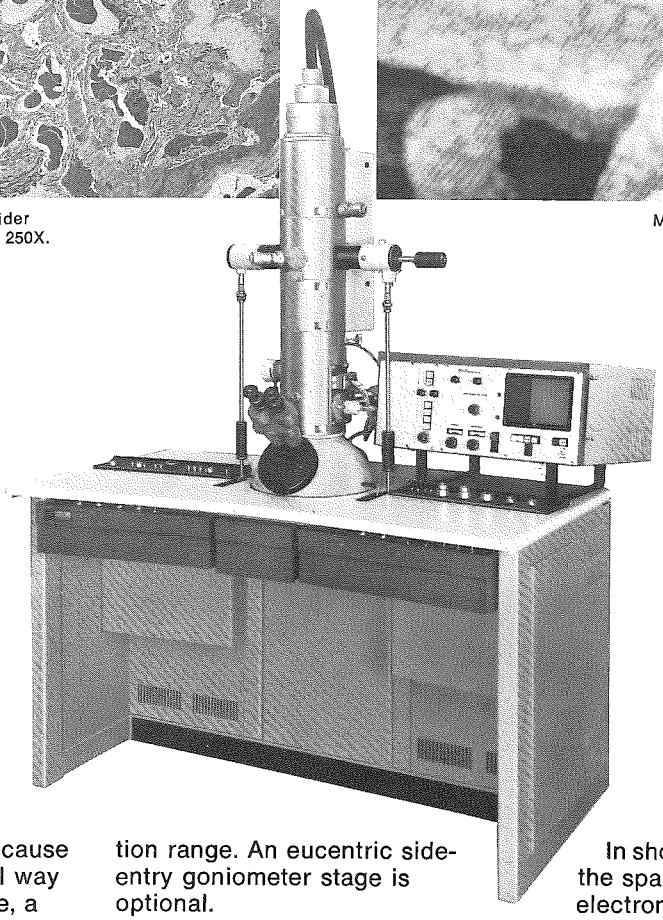
250X-100,000X



It's a SEM

100Å guaranteed
20 kV
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We call the H-300 a UEM because there's simply no conventional way to describe it. For the first time, a full-fledged TEM and a full-fledged SEM are packaged in a common console—without compromising performance in either mode.

The TEM mode, for example, includes a wide field, low mag image free of peripheral blur. This eliminates tedious montaging. You also get zoom over the entire magnification range. An eucentric side-entry goniometer stage is optional.

As an SEM, the H-300 is just as versatile, easily handles bulk specimens. You can also add options like Mode Control with EDX interface, an energy dispersive X-ray spectrometer and Automatic Data Display. Plus Hitachi-patented Dual Magnification: using a single CRT, you can switch from a low mag view of your specimen to a high mag view of a specific field of interest.

In short, the H-300 gives you, in the space of one instrument, a total electron microscopy capability.

Best of all, you can get the UEM package for under $70K. Or, buy it as a TEM for under $50K, integrate the SEM later. For full details and/or a demonstration, call or write: Perkin-Elmer, Instrument Marketing Division, 411 Clyde Avenue, Mountain View, California 94043. Phone (415) 961-0461.

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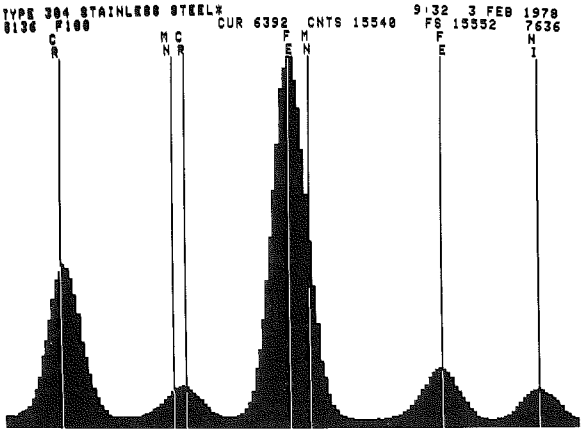

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President's Message

Here is a summary of the state of TSEM. Our membership is 510 including more than 34 corporate members. The membership is up 10 from last year even though 120 individuals have been dropped from the official role and from the mailing list for failure to pay membership fees. This means we have many new members, and I encourage your active participation in society affairs. I am sure that many who dropped out will want to rejoin TSEM. According to the By-Laws, you will have to reapply as new members.

Dr. Ann Goldstein, our newsletter editor, has done an outstanding job. She has improved on the already high quality format and has obtained feature articles par excellance. In addition, she has increased the advertisements needed to produce this quality publication. We anticipate that many scientific libraries in Texas and across the country will soon begin subscription to this publication. All of us thank you, Ann.

Dr. Paul Baur is just completing the second year of his two year term as Treasurer. As an indication of the growth of our Society's business, Paul reports that we will, for the first time, need to file with the IRS because income and dispersement of funds were well over $6,000 in 1978. This means the job of the Treasurer has been time consuming. A recent internal audit of the treasurer's records shows them to be in good order and our total assets remain the same as last year. We have just obtained a sales tax exemption from the State which should save us money. All of us thank you, Paul.

Our Spring meetings in Lubbock and the Fall meeting in Nacogdoches each attracted more than a hundred with more than 20 scientific presentations each. Those who attended know of the fine programs and local arrangements carried out by Mr. Randy Brackeen in Lubbock, and by Dr. Charles Mims in Nacogdoches. Dr. Bruce McKay can be justifiably proud. Unfortunately, much of Bruce's work on future programs will not be apparent until later meetings transpire. So as you benefit from and enjoy next year's programs, say: "Thank you, Bruce".

The transition of secretarial responsibilities required relocating our postal permit, setting up our membership list in new computer facilities and a myriad of time requiring details. Our secretary, Dr. John Hansen, has been of great help to me and is doing a fine job.

During this last year (including the joint meeting in New Orleans), more than 100 presentations were made before the membership. Although this is good, I think we can exchange even more information in the future. One way to accomplish this is to strengthen our material and physical science programs. This will, I think, occur naturally as the field of electron microprobe continues to grow and as new instrumentation relevent to both physical-material and to biomedical sciences are developed and used in Texas. It is a challenge for TSEM to encourage physical-material scientists in the face of the large numbers of biomedical scientists. Although our officers are now biomedical people, we genuinely want to encourage the participation of physical-material sciences in TSEM. It is not so much that we want your participation, it is that we need your participation. Our new program chairman is Dr. Charles Mims, let him know what program you want and we will arrange it.

TSEM continues to encourage and support student participation with over $1,000 set aside for student travel this year. Many students give their first papers at TSEM meetings. The new student representative to the council is Thomas Drier of Texas A & M.

An exciting program for the Dallas meeting on May 4 - 5, is now formalized. The emphasis is on Nervous System. The local arrangements are under the able hands of Drs. Leonard Selig and Jerry Shay. Our following TSEM meeting will be held in conjunction with the EMSA meeting, August 13 - 17, 1979 in San Antonio. The EMSA program looks terrific and TSEM will hold a business meeting some time during the week. TSEM is hosting the EMSA council meeting just prior to the EMSA meeting. Dr. Bill McCombs has made the arrangements.

The Society will be in good hands during the tenure of President Bill McCombs.

For my part, the year has gone fast and smooth. This reflects best on the efforts of your elected officers. To all of the officers and members, I thank you for the honor of serving and participating in TSEM. I seem to get so many benefits from this Society.

Ivan Cameron
President

Editor's Comments

Thanks go again to our advertisers and to our regional editors. I have received a few more questionnaires. Next issue I will summarize the comments made most frequently and tabulate the results.

With the help of Dr. Eugene Garfield of the Institute for Scientific Information, I have applied to the Library of Congress for an ISSN (International Standard Serial Number) for the Texas Society for Electron Microscopy Newsletter. He identified the person who knew the person at the Library of Congress in the right division and she supplied me with the correct form for requesting the number. Believe it or not they wanted only two copies of the form. We are now distributing a free copy of the TSEM Newsletter to libraries in Texas in hopes that they will want to subscribe to future issues.

Remember I still need feature articles for the next issue. Previous articles have been reviews, papers on techniques, papers on methodology and specific research reports of general interest. Send the articles to me by July 1, 1979.

Ann Goldstein
Editor

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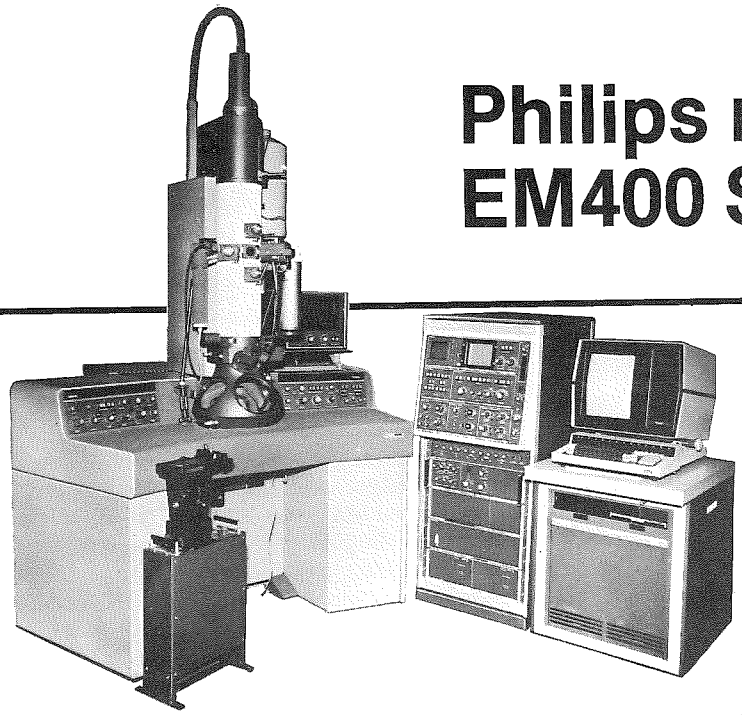

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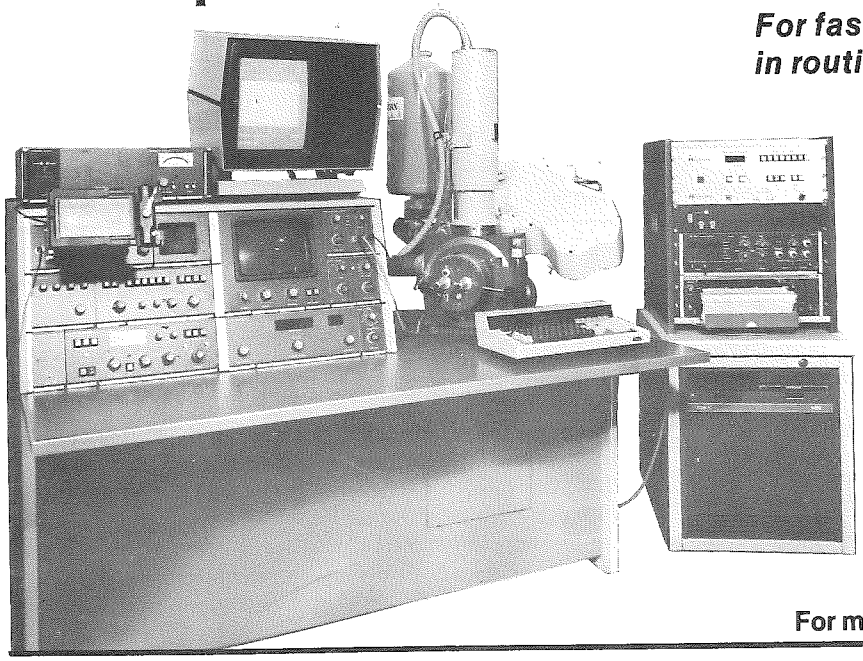

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Job Opportunities

Position Available — Research Associate: To work in all techniques dealing with TEM including thin sectioning and routine electron microscope maintenance. Experience with SEM, X-ray microanalysis, and/or freeze fracture is highly desirable. Requires degree and five years of related experience or equivalent. Contact Dr. Ruth Bulger, Department of Pathology & Laboratory Medicine, The University of Texas Health Science Center at Houston, 6431 Fannin, Houston, Texas 77030; phone 792-5200.

Position Available — Research Assistant II: Immediate opening for individual to perform animal experimentation on pathophysiology of kidneys including transmission and scanning microscopy and X-ray microanalysis. Requires degree and two years related experience or equivalent. Contact Dr. Ruth Bulgar, Department of Pathology and Laboratory Medicine, The University of Texas Health Science Center at Houston, 6431 Fannin, Houston, Texas 77030; phone 792-5200.

Position Available — The Biology Department at Baylor University is seeking a person to teach Histology and Electron Microscopy. The position will begin in August of 1979. Applicants should have Ph.D. Degree. Send C. V. and three letters of recommendation. Write to: Search Committee, Biology Department, Baylor University, Waco, TX 76706. Baylor is an equal opportunity employer.

Position Open — Neuroanat, Lab — University of Texas Medical Branch, Galveston.

Summer position (3-4 months, 1979) for Junior, Senior, or Graduate student in the Biological sciences in a neuroanatomical laboratory investigating peripheral and central vestibular pathways using the neuronal tracers horseradish peroxidase and ^{3}H-fucose. The position involves small animal surgery (pigeon, gerbil), tissue fixation and processing, frozen sectioning of the brain, as well as tissue embedding for electronmicroscopy. All techniques and routines are established and will be taught to the student. Salary competitive and depends upon credentials. Housing available on-campus for single individuals. Send Transcript and two references to: Avrim R. Eden, M.D., Department of Otolaryngology, University of Texas Medical Branch, Galveston, TX 77550, Tel: (713) 765-2721.

