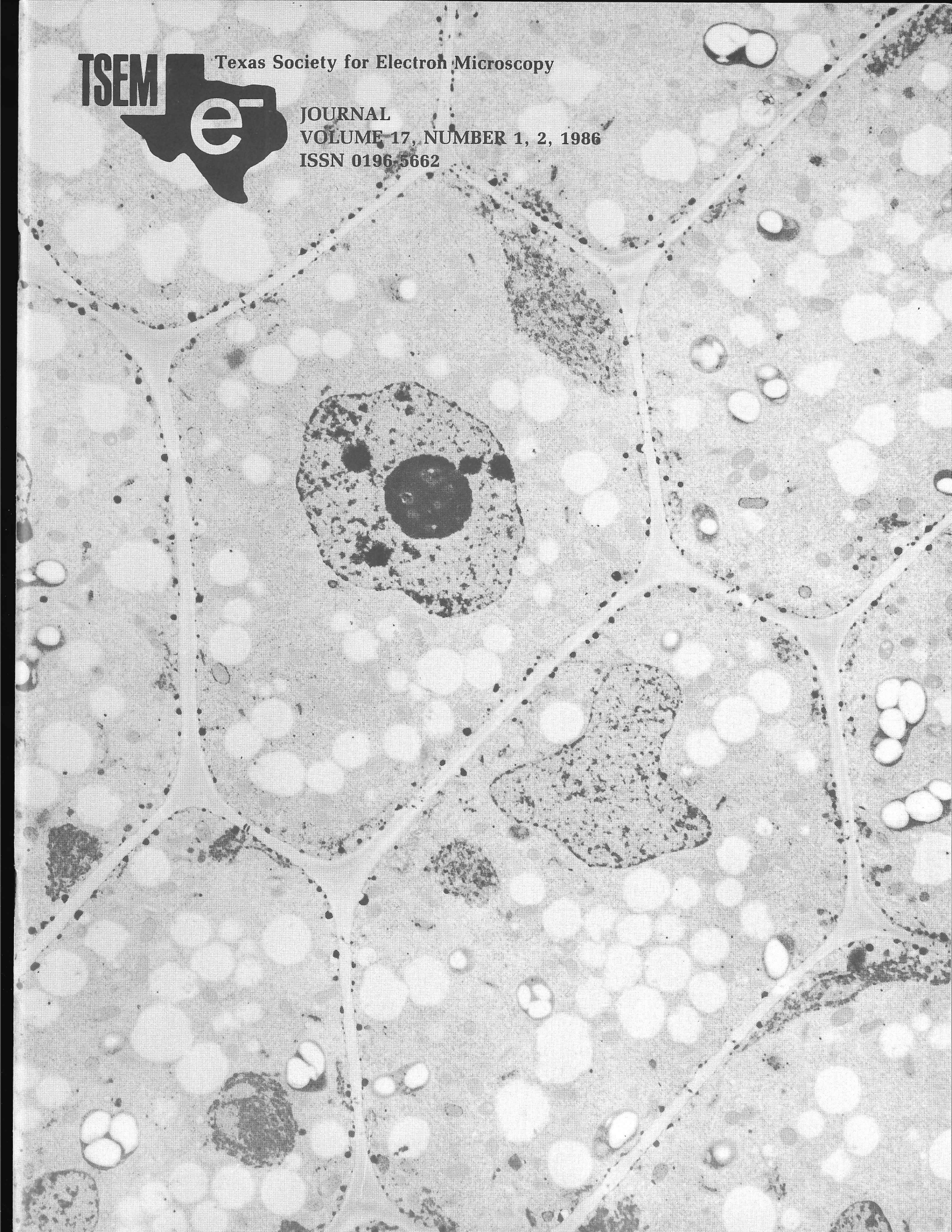

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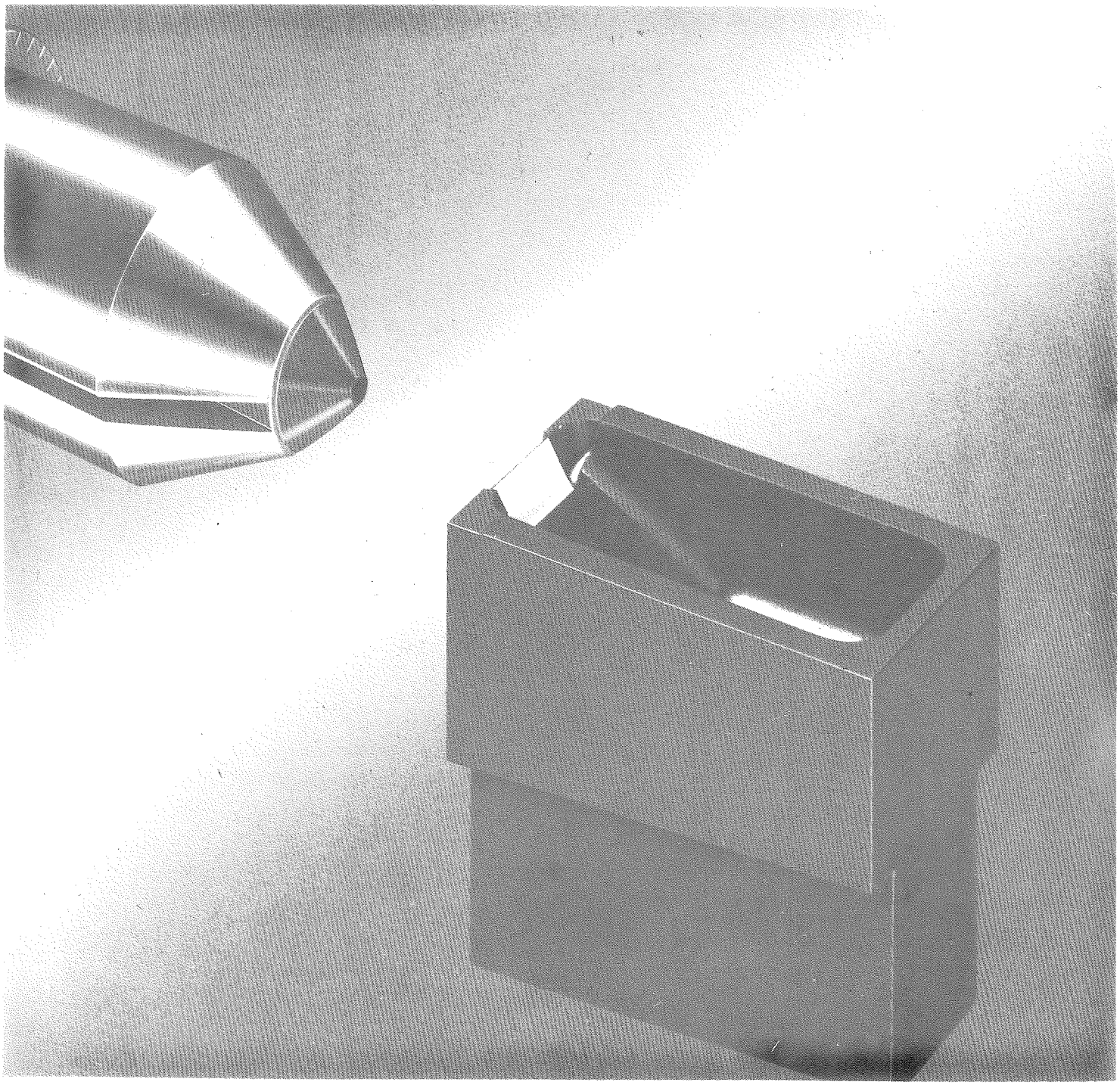