TSEM FINANCIAL REPORT
Period Ending January 31, 1979

Total Assets (9/29/78)		$ 4,992.22
Certificate of Deposit (University National Bank)		2,000.00
Certificate of Deposit (University Bank)		1,329.62
Savings Account		309.26
Balance in Checking Account as of 9/20/78		353.34
RECEIPTS:		
Income from Registration (Nacogdoches)	$ 1,405.00	
TSEM Dues Payments (1,751.00/1979)	1,939.00	
Interest on Cert. of Dep. No. 1099	62.50	
Interest on Cert. Dep. No. 17864	18.27	
Cash-in Cert. Dep. No. 4470	1,349.71	
Return of Cash (Charles Mims)	97.50	
Residual Revenue — Lubbock	13.50	
Residual Revenue — San Antonio	100.00	
Secretary Fund Return (McCombs)	71.79	
Total Income	5,057.27	
DISBURSEMENTS:		
Mel Fuller (Honorarium) Nacogdoches	$ 100.00	
East Texas Strings Ensemble Nacogdoches	120.00	
Bill Brinkley (Honorarium) Nacogdoches	100.00	
Bill McCombs (Ex. Com. Exp.) Nacogdoches	26.50	
Fredonia Inn (Cash) Nacogdoches	550.00	
Fredonia Inn Nacogdoches	442.25	
Carter Dist. Co. Nacogdoches	41.08	
S. F. Austin University Nacogdoches	10.75	
Student Travel Nacogdoches	200.00	
John Hansen (Sec. Expenses)	300.00	
Ivan Cameron (Travel Expense-EMSA)	200.00	
Seinsheimer Ins. Agency (Treas. Bond)	28.00	
Ann Goldstein (Newsletter Expense)	300.00	
U. S. Postal Service (1979 Permit)	40.00	
Total Disbursements		(−) 2,458.58
Balance in Checking Account as of 1/31/79		2,952.03
Certificate of Deposit (Univ. Bank No. 1099)	2,000.00	
Certificate of Deposit (Fannin Bank No. 17864)	1,000.00	
Savings Account (No. 01-7420-3 U.N.B.)	317.03	
Total Assets		6,269.06
ENCUMBRANCES — 1979		
EMSA Meeting	$ 1,500.00	
Student Travel (Usual)	1,000.00	
Publications (Estimated)	1,200.00	
Secretarial Expenses (Estimated)	750.00	
Presidential Expenses (Estimated)	200.00	
Treasurers Expenses (Estimated)	100.00	
Total Encumbrances		4,750.00

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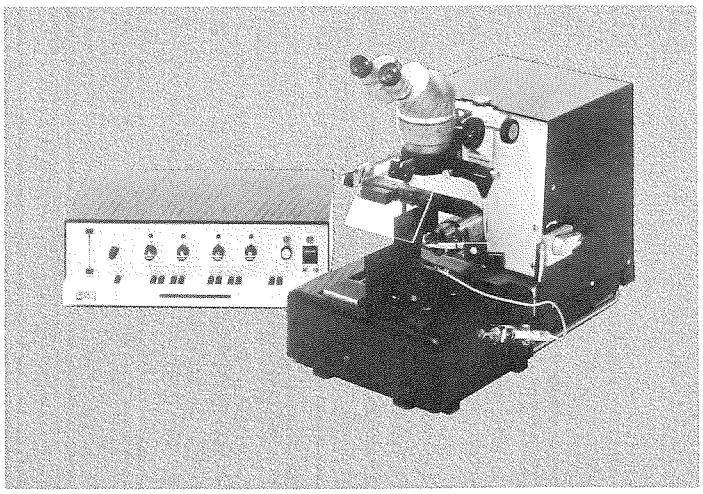

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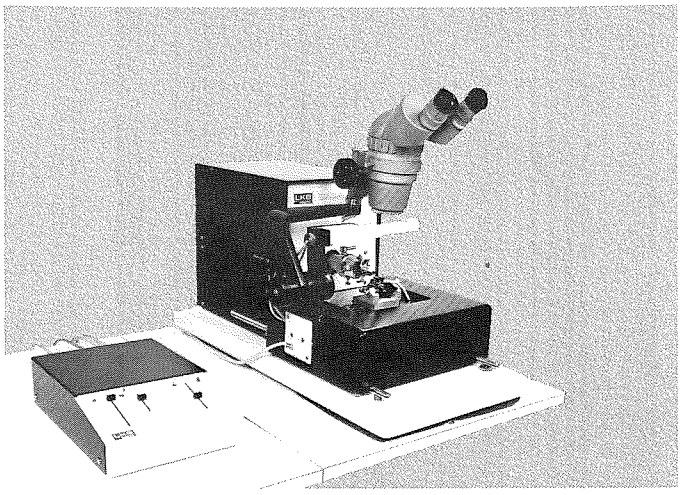

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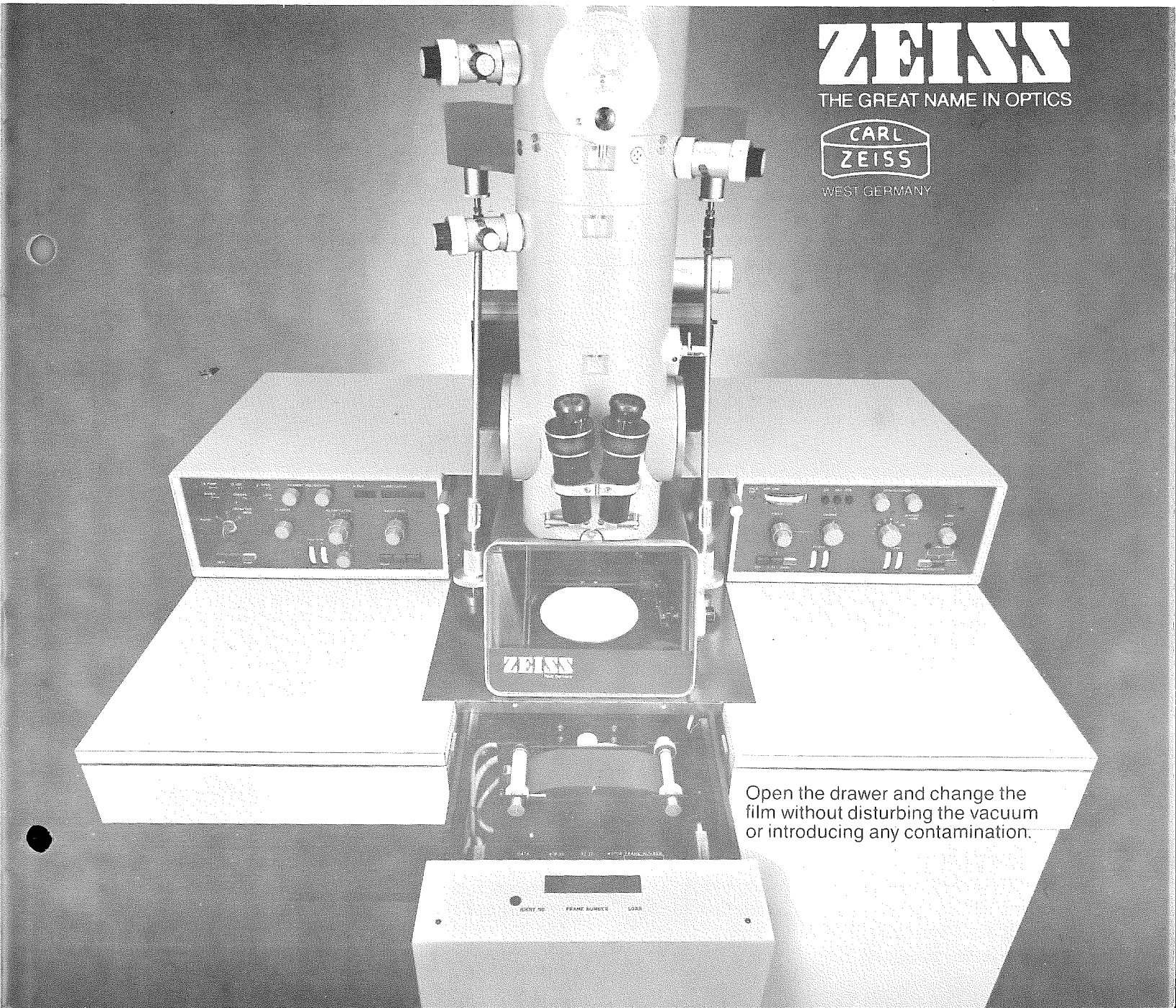

Open the drawer and change the film without disturbing the vacuum or introducing any contamination.

A Review Of Neurotransmitters In Retina

by
Dianna A. Redburn, Ph. D.
Department of Neurobiology and Anatomy
The University of Texas Medical School at Houston
P. O. Box 20708
Houston, Texas 77025

The unique morphology and physiology of the retina offers excellent opportunities for integrative studies of the morphology, biochemistry, and physiology of a specific, well defined neuronal system. The retina can be considered as an externalized brain slice with relatively few cell types (compared to whole brain), arranged in laminar array with an essentially unidirectional information flow, from receptor to bipolar to ganglion cell, with lateral integration by amacrine and horizontal cells at the two synaptic interfaces (Dowling, 1970; Werbling and Dowling, 1969). Systematic investigations have been possible in this comparatively simple tissue which would otherwise be prohibited by the greater complexity of other central nervous system components. One point of particular interest from such studies is that only graded potentials are recorded from all the cells of the outer retinal cells layers, including the bipolar cells (Witkovsky, 1971). The absence of spike activity at several synapses in retina minimizes the contribution of electrical transmission and maximizes the importance of neurotransmitter (NT) systems in retinal physiology.

Most neurotransmitter studies have been based upon the specific criteria by which transmitters are identified as established by Werman, (1966): (1) The compound must be highly localized in the presynaptic terminal of the appropriate neuron; (2) it must be synthesized in that neuron; (3) it must be removed from the cleft either by a high affinity uptake system or by enzymatic degradation; (4) the compound must also elicit an appropriate action on the postsynaptic membrane; (5) it must be released under physiological conditions.

A question remains as to the critical value of some of these classical criteria for identifying NTs. Clearly some of these criteria lack specificity, and, certainly, no single criterion is sufficient. For example, the demonstration of the presence of compounds which evoke an electrophysiological response may provide a necessary prerequisite for the establishment of this compound as a NT, but it is by no means sufficient in this respect. Many of the NT candidates are amino acids which not only play a role as transmitters, but are also involved in general neuronal metabolism. Thus, the presence of an amino acid within a neuron may or may not be an indication that it is functioning as a NT.

Demonstration and localization of a high affinity uptake system for a suspected NT compound was once considered a strong indication that a compound was the native NT within a given system. Such is no longer the case. It has been demonstrated that the high affinity uptake system for a given NT is *not* limited to a specific set of presynaptic terminals. A number of high affinity uptake systems have been demonstrated in glial compartments (Bowery and Brown, 1972; Hutchison et al, 1973; Neal and Iversen 1972). In addition, the synaptosomal high affinity uptake systems for catecholamines do not discriminate between norepinephrine and dopamine and thus are not entirely specific for their native transmitter (Coyle and Snyder, 1969).

Iontopheretic studies can also be open to question since many compounds have nonspecific or bizarre effects on excitable membranes and yet have no physiologically important role as a NT.

An especially difficult criterion to demonstrate which is also highly specific for individual transmitter substances is that a given substance elicits the appropriate action on the postsynaptic membrane. Heretofore, this criterion was tested only in electrophysiological studies. More recently, a biochemical receptor assay has been developed which allows the biochemical demonstration of the appropriate postsynaptic action, i.e., stereospecific, high affinity binding (Enna and Snyder, 1976). Some neurotransmitters such dopamine, bind to receptor complexes which contain an adenylate or guanylate cyclase activation site (Clement-Cormier and Robison, 1977). In these cases, NT stimulation of cyclase activity can also be used as a measure of the appropriation postsynaptic membrane action.

Highly specific ligands have recently been developed for some of the neurotransmitter receptor sites. Many of the compounds are now available in the radiolabeled form and thus can be in biochemical receptor binding studies and in autoradiographic localization of receptor sites in intact tissue.

The demonstration of physiological release is also a critical test for a NT, and is classically most difficult to demonstrate in CNS. The major problem involved is that minute amounts of the NT are released under physiological stimulation, and the release usually occurs within a millisecond after stimulation. At the same time, re-uptake systems and/or catabolic enzymes minimize chances for collection of released NTs in the extracellular fluid.

Techniques have been developed in our laboratories for assaying chemical transmission *in vitro* (Redburn et al, 1975; Levy et al, 1973; Redburn and Cotman, 1974). Results of these studies show that subcellular fractionation of secreting tissue does not impair the functional integrity of the mechanism of stimulus secretion coupling. The secreting unit in neuronal tissue, the synaptic area, is sheared from the rest of the neuron during homogenization and reseals to form a sac, or synaptosome. Synaptosomes thus derived from whole rat brain or retina are osmotically-sensitive, metabolically active, and retain their ability to takeup, synthesize and metabolize compounds under conditions comparable to those described for intact tissue (Bradford, 1973; Whittaker, 1972; Redburn, 1977). Of particular significance is the ability of mixed synaptosomal fractions from whole brain to takeup, store, and release upon stimulation, a variety of NTs, such as ACh, GABA, NE, glutamate, and aspartate (Levy et al, 1973; Levy et al, 1974). Synaptosomal fractions placed on a filter and continuously perfused with buffers containing stimulatory compounds, release endogenous, as well as exogenously loaded, NT stores. The conditions under which NTs are released mimic the conditions of stimulus secretion coupling in neuromuscular junction and adrenal medulla.

During the past two years, retinal subcellular fractionation techniques were developed in our laboratory which yield two morphologically distinct synaptosomal fractions. One fraction (P_1) was enriched in photoreceptor cell synaptosomes (PCS); the second fraction (P_2) contained small synaptosomes derived from conventional sized synapses which are most abundant in the innerplexiform layer. An extensive study was made of fractionation techniques which would yield the most highly enriched synaptosomal fractions. Osmolarity, pH and ionic content of the homogenizing medium were systematically studied; gradient centrifugations using sucrose, sucrose-ficoll, sucrose=Na-diatrizoate and BSA were tested. Contents of each pellet were determined and evaluated using analytical EM techniques. The most suitable isolation procedure is shown in Fig. 1. P_1 and P_2 fractions derived by this method are discribed below. PCS were identified by their large size (3 μ diameter), presence of many synaptic vesicles and synaptic ribbon with an associated halo of synaptic vesicles. In suitable planes of section, invaginations (triads) were observed which contained processes presumably from horizontal and bipolar cells. Contaminants of the P_1 fraction include some nuclei, rod inner and outer segments, and Mueller cell fragments which were often attached to the periphery of the PCS. Few conventional synaptosomes were seen in this fraction. The general composition of the retinal P_2 fraction was similar to P_2 fractions obtained from whole brain. In addition to the many conventional synaptosomes, the P_2 contained free mitochondria and empty membrane sacs. The many small mitochondria were probably derived from photoreceptor inner segments which were sheared during homogenization. By comparison with the morphology of intact retina, these small synaptosomes can be tentatively identified as components of the inner plexiform layer. The small synaptosomes were identified by their small size (1μm or less in diameter) and presence of synaptic vesicles. Some of these had retained portions of the postsynaptic membrane attached to the junctional complex. The P_2 fraction probably contains the somewhat larger synaptosomes derived from bipolar cells, although few were actually seen in any fraction.

One unique feature of the retinal P_2 fraction as compared to brain was the relatively large number of serial or reciprocal synaptic profiles. Such configurations are rarely observed in brain. Thus, the complex synaptic arrangements seen in intact retina and, in particular, in the inner plexiform layer is reflected in more "complex" synaptic profiles in the P_2 fraction.

The relative enrichment of the synaptosomal fractions was estimated by counting the number of profiles of PCS and conventional synaptosomes per grid square (137μ^2 area). The PCS had a diameter of 3.01 $\pm$ 0.71 microns (M $\pm$ SEM). Characteristic triads were present in 68% of the PCS. Only 33% of the PCS showed the typical synaptic ribbon. There were 18.1 $\pm$ 5.0 PCS per grid square. Compared with the P_2 fraction, the P_1 fraction showed 117 fold enrichment in the number of PCS. Contamination of the PCS fraction with small synaptosomes was only 11% by comparative particle count. This contamination is negligible considering that the small synaptosomes have a diameter of 1μm or less and thus the volume occupied by these particles would be quite small in proportion to the

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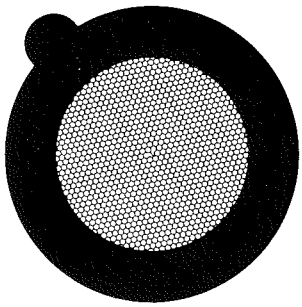

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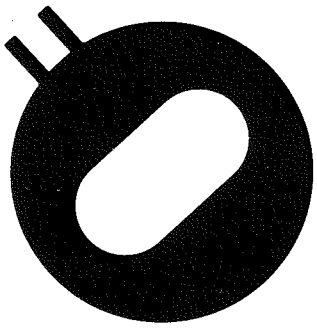

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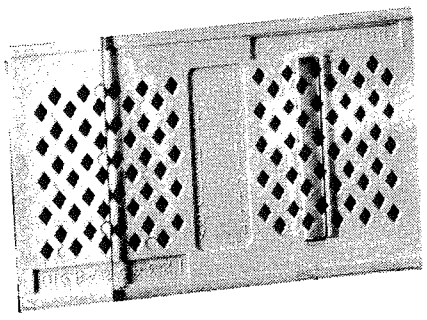

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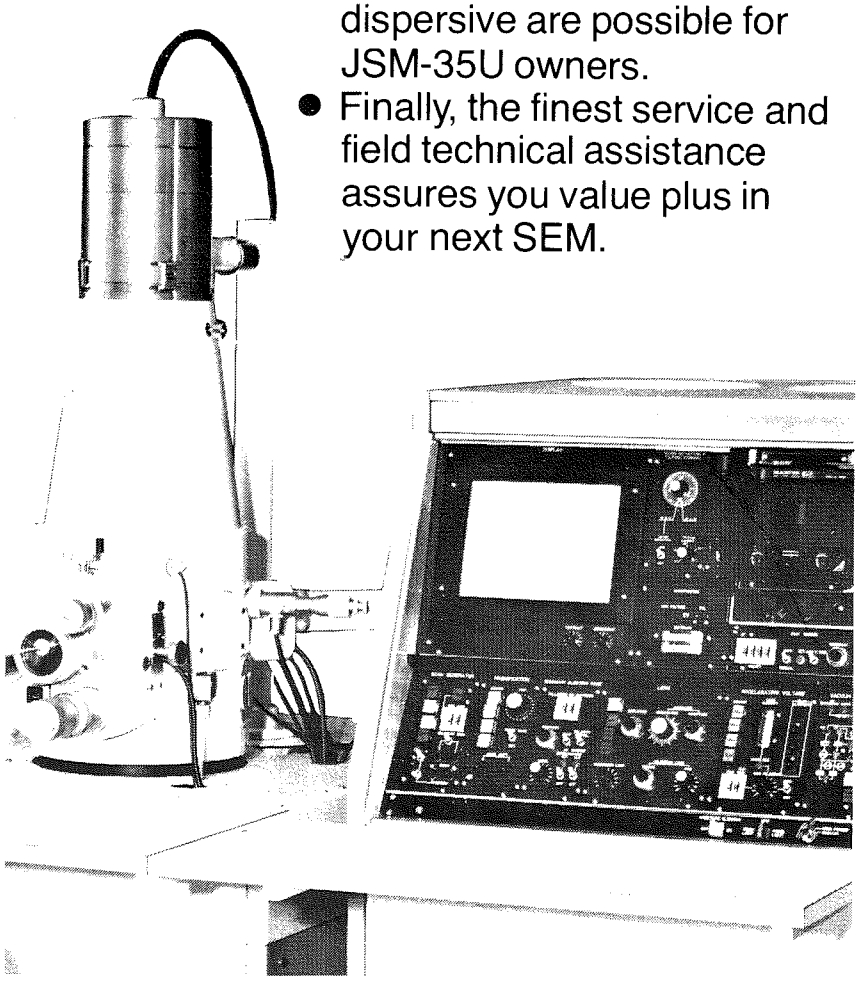

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larger PCS which in fact occupies approximately 50% of the total particulate volume of the pellet.

The P_1 fraction yielded 81 ± 7 mg protein per rabbit retina. The P_2 fraction contained 238 ± 28 mg protein per rabbit retina. Both synaptosomal fractions appear to be functionally active since they are able to take up, store and release several putative retinal neurotransmitters.

These new tools for neurotransmitter studies have been utilized by several laboratories and have produced significant new data concerning retinal transmitters. Nine putative retinal NTs (acetylcholine (ACh), dopamine (DA), γ-aminobutyric acid (GABA), glutamate, glycine, β-alanine, 5-hydroxytryptomine (5-HT), aspartate, and taurine, have been studied in some detail and demonstrate one or more of the five criteria required for NT identification. The seven listed below are of particular interest.

A. *Acetylcholine* - The retina has an unusually high content of ACh synthetic enzyme (choline acetyltransferase) (Hebb, 1963). In addition, electrophysiological studies using cholinomimetics suggest that acetylcholine is acting as both an excitatory and inhibitory NT within the synaptic layers of the retina (Ames and Pollen, 1969). In a very elegant study by Lam (1972), isolated rod cells were shown to synthesize acetylcholine but not other putative retinal NTs such as DA and GABA. Masland and Livingstone (1976) have shown that ^{3}H -ACh synthesized from exogenous ^{3}H -choline is released from rabbit retina in response to light stimulation. Vogel and Nirenberg (1976) have shown autoradiographically the ^{125}I - αbungarotoxin binding is localized in both plexiform layers. Unpublished studies from this laboratory have demonstrated a high affinity, sodium dependent uptake of ^{3}H -choline and subsequent release of synthesized ^{3}H -ACh from retinal P_2 fractions. Cholinergic activity is less pronounced in the P_1 fraction however, it may represent an important system since ACh and its receptor agonists stimulate ^{3}H -GABA uptake specifically in this subcellular fraction.

B. *Dopamine* - Light microscopic studies using fluorescent techniques for specific localization of catecholamines have demonstrated the presence of DA-containing neurons in the retina (Laties and Jacobowitz, 1966; Ehinger and Falck, 1969; Haggendal and Malmfors, 1965; Drujan, et al, 1965). While there is some species variation in the localization of dopaminergic neurons, in general, the cells bodies lie in the inner part of the inner nuclear layer (the amacrine cell layer) with synapses in the inner plexiform layer. These DA cells represent approximately 10% of the cells within the amacrine cell layer. Kramer (1971) has shown that the five criteria for the establishment of DA as a NT in retina have, at least superficially, been met. He shows (directly or indirectly) that (1) DA is synthesized in the retina, (2) its degradative enzymes are present, (3) DA has an inhibitory effect on the elctrical activity of the retina, and (4) when the retina is stimulated by flashes of light, DA is released in increasing amounts as the frequency of the flash increases. Other studies demonstrate that total levels of DA decrease during retinal exposure to light (Drujan, et al, 1965). Radioactive DA is taken up by retinal cells whose general location appear to be the same as for those which show fluorescence for endogenous DA (Kramer et al, 1971).

Recently, Dowling and Ehinger (1975) have shown by histoflourescence a dopaminergic cell, the interplexiform cell, with cell bodies in the inner nuclear layer and terminals in both plexiform layers.

Studies from this laboratory have detailed the biochemical characteristics of the dopamine system in two species; cow and rabbit (Thomas et al, 1978; Clement-Cormier and Redburn, 1978). The dopamine system is highly localized in the P_2 fraction of rabbit retina as demonstrated by uptake, release, dopamine receptor binding and dopamine stimulated adenylate cyclase activities. In the cow, dopamine receptors are equally distributed between P_1 and P_2 fractions. These data suggest that in rabbit, dopamine terminals may be limited to the inner plexiform layer and associated with amacrine cells. In cow, dopamine may be associated with terminals of interplexiform cells in both plexiform layers.

C. *GABA* - High levels of endogenous GABA, its systhetic enzyme, GAD and its catabolic enzyme, GABA-T, are localized in the amacrine cell layer of most species (Graham, 1972; Kuriyama, et al, 1968). In addition, a high affinity uptake system for GABA in retina has been well characterized and is similar to the uptake of GABA in brain (Tunnicliff et al, 1974; Voaden et al, 1973). The location of the GABA uptake sites, however, varies according to species. In rat, GABA is taken up almost exclusively by glial elements, namely the Mueller cells (Neal and Iversen, 1972; Marshall and Voaden, 1974a). In rabbit, it is taken up by Mueller cells, as well as some amacrine cells (Ehinger and Falck, 1971). In most other species, GABA is taken up by either amacrine cells, or, in some cases, by horizontal cells (Marshall and Voaden, 1974b). Radioactive GABA which has been taken up by retina can be released either by a direct electrical stimulation of isolated retina or by exposure to depolarizing levels of potassium (Kennedy and Voaden, 1974; Voaden and Starr, 1972). Since this release occurs in rat as well as other species, the physiological significance of this release has yet to be determined. The release of GABA from those species in which GABA is retained specifically by neuronal elements has not been shown to be calcium dependent. Graham et al (1970) report a decrease in the total amount of GABA in dark adapted retinas. The decrease may result from an inhibition of the GABA uptake system in dark adapted retinas (Lam and Steinman, 1971; Lam, 1972; Starr, 1973). GABA has a specific inhibitory effect on the electrical activity of the retina (Noell and Lasansky, 1960).

Data recently published by the laboratory has further characterized and localized the GABA system in cow and rabbit retina (Redburn, 1977; Ferkany et al, 1978). The P_2 fraction from rabbit and cow contains a prominent GABAnergic system with uptake and release rates among the highest reported for any neuronal tissue. The P_1 fraction in rabbit demonstrated a much lower rate of uptake and release. In the cow, these GABA activities are evenly distributed between P_1 and P_2 fractions.

Two types of GABA receptor sites were found in bovine retina: one site had a much higher affinity (Kd =

38nM) than the other (Kd = 400nM). The P_1 fraction contained both sites; the P_2 fraction contained only the lower affinity receptor sites.

D. *Glutamate/Aspartate* - Both glutamate and aspartate are present in retina (Levi and Morise, 1971; Cohen et al, 1973). A high affinity uptake system has been demonstrated in whole retina for the compounds (Neal and White, 1971: Ehinger, 1972). In addition, electrophysiological studies have shown a marked electrophysiological effect of both aspartate and glutamate on retinal neurons. Thomas and Redburn (1978) characterized the uptake system for aspartate and glutamate in retinal subcellular fractions from rabbit retina. The rate of maximum uptake/mg protein was highest in the P_2 fraction for both compounds. Glutamate uptake sites had a significantly higher affinity (lower Km) and a significantly lower capacity than aspartate in P_1 fractions. Glutamate was shown to be a competitive inhibitor of aspartate uptake. Aspartate was a competitive inhibitor for a large portion of glutamate uptake; however a significant portion of glutamate uptake was not inhibited by aspartate.

Unpublished radiographic data from this laboratory also indicate a difference between glutamate and aspartate uptake sites. There is a predominantly neuronal (cone and amacrine cells) localization of sequestered ^3H -glutamate and a predominantly glial (Mueller cell) localization of ^3H -aspartate.

E. *5-Hydoxytryptamine (5-HT)* - Ehinger (1976) details evidence for the presence of a 5-HT cell in the amacrine cell layer of rabbit retina. Thomas and Redburn (1978) have demonstrated that bovine retina contains appreciable amounts of 5-HT and its major metabolite by direct biochemical analysis. Uptake and release systems for 5-HT are also present in whole retina. These fractions exhibit a high degree of pharmacologic specificity since they are inhibited by the 5-HT uptake blockers chlorimepramine and the storage blocker, reserpine.

In summary, several functional neurotransmitter agents have been identified and partially localized in the vertebrate retina. Candidates for photoreceptor neurotransmitters include acetylcholine, aspartate and glutamate. One class of horizontal cells appears to be GABAnergic. A variety of neurotransmitters including acetylcholine, GABA, glycine, dopamine, glutamate and serotonin are associated with various types of amacrine cells. The identities of bipolar and ganglion cells are unknown. Many of the recent and future neurotransmitter studies rely on *in vitro* systems in order to obtain direct biochemical evidence for neurotransmitter activities. EM analytical analysis of retinal subcellular fractions has aided substantially in establishing these *in vitro* systems as suitable preparations for neurotransmitter studies. In combination with autoradiographic and cytochemical techniques, complete neurotransmitter mapping of the retina should be possible and will, when complete, enlarge significantly the present level of understanding of the unique structure and function of the retina.