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JOURNAL
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Ronald W. Davis, Editor
Department of Medical Anatomy, Texas A&M Univ., College, Station, TX 77843

Texas Society for Electron Microscopy

"For the purpose of dissemination of research with the electron microscope."

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ON THE COVER

Columella (i.e., putative graviperceptive) cells of corn (Zea mays) roots grown in microgravity aboard the space shuttle *Columbia*. Amyloplasts are distributed randomly, and endoplasmic reticulum is in elipsoidal masses. x 4,500. Photograph by Randy Moore, Department of Biology, Baylor University, Waco, Texas 76798.

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President's Message

TSEM has been very busy since you last received an issue of the *Texas Society for Electron Microscopy Journal*. Our Spring meeting in Nacogdoches was one of our finest — excellent papers, interesting speakers, and the usual fun that occurs at our meetings (wasn't the fish-fry nice?). It also provided many of us with an opportunity to bid farewell to Charles Mims, a past-president of TSEM who has taken a position at the University of Georgia. Good luck Charles! Many thanks to all of the people who helped with that meeting.

As you've probably noticed, Ron Davis is the new editor of *Texas Society for Electron Microscopy Journal*. And as you can see from this issue, Ron is already doing a fine job with the *Journal*. But as is the case for any journal, he needs your help — *TSEMJ* continues to suffer from a lack of quality manuscripts. This lack of papers resulted in our having to cancel publishing a Spring issue of the *Journal* — in simplest terms, there simply weren't enough papers submitted to warrant publication. Ron is open to new ideas for papers, and will work with you toward getting your contribution published in the *Journal* . Surely you have a favorite technique, helpful hint, etc. that you could share with the membership!

The Fall meeting in Houston also looks like it is going to be among our best. We're meeting jointly with the Biological Photographers Association — we''ll have joint sessions with them as well as our "own" sessions. Bob Blystone, our program director, has put together what looks like a first-class agenda, and we should also have an increased number of commercial distributors (thanks largely to the work of Bob Turner). The efforts of Bob Blystone and Bob Turner were augmented with a large dose of hard work by Joiner Cartwright (local arrangements) and Wayne Sampson (mailouts, etc.) Producing a TSEM meeting is a real job, and I'm deeply grateful to all those individuals who helped with the arrangements, program, etc.

We also have some activities planned in addition to our regularly scheduled meetings. For example, we hope to have a **TSEM Membership Directory** to each TSEM member before the Fall meeting. In light of this, please be sure that 1) your dues are paid (only "paid up" members will be included in the Directory), and 2) the TSEM secretary (Wayne Sampson) has your current address, phone number, mailing address, etc.

Plans are now being finalized for the Spring meeting of TSEM. It will be held 5-7 March 1987 at the Sheraton Inn in Waco. We're in the early stages of piecing together the program for that meeting — we're planning hands-on workshops, invited speakers, paper and poster sessions, a banquet, and (hopefully) a business meeting/mixer on the Brazos Queen II River Boat. Make plans to be there!

I'm very proud to be able to serve as President of TSEM. Please do not hesitate to contact me or any other officer if you have any suggestions, complaints, or ideas for our society.

Sincerely,

Randy

Randy Moore, President
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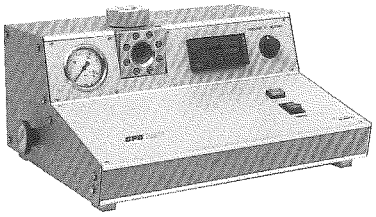

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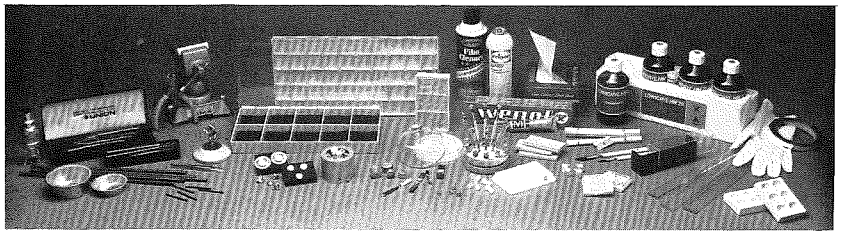