ACKNOWLEDGEMENTS

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ISOLATION PROCEDURE

1. Remove ORS — Vortex Retina in 0.32M Sucrose, Decant

2. Homogenize by Hand in Glass — Teflon Homogenizer.

3. Remove Cellular Debris — Centrifuge 650 x g, 10 Min.

4. Pellet PCS Fraction — Centrifuge 2000 x g, 10 Min.

5. Pellet P_2 (Conventional) Synaptosomal Fraction — Centrifuge 15,000 x g for 12 Min.

Figure 1

TSEM e-

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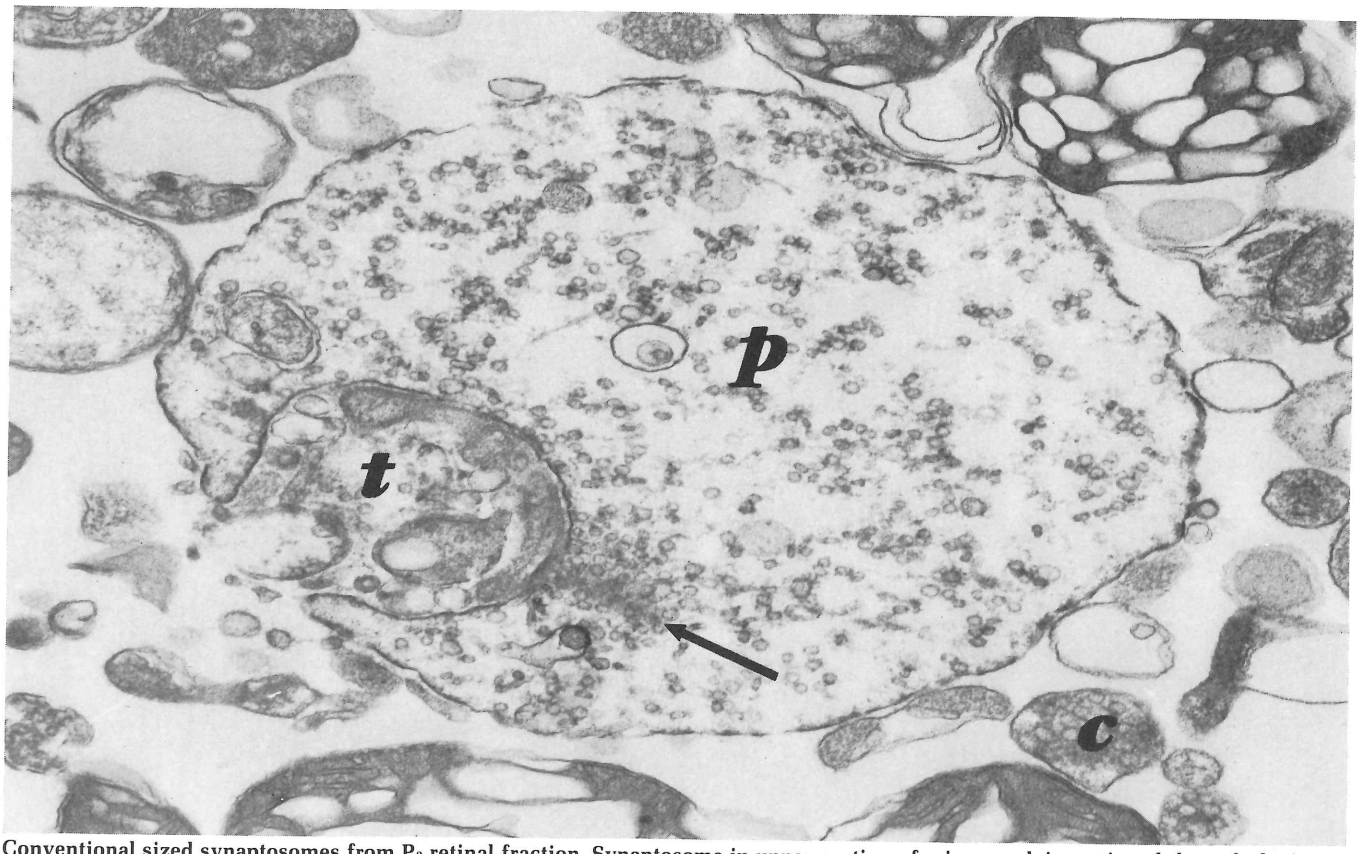
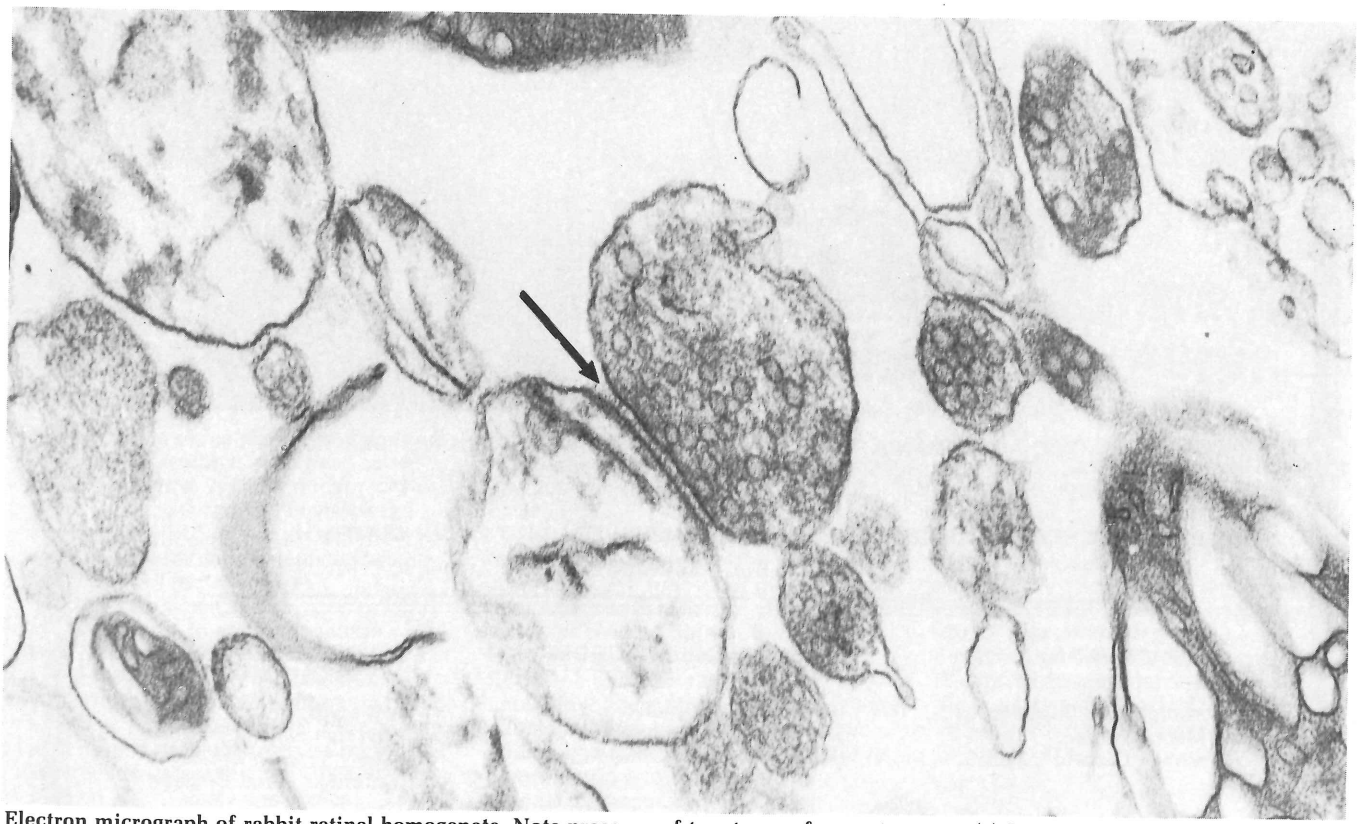


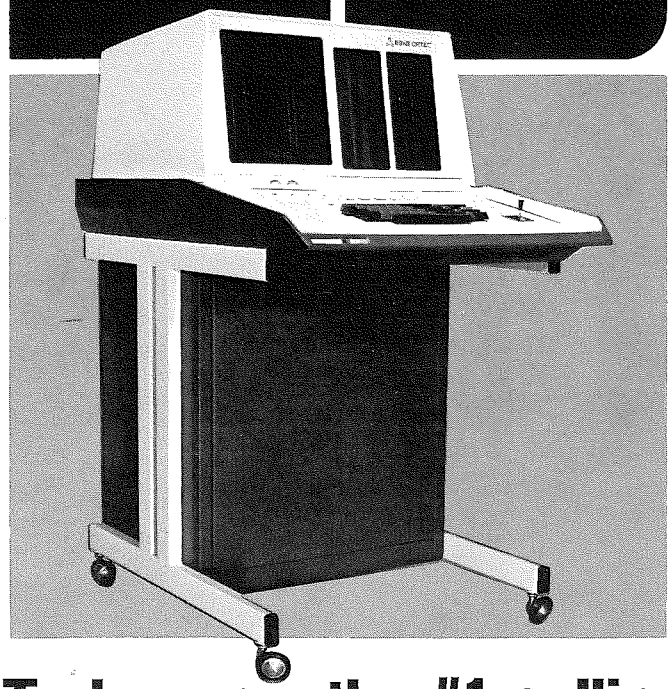
Conventional sized synaptosomes from P_2 retinal fraction. Synaptosome in upper portion of micrograph is sectioned through the junctional complex (see arrow). Pre- and postsynaptic membranes are dark and appear to be connected to filamentous material in the cleft. Morphology is characteristic of comparable fractions obtained from whole brain. ×90 000.



Electron micrograph of rabbit retinal homogenate. Note presence of two types of synaptosomes. (1) Large synaptosome (p) 2·5 μm in diameter with complex postsynaptic element (t) and synaptic ribbon (see arrow) characteristic of photoreceptor terminals. (2) Small synaptosome (c) 0·3 μm in diameter. ×83 000.

Figure 2

EEDS II



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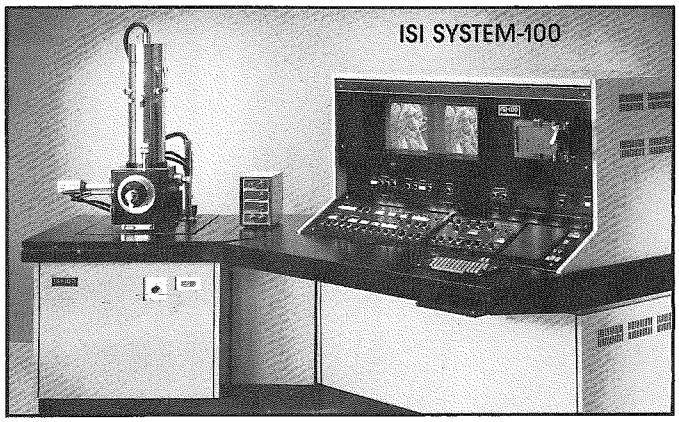

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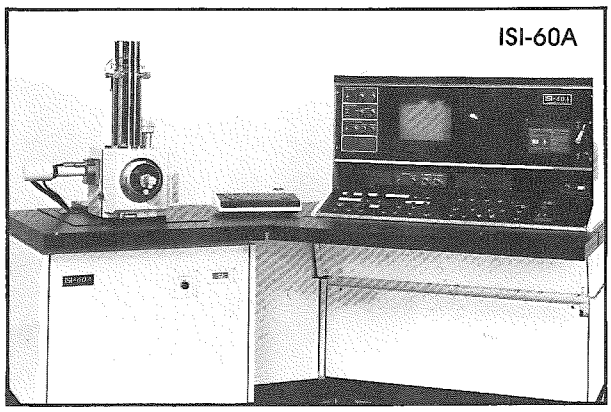

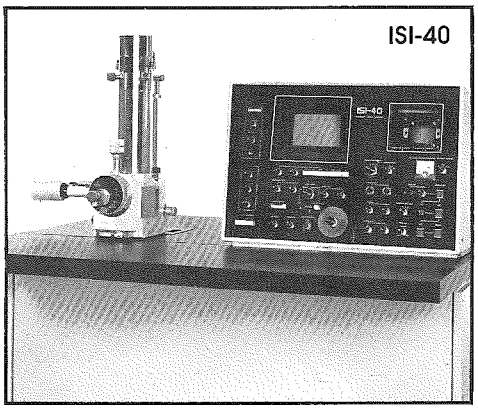

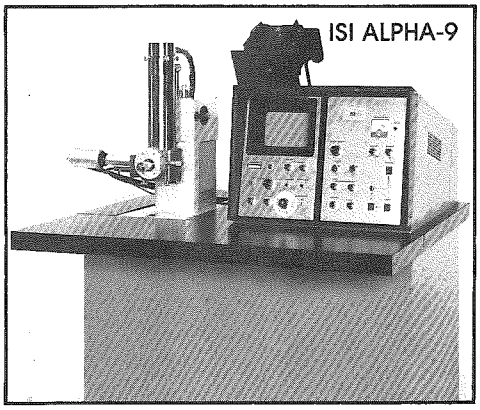

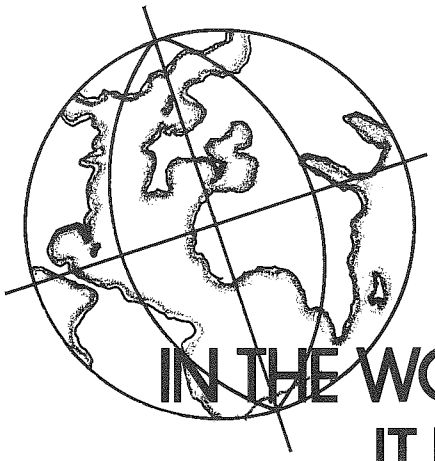

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Abstracts

SPURIOUS SILICON PEAKS IN ENERGY DISPERSIVE X-RAY SPECTRA OF BIOLOGICAL SPECIMENS. Nancy K. R. Smith, Department of Anatomy, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284.

In order to garner some of the wealth of information available from electron probe elemental x-ray microanalysis of biological specimens, one must be ever aware of the possibility of observing spectral artifacts which could lead to incorrect conclusions regarding the qualitative and/or quantitative composition of the specimens. Spectral artifacts may result from the process of x-ray detection, from signal processing, from microscope and specimen holder components, and from specimen contamination. The microanalyst should be very suspicious of a Si peak obtained from biological specimens, particularly if the intensity of the peak increases with time of electron beam exposure. Such a condition completely invalidates quantitative analysis of the elements of interest in the specimen. The investigator must consider each step of his particular specimen preparative procedure in order to isolate sources of Si contamination. Specimen contamination may be acquired at any point during specimen preparation but is most likely to result from either direct or vapor phase exposure of the specimen to silicone based oils and greases. Silicon contamination may originate from vacuumborne contaminants in the electron microscope itself. In the total absence of any Si contamination, a Si peak may still be observed in energy dispersive x-ray spectra due to the internal fluorescence detector peak.

CELL KINETICS AND TRANSEPITHELIAL MIGRATION OF LEUKOCYTES IN THE MAMMARY GLAND. L. L. Seelig, Jr. The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Cells with morphological characteristics of leukocytes residing in the mammary alveolar epithelium were studied with special reference to their migration to, presence in, and transit through the epithelium to become normal components of milk. During alveolar development the intraepithelial leukocyte population was constant (3.5%); however, it increased significantly (8.7%) during lactation. Although the total number of leukocytes was increased, the percentage of labeling was unchanged at the onset of lactation and was significantly decreased between 2 and 6 days postpartum. Intraepithelial leukocytes were typically located in the basal portion of the alveolar epithelium and were usually observed in the vicinity of myoepithelial cells. In certain areas, a complete loss of apical junctional complexes between epithelial cells was observed, and the resulting epithelial gaps were filled with alveolar secretions in direct contact with an expanse of nude basal lamina. Of major interest was the finding that leukocytes were observed both within the gaps and in the efferent ductal system. These morphological observations suggest a possible pathway for the migration of leukocytes from the alveolar connective tissue into the exosecretions of the mammary glands, there to be passed to the immunologically naive suckling infant.

ABNORMALITIES IN AORTAS OF COPPER DEFICIENT RATS. E. J. Root and J. B. Longenecker. Graduate Nutrition Division, The University of Texas at Austin. Austin, Texas 78712.

Aortas of albino rats fed diets low or deficient in copper have been examined by light and electron microscopy and two quite different types of abnormalities have been found.

One defect is a separation of the elastic laminae of the arteries, probably caused by inadequate cross-linking of the elastin component. Incidence of this defect suggests that it is dependent upon a genetic susceptibility. In addition the defect is more prominent if vitamin B_6 or essential fatty acids are absent from the diet, and less prominent if the diet contains cholesterol in butter.

The other abnormality is the loss of smooth muscle cells in localized areas of the artery, leading to rupture, hemorrhage and death of the animal. Cells adjacent to the lesion show mitochondrial swelling, clumping of nuclear chromatin, disorganization of myofilaments and enlargement of the endoplasmic reticulum with dissociation of ribosomes.

Appearance of the tissues is consistent with changes which might be expected to follow loss of activity of two copper-dependent enzymes found in arteries. Decreased lysyl oxidase activity may be responsible for the elastic tissue defect, and loss of cytochrome oxidase activity could cause the intracellular changes. Reasons for the varied effects of copper deficiency are not known but may be related to both genetic and dietary factors.

ULTRASTRUCTURE OF TELIOSPORE FORMATION IN THE RUST PUCCINIA PODOPHYLLI. Charles W. Mims and E. Laurence Thurston. Department of Biology, S. F. Austin State University, Nacogdoches, Texas 75962, and Department of Biology, Texas A&M University, College Station, Texas 77843.

Puccinia podophylli is an autoecious rust parasitizing **Podophyllum peltatum**. Infected leaves of the host bearing telia of the fungus were collected and prepared for study with both TEM and SEM.

Teliospore initials of **P. podophylli** develop from binucleate sporogenous cells lining the base of the telium. The nuclei of the sporogenous cell divide conjugately and two of the daughter nuclei move into the teliospore initial while two remain in the sporogenous cell. The initial elongates and is delimited from the sporogenous cell by the formation of a septum. The nuclei within the initial then divide conjugately and a septum develops separating the initial into two binucleate cells. The basal cell becomes the pedicel of the spore while the apical cell continues to develop into a two-celled teliospore.

Mature teliospores of **P. podophylli** are oblong or clavate and are sparingly covered with rather long straight or slightly curved spines. These spines initially appear as slight bulges on the surface of the young spore. The spore wall in such a region then evaginates to form a slender projection filled with cytoplasm. A central electron-translucent lumen devoid of cytoplasm is visible in older spines viewed in thin section. Little substructure is evident in the extremely electron-opaque wall of older teliospores

ULTRASTRUCTURE OF THE AECIAL AND TELIAL HAUSTORIA OF THE AUTOECIOUS RUST PUCCINIA PODOPHYLLI ON PODOPHYLLUM PELTATUM. Jane Borland, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

Intracellular absorbing organs or haustoria produced in rust fungi are of two basic types; those characteristic of pycnial-aecial infections and those characteristic of uredial-telial

infections. These two types of haustoria were examined in the aecial and telial stages of the autoecious rust **Puccinia podophylli** on **Podophyllum peltatum**, and their structural differences may be summarized as follows:

Characteristic	Aecial	Telial
(1) HMC wall thickenings in penetration zone	−	+
(2) Amorphous electron dense material present between HMC wall and host wall	−	+
(3) Entire HMC wall enters haustorium	+	−
(4) Presence of a collar	+	−
(5) Neck with dark staining neck ring	−	+
(6) Presence of septations	+	−
(7) Haustorium has smooth outlines	−	+
(8) Haustorium highly vacuolate	+	−

Since **Puccinia podophylli** is an autoecious rust, these differences cannot be the result of differences in host tissues as could be the case in heteroecious species. These absorbing organs should also be justly referred to as haustoria rather than intracellular hyphae because all of the organs observed terminated in the host cell and none of the host cells appeared to be necrotic.

ULTRASTRUCTURAL INVESTIGATIONS OF PROCESS TRANSECTION ON NB41A3 MOUSE NEUROBLASTOMA CELLS VIA FOCUSED UV LASER PULSES. Marilyn N. Smith, M. Louise Higgins and Guenter W. Gross, Department of Biology, Texas Woman's University, Denton, Texas 76204.

The investigation of neuronal network information storage requires the monitoring of all electrical activity in known anatomical circuits. To approach this condition the growth of two-dimensional, ordered neuronal networks on multimicro-electrode surfaces is being investigated. A new method of laser microbeam cell surgery allows the removal of undesired cells or unwanted neuronal processes in closed chambers. A UV microbeam laser producing 10 nsec pulses at a 337 nm wavelength, a maximum energy flux of 200 microjoules/μm^2 and a minimum focus diameter of 0.7μm is being utilized. Cells survive extensive surgical manipulation but the limits of this manipulation are not known. Irradiated cells, marked for identification by laser shots into the surrounding substrate, are fixed conventionally. Transected cell processes show a resealing of membranes at the site of transection and remarkably little ultrastructural change in adjacent, unirradiated regions. Variation of temperature or pH (23-38°C; 7-7.6) during working periods with the cells causes no identifiable changes, whereas low serum growth media result in pale membranes. Process transection can be achieved by direct vaporization of cytoplasm or indirectly by small shock waves resulting from vaporizing substrate. At high energy densities, shock waves appear most deleterious and cause somal blebbing, vesiculation of ER and margination of chromatin.

CALCIFICATION OF NECROTIC TISSUES IN THE BRAIN. J. B. Kirkpatrick, H. Hagler and L. Gilbert, Departments of Pathology and Neurology, University of Texas Health Science Center at Dallas, Dallas, Texas.

The encrustation of necrotic tissues by calcium salts is a well-known phenomenon in general pathology. Except for the "ferruginated" neuron, Fahr's disease and vessels of the basal ganglia, the calcifications which occur in the brain have received only sketchy attention in the laboratory. Now newly available techniques, including energy dispersive spectroscopy, make it possible to examine minute deposits of crystals, with direct electron microscopic control of the sample assayed. The new technique has been applied most successfully to the phenomenon of calcific crystals which develop within hypoxic myocardial mitochondria. This paper records similar observations from the brains of patients who died of cerebral hypoxia or other necrotizing conditions. The cerebral calcifications develop on a more leisurely time scale than the myocardial deposits — days, compared to hours — but the localization to subcellular organelles and the required proximity to intact circulation are similar. The significance of these observations is more than merely theoretical, since it may become possible to visualize the deposits in still living patients through the use of calcium-seeking radioactive or augmented radiocontrast probes.

NEUROENDOCRINE RELATIVES: THE PARANEURON CONCEPT. John T. Hansen, Department of Anatomy, The University of Texas Health Science Center, San Antonio, Texas 78284.

Paraneurons are cells which share certain morphological, physiological and metabolic characteristics with neurons and amine and peptide producing endocrine cells. As originally proposed by Fujita (1976), paraneurons must meet the following criteria: (a) produce a neurotransmitter substance or polypeptide hormone; (b) possess synaptic vesicle-like granules; (c) possess a recepto-secretory function, and; (d) be of neuroectodermal origin. Some paraneurons are primarily endocrine in function while others are sensory. Examples of members of the paraneuron classification include: chromaffin cells, SIF cells, arterial chemoreceptor cells, pancreatic islet cells, gustatory cells, hair cells of the inner ear, pinealocytes, Merkel cells, and several others. An explanation of the paraneuron concept will be given, using the glomus cells of the arterial chemoreceptors as examples.

(Supported by a Grant-in-Aid from the American Heart Association)

THREE DIFFERENT SYSTEMS OF INTERMEDIATE FILAMENTS IN BRAIN CELLS. Howard Feit, John Fuseler and Jerry Shay, The University of Texas Health Science Center, Dallas, Texas.

Intermediate filaments isolated from bovine white matter and peripheral nerves are each composed of proteins which can be distinguished from each other and from the intermediate filaments of smooth muscle (desmin) on the basis of electrophoretic and antigenic properties. The heterogeneity of the intermediate filaments of central and peripheral axons was confirmed by comparing the protein composition of white matter, dorsal roots and femoral nerves by two-dimensional electrophoresis. Immunofluorescence studies of cerebral explants and developing cardiac myoblasts obtained from neonatal rats using antibodies against central and peripheral axonal intermediate filaments and against desmin showed that both brain cells and developing myoblasts contain at least three different systems of intermediate filaments.

IMMUNOFLUORESCENT AND ULTRASTRUCTURAL OBSERVATIONS ON INTERMEDIATE FILAMENTS LOCALIZED WITHIN RAT BRAIN CELLS TREATED WITH ANTIMITOTIC AGENTS. John Fuseler, Howard Feit, and Jerry Shay, The University of Texas Health Science Center, Dallas, Texas.

Investigations utilizing indirect immunofluorescence with monospecific antibodies against 51,000 dalton neurofilament protein and transmission electron microscopy has revealed an

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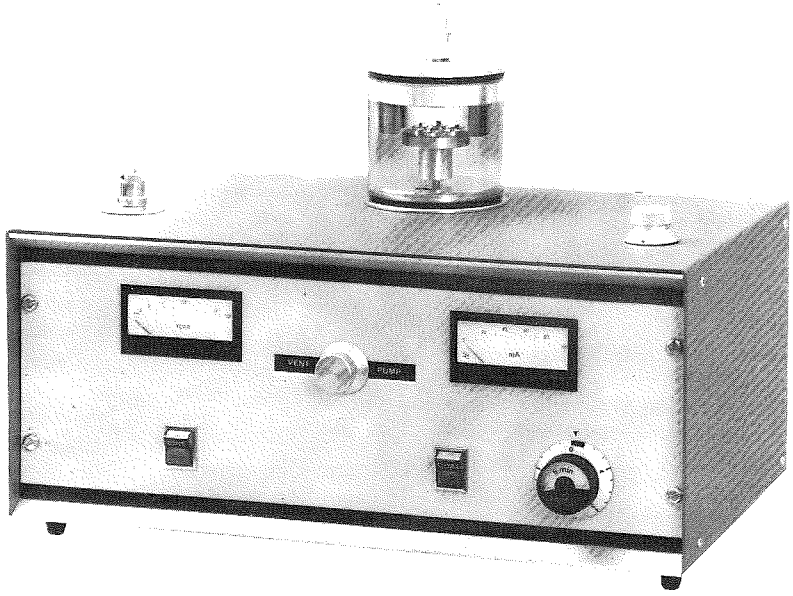

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elaborate network of intermediate filaments in cells derived from neonatal rat cortex. This class of neurofilament are not altered by low temperature (0°C) treatment for up to four hours. The distribution and morphology of these intermediate filaments are highly altered by the antimitotic drugs Colchicine (0.01mg/ml), and Vinblastine (0.01mg/ml) after 24 to 48 hours of treatment to form massive filament bundles or cables closely associated with the nucleus. These filament bundles are not altered by low temperature treatment, and remain present in the cell for up to three days following removal of the drug. These observations suggest that chemical agents which depolymerize microtubules can also effect the state of organization of intermediate filaments in brain cells. It is, however, not clear at this point if the state polymerization of microtubules and the orientation of intermediate filaments are coupled.

TRANSMISSION AND X-RAY ANALYTICAL ELECTRON MICROSCOPY OF PATHOLOGICAL CALCIFICATION IN THE HEART. Linda E. Lopez, Herbert K. Hagler, Vincent DiMaio, L. Maximilian Buja. Department of Pathology, The University of Texas Health Science Center at Dallas, Dallas, Texas.