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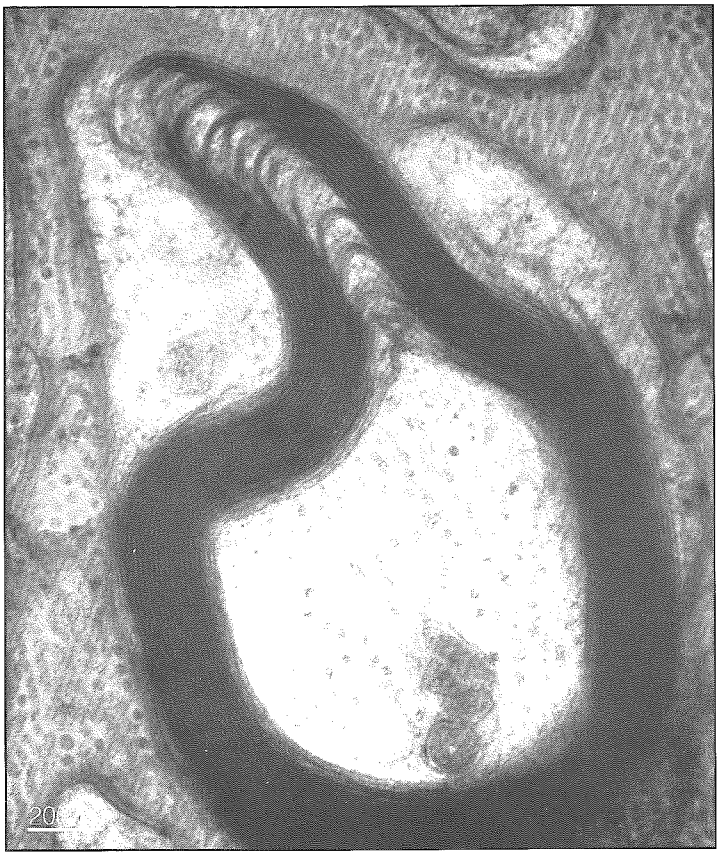

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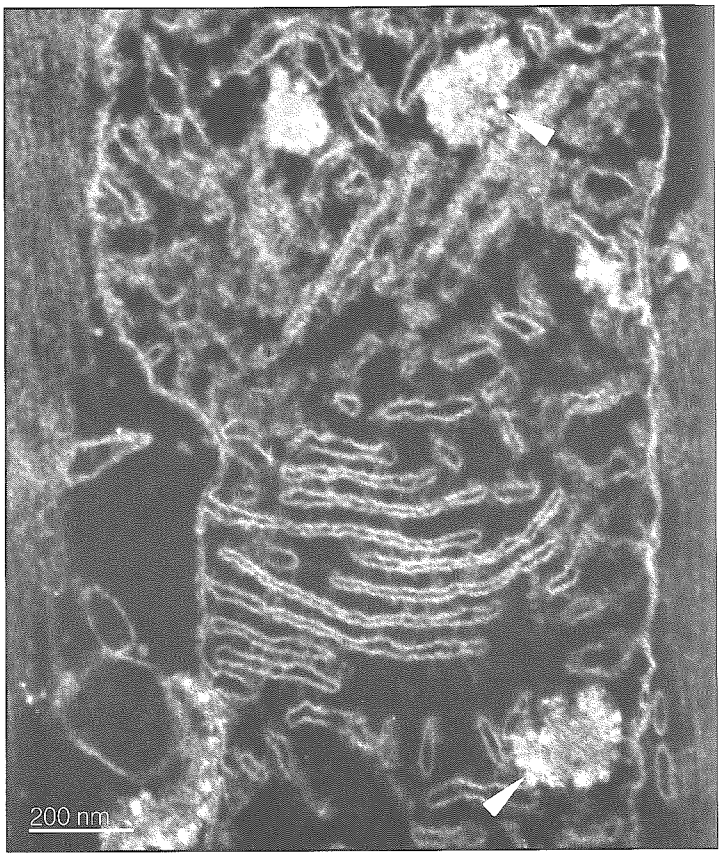

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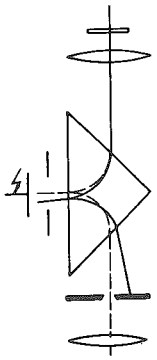

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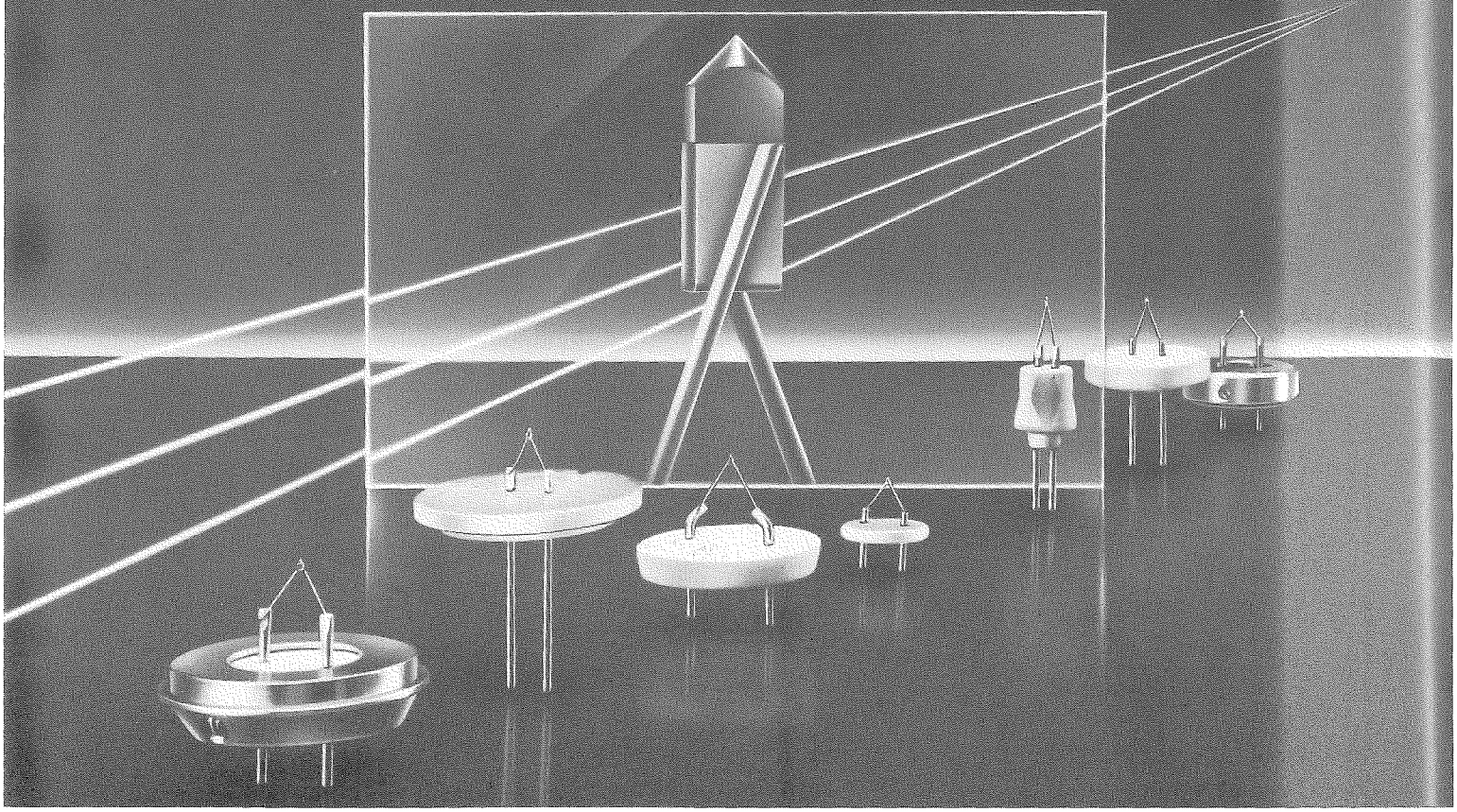

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ULTRASTRUCTURAL RESPONSES OF RATS TO LASALOCID PRELIMINARY RESULTS

By

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INTRODUCTION

Ionophorus compounds were first isolated and characterized in 1951 (9). They are antibiotics that induce alkali ion permeability in mitochondria and other membrane systems and they function by carrying ions across lipid barriers as lipid soluble complexes. Ionophores were recognized immediately as potential probes of biological function (8, 9) but major economic importance was not forthcoming until the discovery of monensin in 1967 and the recognition of its potential in the poultry industry as a coccidiostat (14). Since that time, ionophores have become of great commercial value in both broiler chicken (for controlling coccidia) and beef (for promoting weight gain) production (10) and are a constituent of virtually all feed used for those purposes. Currently, three ionophores are licensed for use with poultry and cattle — monensin, lasalocid, and salinomycin. Monensin is the most used of the licensed ionophores and, also, the most thoroughly studied. Lasalocid is purported to be less toxic to both poultry and cattle than monensin but otherwise functions similarly.

In addition to its uses in agriculture as coccidiostat and weight promoter, monensin is used in biology as a perturbant of cellular secretion. Monensin is active against the trans part of the Golgi apparatus and has become a valuable probe for localizing and indentifying the molecular pathways of the sectetory products (3, 12). Lasalocid also appears to affect cellular secretion in the same manner as monensin though little has been published regarding this potential activity.

There are several unresolved problems associated with ionophores in regard to their toxic effects in animals and their mode of action in cells. For example, the expression of toxicity in an animal is different from that observed in cultured cells and plants; e.g., striated muscle mitochondria are affected in animals that have ingested the ionophore monensin (5, 6, 7, 13) whereas the Golgi apparatus is the primary target in cultured cells and plants that have been exposed to monensin (2, 4, 5, 12). This implies that there are either marked differences in the mode of action of ionophores as related to target or that their mode of action in an animal is different from that in cultured cells and plants. The latter seems unlikely and it is our belief that the mode of action of ionophores in animals is not yet adequately defined. It is also of interest to us that many of the ionophore-induced cellular changes seem to be semi-independent of the type of ion that is sequestered by the ionophore for transfer into the cell. This is important in trying to understand the differences and/or similarities of cellular responses following exposure to monensin (a strong sodium ionophore) and lasalocid (a weak calcium ionophore).

This study was initiated to determine the response of an animal to lasalocid by examination of selected tissues at the ultrastructural level. Though the *in vitro* toxic effects of lasalocid have been documented (1, 11), no report on lasalocid vs ultrastructure in intact animals has been indentified. In this report we show that the toxic effects of lasalocid are essentially the same as those of monensin (6, 7, 13). Also, this study establishes suitable dosage levels of lasalocid for use in further studies.

MATERIALS AND METHODS

Lasalocid (Cal Biochem), dissolved in DMSO and diluted with sterile distilled water, was administered subcutaneously. The rats were Sprague-Dawley outbred from TIMCO (Houston, Texas.) Seven rats were dosed at a level of 25 mg/kg lasalocid, 2 at 40 mg/kg lasalocid, and 2 at 10 mg/kg lasalocid. Two rats were used as controls receiving only comparable levels of DMSO. The rats were to be sampled at 13 days post treatment: however, because of the severe adverse affects of lasalocid, some had to be sacrificed at intermediate periods just prior to their estimated demise. Actual sampling times are given in Table 1.

The rats were anesthetized with xylazine hydrochloride and ketamine hydrochloride (IM) after which tissue samples from the left ventricle of the heart and from the diaphragm were excised and processed for electron microscopy. The fixative contained 3% glutaraldehyde in 0.1 M PIPES buffer at pH 7.3 and 10% (V/V) saturated picric acid, and fixation was at room temperature for 90 minutes. Tissues were postfixed for 90 minutes at ice bath temperature in a fixative containing 1% osmium tetroxide, 0.1 M PIPES buffer at pH 7.3, 0.05 M

TABLE 1: Listing of dose levels vs sampling times for the rats used in this study.

Sampling Times, days (approx.) except for rats # 3, 5				
Rat #	High dose 40 mg/kg	Med. dose 20 mg/kg	Low dose 10 mg/kg	Control
1	1*			
2	2			
3		1 hr*		
4		3		
5		2 hr*		
6		13		
7		13		
8		2		
9		13		
10			1*	
11			7	
12				7
13				13

*These rats died unexpectedly and no tissue samples were taken for electron microscopy.

sucrose, and 0.05 M potassium ferricyanide. After postfixation, tissue samples were rinsed in deionized water, block-stained in aqueous 1% uranyl acetate, dehydrated in an ethanol and acetone series, and embedded in epoxy resin. Sections were examined with a Philips EM 300 electron microscope.

RESULTS AND DISCUSSION

Most subcellular changes were associated with mitochondria and included condensation of matrix substance (Figs. 1, 3), swelling (Fig. 1), and formation of granules (Fig. 5). Swollen mitochondria had the same general morphology as those of monensin-treated animals (6, 7). Some muscle fibers were greatly altered (Fig. 1), others only mildly so. These effects were present in both heart and diaphragm, but were most conspicuous in heart. Other aspects of muscle architecture (e.g., fibril organization) sometimes appeared altered (Fig. 1) and sometimes appeared normal (not illustrated).

In control rats, none of the mitochondrial aberrations described above were present, and the heart and diaphragm appeared normal (Figs. 2, 4).