In order to study the process of pathologic calcification in the heart, tissue was obtained from: (a) dog heart subjected to 40-60 minute temporary coronary artery occlusion and reflow; (b) dog hearts with 24-hour permanent coronary occlusion; (c) a human case of idiopathic multifocal myocardial necrosis. In the short-term temporary ischemia model, calcification was localized in the mitochondria in the form of small granular inclusions. The 24-hour ischemic tissue showed more extensive mitochondrial calcific deposits which frequently had a spicular appearance. Mitochonrial calcification was localized to the infarct periphery which received some collateral blood flow. Similar spicular crystals were seen in the human heart in the mitochondria, the cytoplasm, as well as the extracellular spaces. Electron probe x-ray microanalysis has confirmed that both types of aggregates are composed primarily of calcium and phosphorus. Thus, pathological calcification can be a prominent feature of myocardial damage.

ULTRASTRUCTURE OF THE CHLAMYDOSPORE OF CANDIDA ALBICANS. Sara A. Michie and Daniel F. Cowan, Department of Pathology and Laboratory Medicine, The University of Texas Medical School at Houston, Houston, Texas, 77025.

Candida albicans, a fungus which causes a wide variety of human and animal diseases, is a dimorphic organism capable of growing as a budding yeast or a pseudomycelium. In addition, it will produce thick walled chlamydospores when grown in nutrient deficient culture. These highly characteristic chlamydospores are of great importance in the laboratory identification of the organism. This study deals with the ultrastructural aspects of the chlamydospore cell wall and cytoplasmic structures. This information will be used as a baseline for further ultrastructural studies on the formation and germination of the chlamydospore.

TUMORS OF VASOFORMATIVE CELLS. Bruce Mackay, M. D. Anderson Hospital & Tumor Institute, Houston, Texas.

Benign and malignant tumors that develop from the cells that form the walls of blood and lymphatic vessels present a range of histologic appearances. The benign tumors can usually be identified by light microscopy with little difficulty, but when lumens are poorly formed within solid groups of cells, electron microscopy is useful: the ultrastructural features of endothelial cells are not completely specific, but integration of the clinical and morphologic findings should lead to the correct diagnosis. A curious formation in some vascular tumors is the occurence of papillary projections consisting of connective tissue cores covered by a layer of endothelial cells: so-called papillary endothelial hyperplasia. Angioma and lymphangioma differ in minor degree in their fine structure. The glomus tumor arises from cells of the Suquet-Hoyer canal and the round tumor cells have smooth muscle features. Angiosarcomas present a variety of ultrastructural appearances that depend to some extent on the differentiation of the tumor: the cells may form crude vascular channels, but cell junctions and basal lamina are sparse or absent. The spindle cells of Kaposi's sarcoma simulate fibroblasts and have some phagocytic activity, but they form small vessels whose walls may be incomplete allowing extravasation of red blood cells. The hemangiopericytoma is thought to be derived from pericytes, but cytoplasmic filaments are not always present, and light and electron microscopic criteria for its identification are not well defined.

ULTRASTRUCTURE OF CONIDIOGENESIS IN THE FUNGI DORATOMYCES MICROSPORUS AND GRAPHIUM PUTREDINIS. Nancy Wagner and Darrell Hudson. Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

Conidial ontogeny is one of the most important characteristics used in the taxonomy of fungi belonging to the form-Class Deuteromycetes. Because of the small size of the conidiogenous cells in most of these fungi, it is necessary to use electron microscopy to elucidate the details of conidiogenesis. In this study conidial ontogeny is examined at the ultrastructural level in the synnematal fungi **Doratomyces microsporus** and **Graphium putredinis**.

Conidiogenesis in both species noted above proved to be annellidic. In this mode of conidiogenesis the conidiogenous cell is termed the annellide. An annellide gives rise to conidia in a basipetal fashion and elongates with the formation of each conidium. When successions of conidia occur the conidium proliferations take place through the scar of the most recently formed conidium and the annellide bears a series of ring-like scars, termed annellations.

THE EFFICACY OF USING HUMAN AMNION AS A BURN WOUND DRESSING. Baur, P. S., Thomson, P. D., Brown, G. M. and Browne, M. E. Shriners Burns Institute and the University of Texas Medical Branch, Galveston, Texas 77550.

Human amnion is currently being used as a biological dressing for the treatment of burns. Since this tissue is a continuation of the fetal integument it has many of the physical qualities of skin. Amnion has become a more popular dressing in some institutions than porcine xenograft. The use of amnion as a biological dressing depends in part on the integrity and sterility of the membrane and the cleanliness of the wound to which it is applied. It is now recommended that the amnion be replaced every three days. Electron microscopic studies of this tissue have revealed the near total degradation of amnion by the host as early as three days post-application. This enzymematic degradation is initiated as early as the first 24 hours after application. Collagen filaments in the amnion dermis are first unraveled then dissolved by the enzymatic process. The soft collagen configuration is frequently observed in this tissue. Cellular features of the amnion dermis and epidermis are completely disrupted after 48 hours. The integrity of the basal lamina is similarly compromised. The morphological electron

microscopic evidence gathered in this study suggest that the barrier qualities of the amnion are lost long before the patients' need for barrier protection has ended.

STUDIES ON THE CYTOLOGY AND SYNAPTIC ORGANIZATION OF THE BASILAR PONTINE NUCLEI IN THE RAT EMPLOYING AUTORADIOGRAPHY, HRP AND COMBINED GOLGI-ELECTRON MICROSCOPIC PROCEDURES. Gregory A. Mihailoff, Cell Biology Department, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

On the basis of autoradiographic studies, it has been possible to define those regions within the pontine nuclei which receive input from various portions of the cerebral cortex and deep cerebellar nuclei and subsequently to identify with electron microscopy the particular types of axonal boutons present in those regions which undergo degeneration following lesions of each afferent system. Our results indicate that two distinct types of degenerating boutons are present after cortical or cerebellar lesions, thereby suggesting that each system might be composed of two separate neuronal elements. This hypothesis was tested by injecting horseradish peroxidase (HRP) directly into the pontine nuclei in order to visualize the cell bodies giving rise to the cortical and cerebellar afferents. In the cerebral cortex, labelled cell bodies were segregated into **two** laminae within layer Vb while in the cerebellar nuclei, **two** classes of reactive somata were easily distinguished on the basis of size. In order to analyze the synaptic interactions of cortical and cerebellar afferents with **individual** pontine neurons, the combined Golgi-EM degeneration methodology was employed. Single pontine neurons located within cortical or cerebellar terminal zones were identified in Golgi prepared tissue taken from lesioned animals. Sections containing such neurons were trimmed, processed for EM and serially thin-sectioned. Initial observations indicate that **both** types of cortical afferents form synaptic contacts with **spiny** pontine projection neurons.

MORPHOMETRIC QUANTITATION OF RABBIT PMN GRANULES. K. Porter,* W. J. Brown,° W. J. Snell,° and W. A. Shannon, Jr.*° Veterans Administration Medical Center* and Department of Cell Biology, Southwestern Medical School°, Dallas, Texas.

Essentially two populations of rabbit polymorphonuclear (PMN) leukocyte granules exist: azurophilic granules (A), which are relatively larger, more electron dense and contain lysosomal enzymes, and specific granules (S), which are relatively smaller, less electron dense and contain lysozyme. Fractions of A and S granules were prepared from disrupted PMN from pooled rabbit peritoneal exudate (from 3 animals) by centrifugation in a discontinuous sucrose gradient (Brown, Shannon and Snell, Proc. EMSA, vol 37, in press). The areas of the granules in intact cells and isolates were morphometrically quantitated by tracing around the perimeters of the granules using a Bit Pad digitizer and 8080 microprocessor for data analysis.

The average size of A and S granules in the cells was 0.184 u^2 (N=151) and 0.063 u^2 (N=255), respectively. The average number of A and S granules per u^2 of cytoplasmic area (estranuclear) was 0.827 and 1.397, respectively. The A and S granules were found to occupy 13.8% and 8.3%, respectively, of the total extranuclear cytoplasmic area. The ratio of the number of A to S granules was thus found to be 1:1.7. A comparison of the digitizer method using % area and stereological analysis using % volume was made since $A_A=V_V$. For the A granules the % area and % volume was the same but for the S granules the two values were slightly different.

The initial attempt was made to compare the granule sizes found within cells to isolated pellets. Our preliminary data indicate that the granules may undergo a significant size change (up to 14%). The reason for this may be due to either an alteration of granule size during isolation and/or a sampling error. Further investigation is being conducted to resolve the differences.

D-CELL POPULATION AND SECRETION IN HYPOPHYSECTOMIZED DOG PANCREAS AND STOMACH. W. A. Shannon, Jr., Veterans Administration Medical Center and Department of Cell Biology, Southwestern Medical School, Dallas, Texas.

Hypophysectomized (hypoX) dogs were prepared by transtemporal surgical extrication of the pituitary gland and maintained up to 254 d. Splenic pancreas and antral stomach specimens were fixed in Bouin's solution or glutaraldehyde followed by osmium for immunofluorescence and transmission electron microscopy (TEM), respectively. The extent of pituitary remmant was microscopically determined postmortem.

The numbers of pancreatic islets per unit area and cells per islet were decreased (68 vs 152 and 17 vs 31/mm^2, respectively). Immunofluorescence staining for somatostatin, i.e., SRIF, somatotropin (GH, growth hormone) release inhibiting factor, indicated a decrease in the number of D-cells. Likewise, there appeared to be fewer D-cells in the antral stomach (46 vs 97/mm^2). TEM of pancreatic islets indicated fewer D-cells. The cells appeared to be hypersecreting. They contained a number of granules of greater density than in normal D-cells. In addition, the granules contained an atypical dense core.

A relationship is apparent between the pituitary gland, in which GH is regulated by hypothalamic SRIF, and SRIF-containing cells of the stomach and pancreatic islets. Perhaps in the normal animal, feedback from pituitary GH inhibits hypothalamic SRIF secretion which in the hypoX dog produces the described anomalies.

ENDOCRINE CELLS IN OXYNTIC MUCOSA OF ALLOXAN DIABETIC DOGS. W. A. Shannon, Jr. Veterans Administration Medical Center and Department of Cell Biology, Southwestern Medical School, Dallas, Texas.

Diabetic dogs were prepared by a single 70 mg/kg i.v. injection of alloxan. The animals were maintained 4 months to 7 years. Specimens of oxyntic mucosa were fixed in Bouin's fixative for immunocytochemistry or glutaraldehyde and osmium for TEM. The distribution of glucagon- and somatostatin-containing cells was determined by immunofluorescence.

Immunofluorescence indicated an increase in the number of A- (209 vs 115) and D- (251 vs 97) cells per mm^2. Often two or more of the reactive cells would appear adjacent or in the same cross section of gastric gland. At the fine structural level, four endocrine cells were identified: A-, A-like, D-, and ECL. Each of these cell types appeared to have increased numbers present per unit area. In some D-cells, hypersecretion was evident. There were some cells present without granules which appeared to be depleted D-cells or perhaps lymphocytes. Weak immunofluorescence was noted in rare cells in subglandular lymph nodules. TEM revealed rare lymphocytes similar to the cell type observed in the mucosa to be present in the nodules.

The hyperplasia of oxyntic mucosal endocrine cells noted in the alloxan diabetic dog apparently results from the diabetic condition. The hyperplasia may be secondary to the loss of corresponding pancreatic cells or due to the severe loss of B-cells. The parallel increase of these cells may well indicate a functional relationship.

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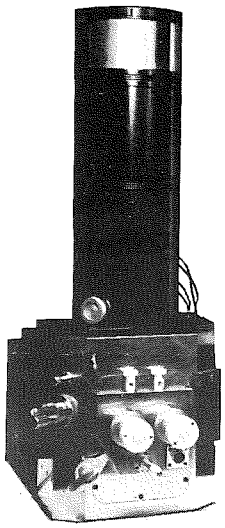

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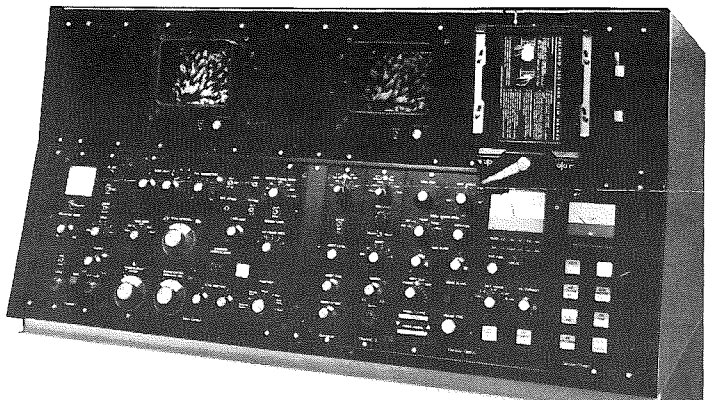