As with monensin, (5, 6, 7, 13), striated muscle seemed to be the primary target of lasalocid toxicity and mitochondria the most affected subcellular component. No Golgi apparatus effects (i.e., swelling of cisternae) were observed in this study although such effects are characteristic responses to both monensin and lasalocid in cultured cells (1, 2, 11).

Several distinct patterns of change were identified. Mitochondrial condensation occurred rapidly and then declined whereas mitochondrial vacuolation remained relatively constant or declined only slightly over the test period. Mitochondrial vacuolation was

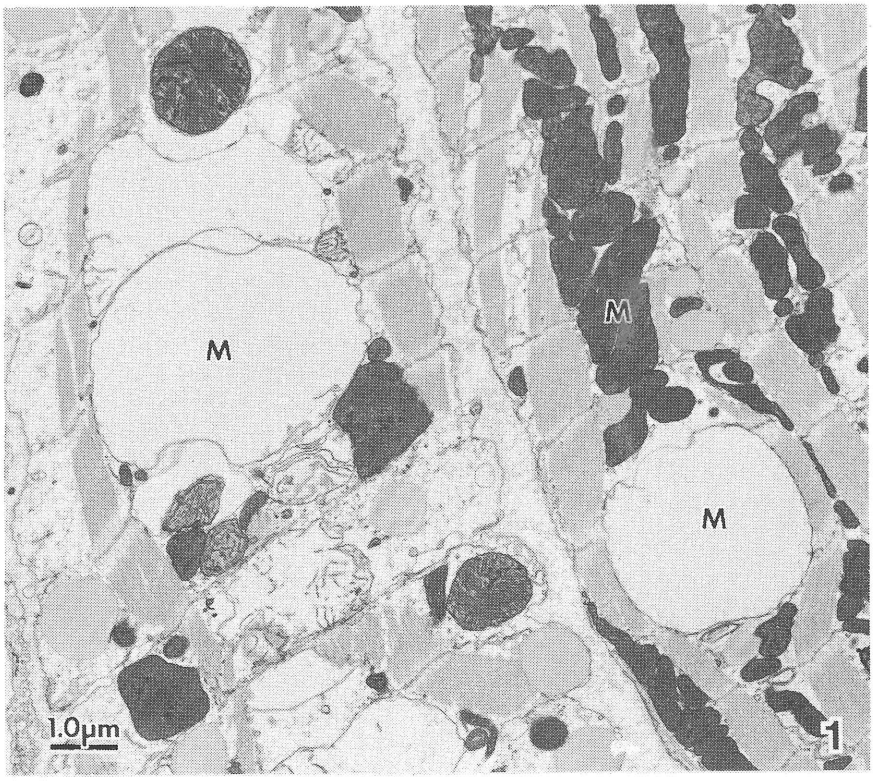

Fig. 1. Heart muscle from rat given 40 mg/kg lasalocid and sampled 2 days post treatment. Mitochondria (M) were either heavily condensed or swollen and vacuolated.

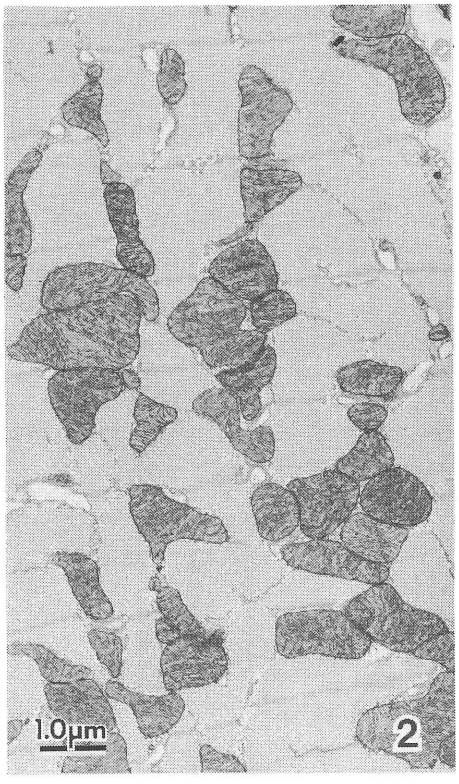

Fig. 2. Heart muscle from a DMSO-treated control rat showing normal appearance of tissue.

more prominent in heart than in diaphragm which is contrary to the subcellular effects in rat observed after monensin poisoning (7). Mitochondrial condensation was much less obvious at 20 mg lasalocid/kg treatment level than at the higher dosage levels but mitochondrial swelling was present at all dosage levels.

In comparison with monensin, the toxic effects of lasalocid appear to be of much longer duration with the possibility of death occurring many days after lasalocid treatment even though the animal may look and act normal. In contrast, rats exposed to monensin seldom die if they survive the first 24 hrs post treatment (unreported results). Studies currently underway indicate similar effects when lasalocid is administered orally.

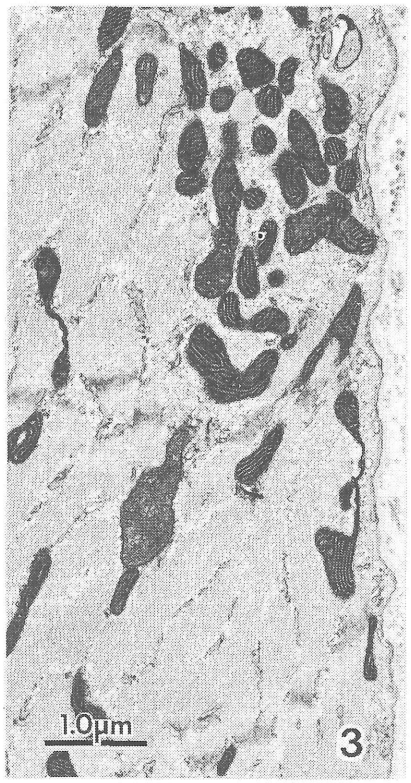

Fig. 3. Diaphragm (red muscle cell) from a rat given 40 mg/kg lasalocid and sampled 2 days post treatment. Mitochondria were condensed and somewhat misshapen but only a few were swollen (not illustrated).