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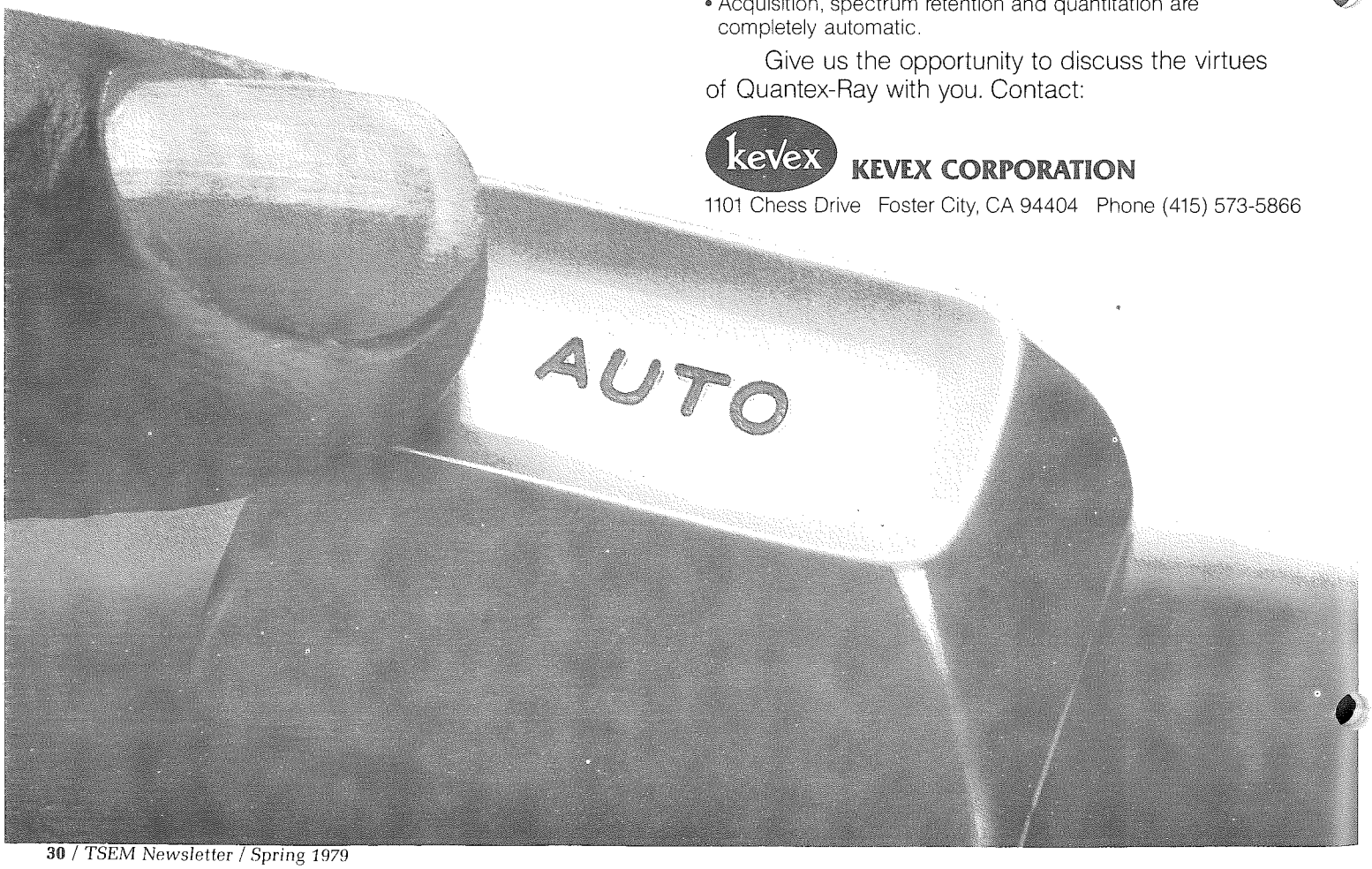

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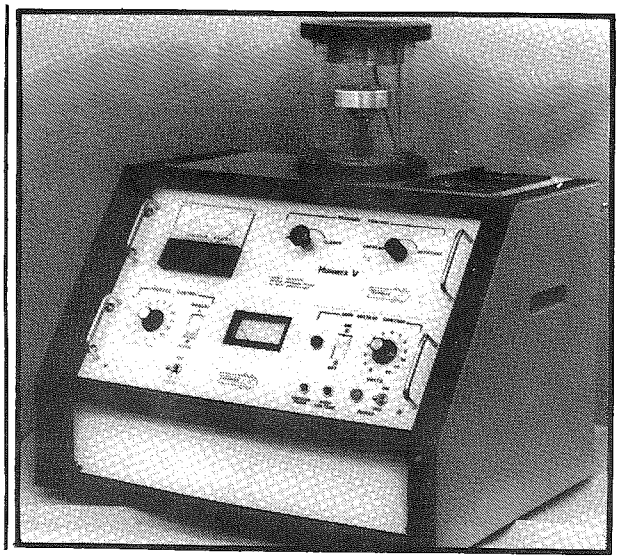

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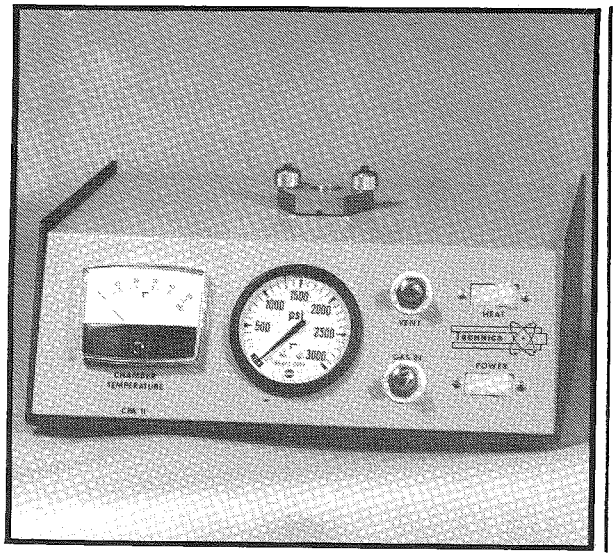

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I hereby apply/nominate for Regular ☐ Student ☐ Corporate ☐ membership in the Texas Society for Electron Microscopy.

Name of institution applicant or corporation nominee person _____

P. O. Address _____

_____

Information as to position, degrees, and qualifications for Membership: _____

_____

_____

_____

This nomination is accompanied by a statement of interest in and contributions to Electron Microscopy and associated fields of science.

One year's dues in the form of a check or money order should be sent with the application for Membership form. (Regular $10.00, Student $2.00, Corporate $75.00)

Signature of one Member making the nomination:

_____

Dated _____ 19 _____

This application to Membership in the Society, or this application for transfer from the grade of Student to Regular Member, signed by one Member should be sent to the Secretary to be presented at the next meeting of the Council or approval by a majority vote of the Council. Notice of approval will be mailed by the Executive Secretary.

_____

Presented to the Council at _____ meeting. Date _____

Action _____

Remarks _____

Send Application to: John Hansen
Univ. of Texas Health Science Center
Dept. of Anatomy
7703 Floyd Carl Drive
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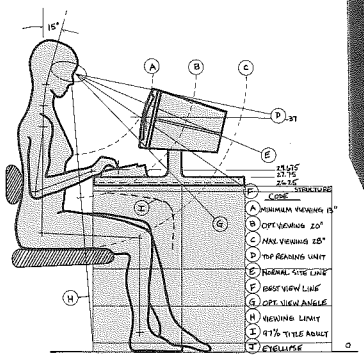

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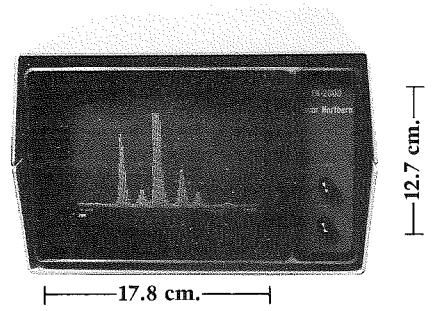

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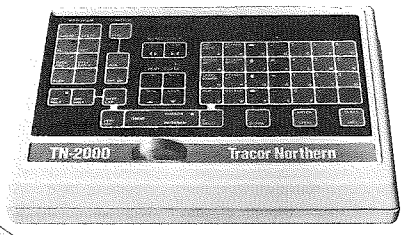

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Regional News

DALLAS: SOUTHERN METHODIST UNIVERSITY, BIOLOGY DEPARTMENT

COURSE OFFERED

Dr. James Martin is offering a course this spring in Advanced Techniques in Electron Microscopy to doctoral students in biology.

PERSONNEL

Dr. R. S. Sohal will be a senior visiting scholar at the Zoology Department, The University of Cambridge, England from June, 1979 to January 1980.

PUBLICATIONS

Sohal, R. S. and Bridges, R. (1978) Associated changes in the size and number of mitochondria present in the midgut of the larvae of the housefly, **Musca domestica. J. Cell Science 13**, 335-341.

Sohal, R. S. and Lamb, R. E (1979) Storage-excretion of metallic cations in the adult housefly, **Musca domestica. J. Insect Physiol.** 119-124.

Sohal, R. and Donato, H. (in press) Effect of experimental prolongation of life span on lipofuscin content and lysosomal enzyme activity in the brain of the housefly, **Musca domestica. J. Geront.**

Ubelaker, J. E. (in press) Biology of the Cysticercoid of **Hymenlopis dimunita.** In **Biology of Hymenlopis dimunita.** Academic Press, New York.

DENTON: TEXAS WOMAN'S UNIVERSITY, DEPARTMENT OF BIOLOGY

GRANTS AWARDED

Dr. Guenter Gross received a 3 year grant from the National Institute of Health for the study of ordered neuronal networks on multimicroelectrode surfaces. Ordered neuronal networks, made possible by laser cell surgery, will be studied by light and electron microscopy to correlate electrophysiological and morphologic data.

PUBLICATIONS

Marilyn W. Smith, S. Donald Greenberg, Harlan J. Spjut, The Clara Cell: A Comparitive Ultrastructural Study in Mammals, American Journal of Anatomy (in press).

DEPARTMENT OF CHEMISTRY

NEW FACULTY

Dr. Janice Barton, Assistant Professor of Chemistry, is interested in the polymerization of cytoplasmic microtubules.

PRESENTATION

Dr. Barton presented a paper at the American Society of Neurochemistry at Charleston, South Carolina and also gave a seminar at Baylor University Department of Chemistry at Waco. The Waco seminar is a part of a speakers exchange program between the chemistry departments of Texas universities.

GALVESTON: UNIVERSITY OF TEXAS MEDICAL BRANCH, DEPARTMENT OF HUMAN BIOLOGICAL CHEMISTRY & GENETICS, DIVISION OF CELL BIOLOGY

PUBLICATIONS

Chang, J. P., Mayahara H., Yokoyama, M., Ubukata, A., Moller, P C., An Ultrastructural Study of Morphogenesis of the Fibrogranular Complex in **ductuli efferentes** of Chinese Hamster, Tissue and Cell (in press).

Mayahara, H., Chang, J. P., Electron Microscopic Study of Acid Phosphatase Activity in Cultured Human Cystic Fibrosis Fibrobalsts, Acta Histochem. Cytochem., **11**, 499-459 (1978).

DEPARTMENT OF ANATOMY

Several TSEM member in the Department of Anatomy have recently presented papers at the Anatomy Meetings in Hollywood, Florida:

Callas, G., Atkinson, V. T., DeGroot, W. J., Pressure Volume Changes in Thyroid of Steroid Treated Rats.

Duncan, D., Morales, R., An Effect of High Tonicity in the **substantia gelatinosa** of the Cat.

Payer, A., Paracrystalline Inclusions in Leydig Cell Cytoplasm of Perfused Human Testes.

PUBLICATIONS

Duncan, D., Morales, R., Relative Numbers of Several Types of Synaptic Connections of **substantia gelatinosa** of Cat Spinal Cord, J. Comp. Neurol., **182**, 601 (1978).

Payer, A., An Ultrastructural study of Schwann Cell response to axonal degeneration, J. Comp. Neurol., **183**, 365 (1979).

DEPARTMENT OF PHYSIOLOGY

PUBLICATIONS

Krauhs, J. M., Andresen, M. C., Baur, P. S., Ultrastructure of **Aplysia** neurons having different degrees of sight sensitivity, J. Neurobiol. (in press).

SAN ANTONIO: UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT SAN ANTONIO, DEPARTMENT OF ANATOMY

INVITED LECTURER

Dr. Ivan Cameron was the feature invited speaker at the recent Rocky Mountain Societies for Electron Microscopy and for Electron Microprobe in Denver. He talked on "Energy Dispersive X-ray Microanalysis of Elements in Thin Sections". Dr. John Hansen has recently returned from the Department of Physiological Sciences, Oklahoma State University, where he presented a seminar entitled "The Peripheral Arterial Chemoreceptors".

NEWS BRIEFS

Dr. V. K. Berry attended the Annual Scanning Electron Microscopy Meeting in April (16 - 20), 1979, in Washington, D. C. and presented a paper entitled "Morphology and Chemistry of Manganese Micronodules From the Northwest Atlantic".

PUBLICATIONS

Berry, V. K., Addy, S. K., McKee, T. R. and Smith, N. K. R. 1979. Morphology and Chemistry of Manganese Micronodules From Northwest Atlantic. SEM (in press).

Kaster, A. G. and Cameron, I. L. Scanning Electron Microscopic Observations of the Selective Habitation of Bacteria on the Lingual Mandibular Gingiva of the Rat. J. Dent. Res. (in press).

Cameron, I. L., Pavlat, W. A. and Jeter, J. R., Jr. Chromatin Substructure: An Electron Microscopic Study of Thin-Sectioned Chromatin Subjected to Sequential Protein Extraction and Water Swelling Procedures. Anatomical Record (in press).

Hansen, J. T. 1978. Development of Type I cells of the rabbit subclavian glomera (aortic bodies): A light, flourescence and electron microscopic study. Am J. Anat. **153**: 15-32.

Hansen, J. T. and Ord, T. 1978. Effects of 6-Hydroxydopamine on rat carotid body chief cells. Experientia. **34**: 1357-1358.

Hansen, J. T. 1979. An ultrastructural stereological analysis of the aortic body chief cell of adult rabbits. Cell Tiss. Res. (in press).

Hansen, J. T. and Smith, N. K. R. 1979. Calcium binding sites in vesicles of the carotid and aortic body chief cells. Cell Tiss. Res. (in press).

Herbert, D. C., Parker, C. R., Jr., and Bartke, A. 1978. Serum estradiol, testosterone and dihydrotestosterone in male monkeys treated with testosterone propionate. Endocrine Res. Commun. **5**: 249-257.

Herbert, D. C., Ishikawa, H., and Rennels, E. G. 1979. Evidence for the autoregulation of hormone secretion by prolactin. Endocrinology **104**: 97-100.

Herbert, D. C. 1979. Intercellular junctions in the rhesus monkey pars distalis. Anat. Rec. (in press).

Contreras, A., Herbert, D. C., Grubbs, B. G., and Cameron, I. L. 1979. Blue-green alga, **Spirulina**, as the sole source of protein in sexually maturing rats. Nut. Rep. Int. (in press).

Sheridan, P. J. and Herbert, D. C. 1979. An autoradiographic and immunocytochemical study of the neonatal rat pituitary gland. **Anat. Rec.** (in press).

SOUTHWEST RESEARCH INSTITUTE PUBLICATIONS

Davidson, D. L. and Gause, E. 1978. Chemical and physical characterization of coal gasification particles and its relevance to inhalation toxicology. **Proceedings of the Microbeam Analysis Society**. May, 59A-59I.