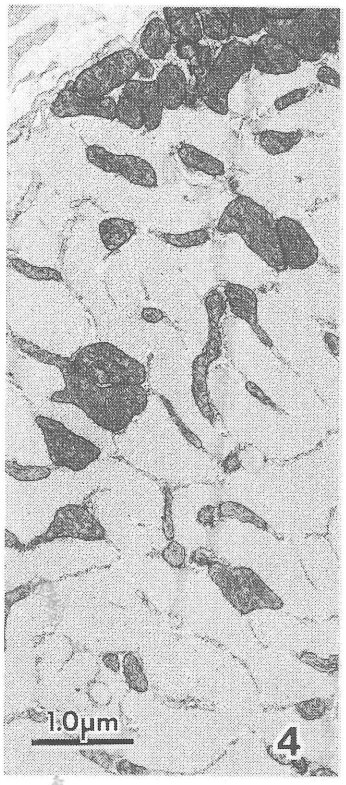

Fig. 4. Diaphragm (red muscle cell) from a DMSO-treated control rat showing normal appearance of tissue (compare with fig. 3).

Fig. 5. Heart muscle from rat given 20 mg/kg lasalocid and sampled 13 days post treatment. Very few mitochondria were condensed but many were still swollen (compare with Fig. 1). Additionally, there were dense granules (arrowheads) in many of the mitochondria.

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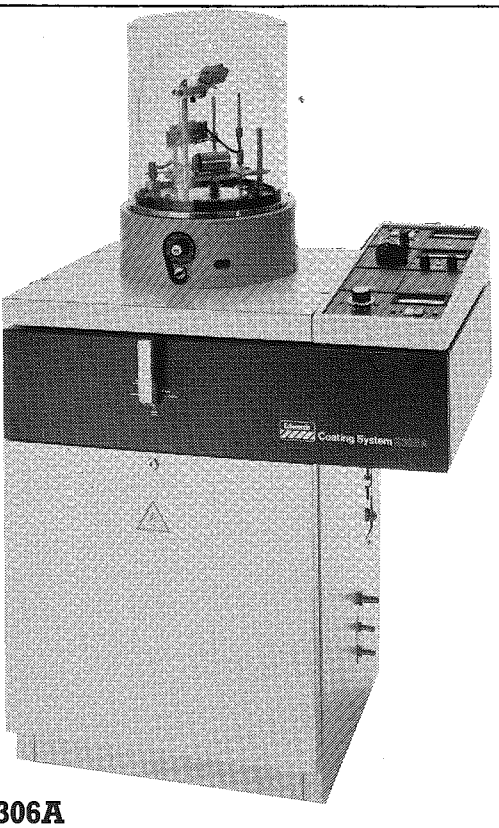

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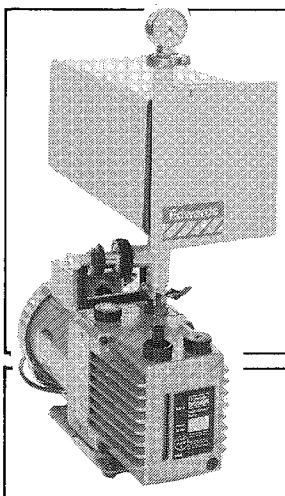

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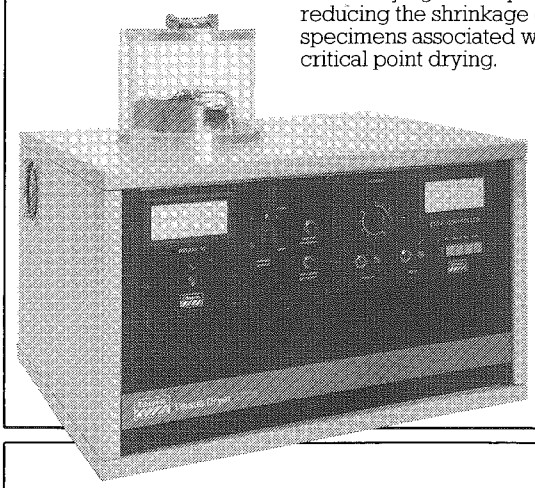

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(1) A. Glauert, Practical Methods in Electron Microscopy. Vol. 2 (North-Holland. Amsterdam, 1974) 82-88.
(2) P.S. Baur, Jr., G.F. Barratt, G.M. Brown and D.H. Parks. Ultrastructural Evidence for the Presence of "Fibroclasts" and "myofibroclasts" in Wound Healing Tissues. J. of Trauma. 19 (1979) 774-756.
(3) D. Gabor. Information Theory in Electron Microscopy, in: Quantitative Electron Microscopy. Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1956) 63-68.

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ACKNOWLEDGEMENTS should appear as a footnote which will appear at the top of the first page of the article.

TSEM MEMBERSHIP DEMOGRAPHICS

By

Ronald W. Davis
Department of Anatomy
College of Medicine — Texas A&M University
College Station, Texas 77843

There has been some concern expressed about how the TSEM membership is distributed throughout the state. This becomes an important consideration for planning future meetings so that they will be accessible to the greatest number of people. The information for this study was taken from a membership list dated January 1, 1986.

When the cities containing three or more TSEM members are plotted on a map of Texas (Fig. 1) it shows that 93% of the membership resides in the central and eastern half of the state. 68% of the membership is in the four areas of Dallas-Fort Worth, Houston-Galveston, College Station, and San Antonio. Table 2 shows a complete list of cities where TSEM members live and the number of members in each city.

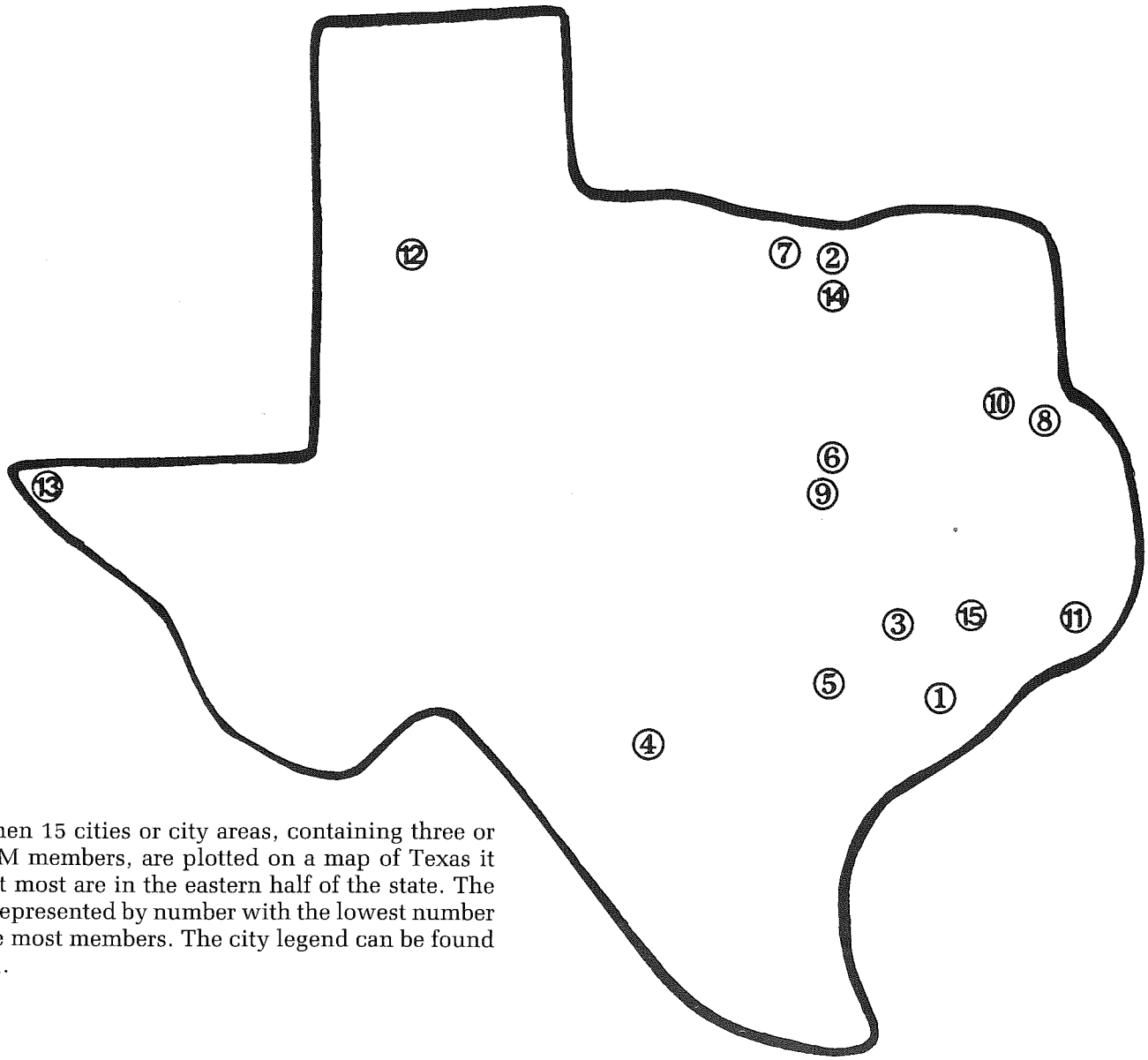

Fig. 1. When 15 cities or city areas, containing three or more TSEM members, are plotted on a map of Texas it shows that most are in the eastern half of the state. The cities are represented by number with the lowest number having the most members. The city legend can be found in Table 1.

TABLE 1 Cities or City areas represented in the map in Figure 1 listed in order of decreasing numbers of TSEM members.

Rank	City	No. of Members
1	Houston, Galveston	125
2	Dallas, Fort Worth, Richardson, Arlington, Garland	118
3	College Station, Bryan, Hearne	56
4	San Antonio	35
5	Austin	27
6	Waco	19
7	Denton	16
8	Nacogdoches	14
9	Temple	12
10	Tyler	12
11	Beaumont	12
12	Lubbock	6
13	El Paso	6
14	Carollton	7
15	Huntsville	3
	TOTAL	468

NUMBERS OF TSEM MEMBERS AND % OF TOTAL MEMBERSHIP (TEXAS) ACCORDING TO CITY

Town	Number	%
Alpine	1	0.2
Amarillo	1	
Arlington	8	1.6
Aubrey	1	
Austin	27	5.5
Barry	1	
Baytown	1	
Beaumont	12	2.4
Bellaire	1	
Borger	1	
Bryan	2	0.4
Carollton	7	1.4
College Station	53	10.9
Dallas	63	12.9
Deer Park	1	
Denton	16	3.3
Douglas	1	
El Paso	6	1.2
Fort Sam Houston	3	0.6
Fort Worth	25	5.1
Freeport	1	
Friendswood	1	
Galveston	30	6.1
Garland	3	0.6
Georgetown	1	
Gonzales	1	
Groves	1	
Hearne	1	
Houston	95	19.5
Huntsville	3	0.6
Irving	1	
Jacksonville	1	
Katy	1	
Killeen	1	
Kingwood	2	0.4
Lackland A.F.B.	1	
Lake Jackson	1	
Lubbock	6	1.2
Mesquite	1	
Nacogdoches	14	2.9
Nederland	1	
Port Neches	1	
Richardson	9	1.8
Richmond	1	
Riviera	1	
San Antonio	35	7.2
Sherman	1	
Spring	2	0.4
Stafford	1	
Sugarland	1	
Temple	12	2.4
Texas City	1	
The Woodlands	1	
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Waco	19	3.9
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Woodlawns	1	