Lankford, J. and Davidson, D. L. 1978. Fatigue crack tip plastic zone sizes in aluminum alloys. Intern. Jour. Fracture. **14**: R87-R90.

Davidson, D. L. and Lankford, J. 1979. Determination of the energy of fatigue crack propagation and alteration by wet air. **Proceedings of the Symposium of Environment Sensitive Fracture of Engineering Materials**, edited by Z. A. Foroulis, AIME. 581-594.

Davidson, D. L. 1979. Fatigue crack tip displacement observations. Jour. of Materials Science. **14**: 231.

HOUSTON: BAYLOR COLLEGE OF MEDICINE, DEPARTMENT OF MEDICINE, SECTION OF CARDIOVASCULAR SCIENCES

GRANTS AWARDED

The National Heart Research and Demonstration Center Grant has been renewed and Dr. Ann Goldstein's project on the Ultrastructural Characteristics of Normal and Diseased Heart has been funded.

PUBLICATIONS

Goldstein, M. A., and Entman, M. L. Microtubules in Mammalian Heart Muscle. J. Cell Biol., 80:183-195, 1979.

SEMINARS AND SPEECHES

Dr. Ann Goldstein presented a seminar on "Making a Model of the Z Band" at the Jerry Lewis Neuromuscular Disease Institute at Baylor College of Medicine.

Dr. Goldstein presented a talk on "Three-dimensional Reconstruction of the Z Lattice" to the External Advisory Committee of the High Voltage Electron Microscopy Laboratory at the University of Colorado at Boulder, Colorado.

Dr. Goldstein presented talks for the American Heart Association on heart research to the Auxiliary of the Harris County Pharmacy Association in February and to the Houston Area PTA Council of the Houston Independent School District in April.

TSEM CORPORATE MEMBERS

American Optical Corporation, L. B. Read, P.O. Box 1929, Dallas, Texas 75221. (214) 747-8361.

Advanced Metals Research Corporation.

Carl Zeiss, Inc., Tom W. Valliere, P.O. Box 977, Lewisville, Texas 75056

Collins Radio Group — Rockwell Instruments, Donald L. Wilson, Mail Station 406-146, Richardson, Texas 75080.

Coates & Welter Instrument Corporation, A. Baron Challice, 777 North Pastoria Avenue, Sunnyvale California 94086. (408) 732-8200.

Cambridge-Imanco, Steve Miller, 9551 Williams Street, Rosemont, Illinois 60018. (312) 671-4270.

DuPont/Sorvall, Jim Gordon, 91 Talmage Hall Drive, Conroe, Texas 77301. (713) 667-6273 or 273-4809.

EDAX International Inc., Charles J. Walsh, P.O. Box 135, Prairie View, Illinois 60069.

Electron Microscopy Sciences, M. J. Oulton, P.O. Box 251, Ft. Washington, Pennsylvania 17034.

Ernest F. Fullam, Inc., I. T. Stoneback, Advertising Mgr., P.O. Box 444, Schenectady, New York 12301.

ETEC Corporation, P.J. Breton, 3392 Investment Boulevard, Hayward, California 94545. (415) 783-9210.

International Scientific Instruments, Richard Lois, 6655 Hillcroft, Suite 100, Houston, Texas 77081.

JEOL, USA, Inc., Paul Enos, 412 Shelmar Drive, Euless, Texas 76039. (817) 267-6011.

Kevex, Carl Meltzer, H. S. Culver, 898 Mahler Road, Burlingame, California 94010. (415) 573-5866.

Ladd Research Industries, Inc., Mr. Ted Willmorth, 1209 Dogwood Drive, Kingston, Tennessee 37763. (615) 376-6358.

L.K.B. Instruments, Inc., Jack Kirten, 5300 Telephone Road, Houston, Texas 77017.

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Montedison USA, Inc., Richard A. Steenrod, Jr., P.O. Box Drawer L, Bridgeton, Missouri 63044.

Olympus Corporation, P.O. Box 44008, Dallas, Texas 75234.

Ortec, Robin Galyan, 4009 Finley, Irving, Texas 75062. (214) 255-7414.

Overall Engineering, Inc., Edsel E. Overall, 15755 Daleport, Dallas, Texas 75248.

Perkin-Elmer Corporation, Mike Mullen, 11110 Los Alamitos Boulevard, Suite 202, Los Alamitos, California 90720. (213) 596-2512.

Philips Electronic Instruments, Bob Peterson, 7302 Harwin Drive, Suite 106, Houston, Texas 77036. (713) 782-4845.

Physical Electronics Industries, Inc., Thomas J. Baum, 324 North Central Expressway, Suite 119, Richardson, Texas 75080.

Polaron Instruments, Inc., Dermot Dinan, 1202 Bethlehem Pike Line, Lexington, Pennsylvania 18732. (215) 822-3364/5.

Polysciences, Dr. David B. Halpern, President, Warrington, Pennsylvania 18976.

Princeton Gamma Tech, Dick Neiman, 21718 Rotherham, Spring, Texas 77379. (713) 225-6160.

Schares Instrument Corporation, Margot Martin, 2600 S. Gessner, Suite 315, Houston, Texas 77063. (713) 468-4460.

Scimetrics, Inc., P.O. Box 42204, Houston, Texas 77042.

SCR Laboratories, Inc., Surface Analysis Section, Robert R. Keith, 4800 West 34th, Suite A-12, Houston, Texas 77092.

Seimens Corporation, Mr. Dietrich Voss, 11506 South Main, Suite 400, Houston, Texas 77025. (713) 661-3601.

Spectrochemical Research Lab, D. Raymond Slovinsky, 4800 West 34th Street, Suite A-12, Houston, Texas 77092.

Technic, Inc., Robert Barr, 5510 Vine Street, Alexandria, Virginia 22310. (703) 971-9200.

Technical Instrument Co., John Meny, 13612 Midway Road, Suite 333, Dallas, Texas 75240. (214) 387-0606.

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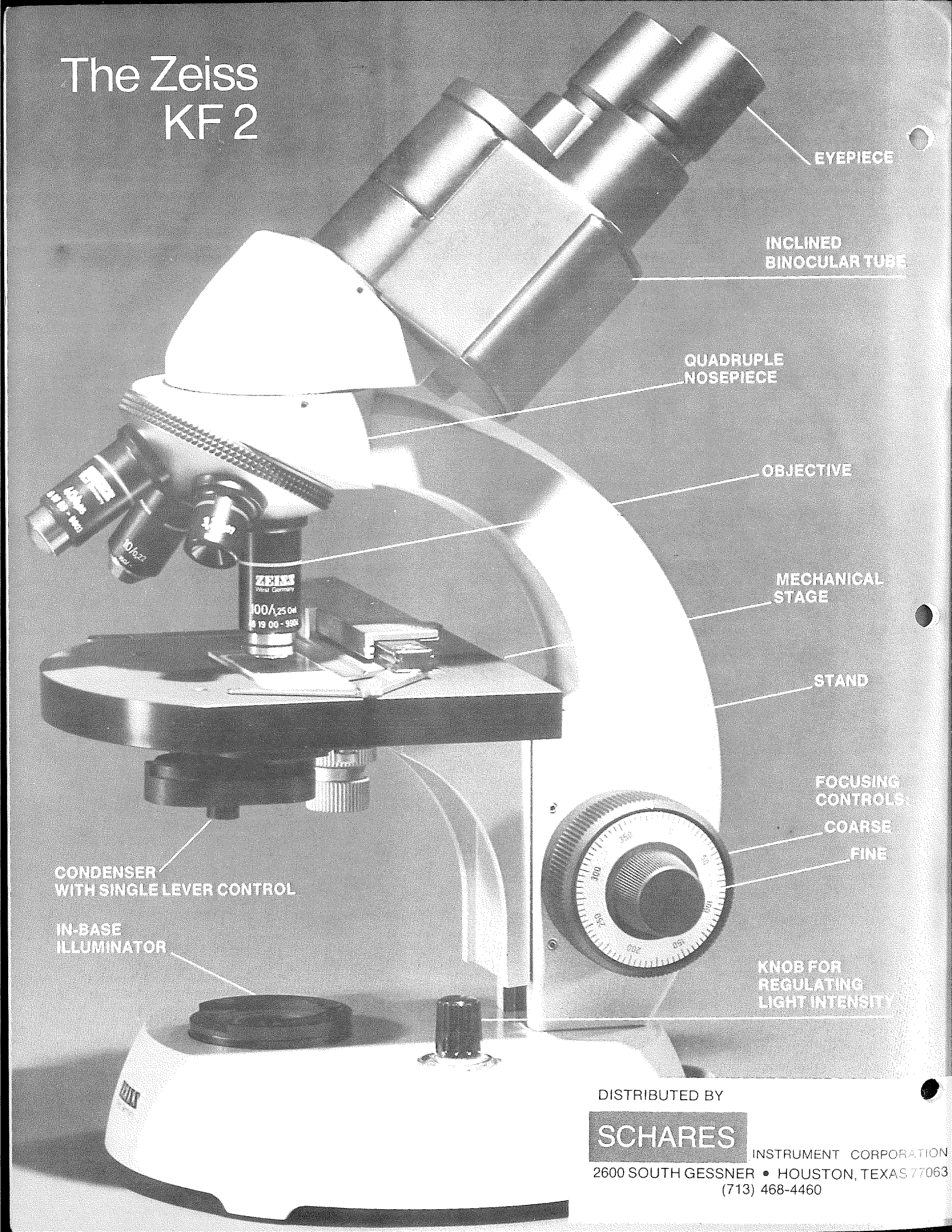

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Questionnaire For Readers of
TSEM Newsletter

1. What do you read in the TSEM newsletter?

   _____ everything
   _____ advertisements
      _____ a. in general — to see what is available
      _____ b. for specific items such as:
         _____ 1. new accessories for equipment you have
         _____ 2. new equipment by your favorite manufacturer
         _____ 3. new types of equipment
   _____ abstracts
   _____ feature articles(s)
   _____ letters to editor
   _____ regional news
   _____ President's letter
   _____ job descriptions and applications
   _____ by-laws
   _____ notices for future meetings

2. What do you look at in the TSEM newsletter?

   _____ advertisements
   _____ featured electron micrographs
   _____ names of new members or new addresses for current members
   _____ news from EMSA

3. What do you like most about the newsletter?

4. What do you like least?

5. What special features would you like to see in future issues?

6. What items would you like to see eliminated?

7. Do you think we should have a Library of Congress number?

8. If so, do you think the name should be changed to Proceedings of the TSEM?

9. If so, do you think that the personal news items should be sent out as a separate mimeographed sheet?

10. Specific comments you wish to see in print:

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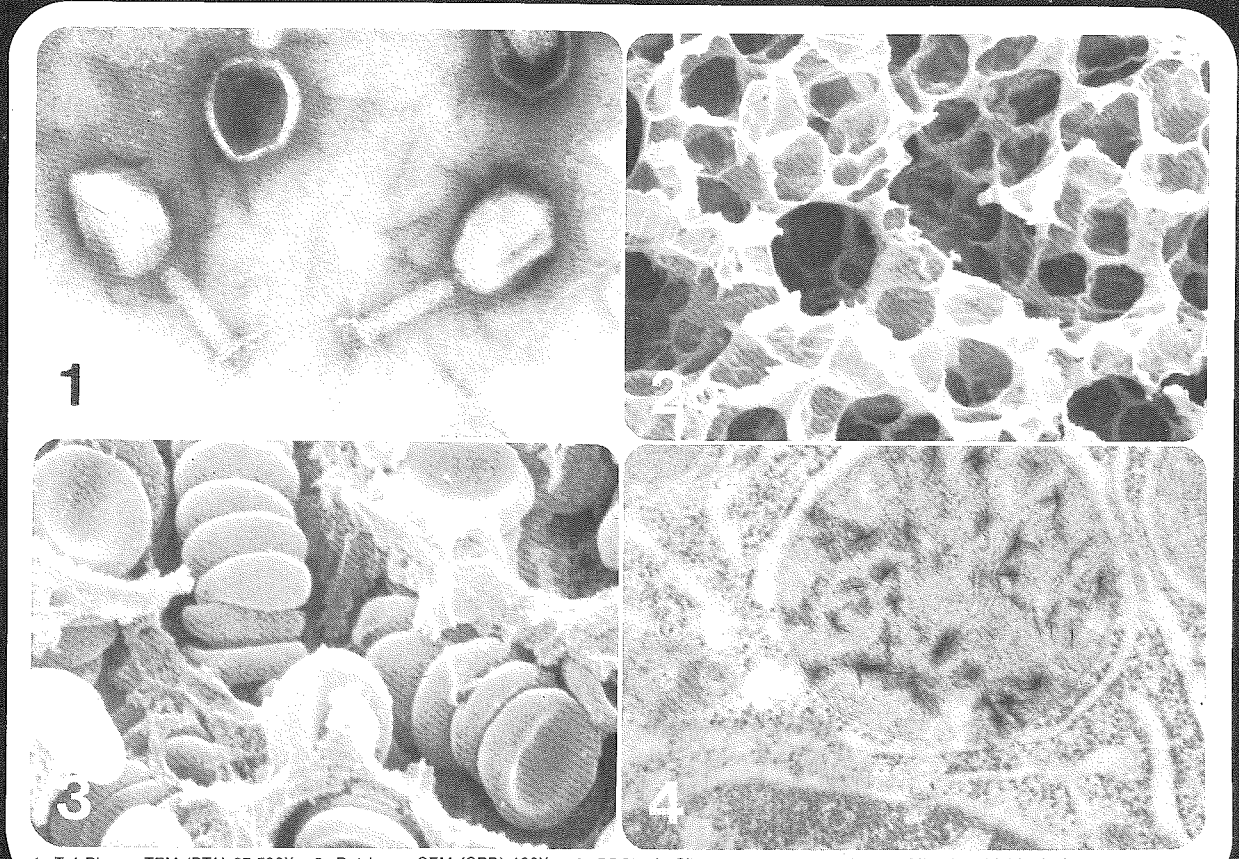

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