TOTAL: 498 Members
58 Towns

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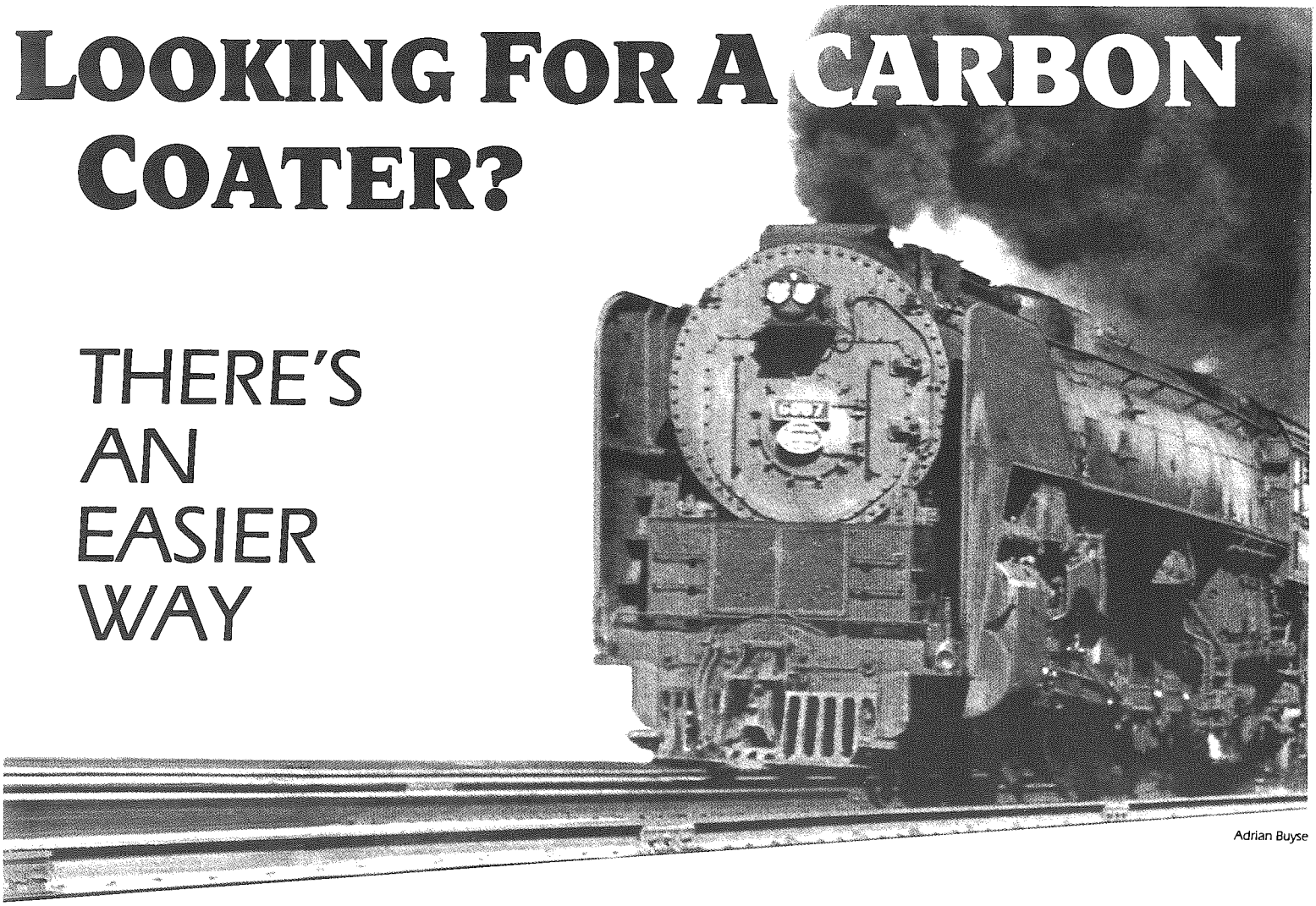

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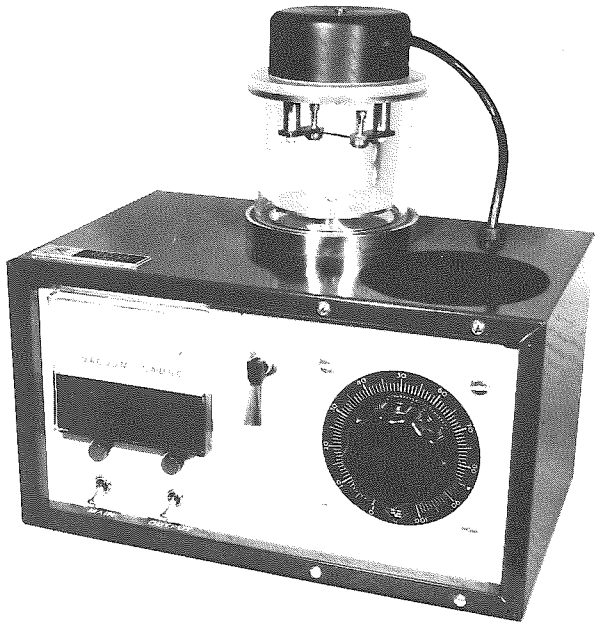

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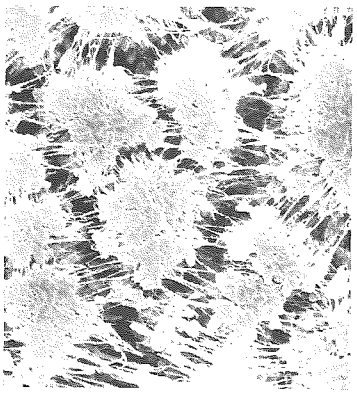

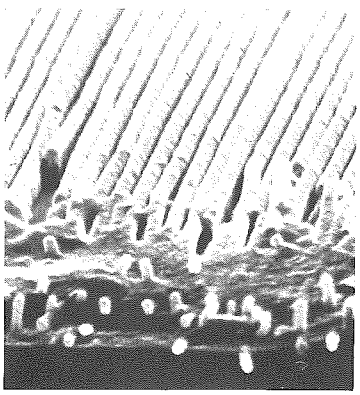

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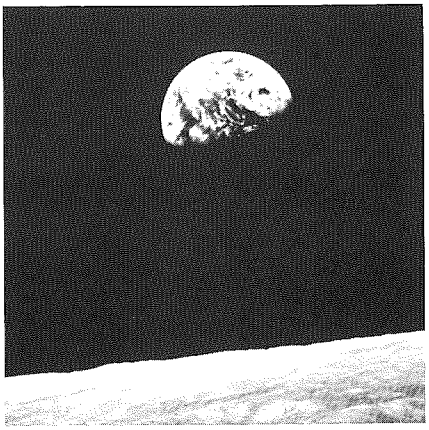

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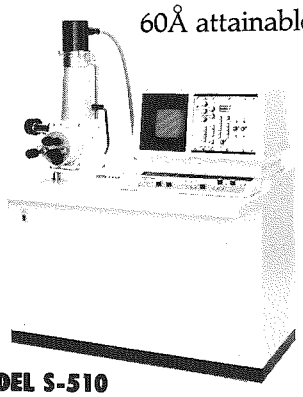

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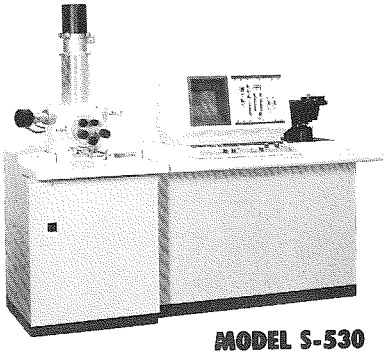

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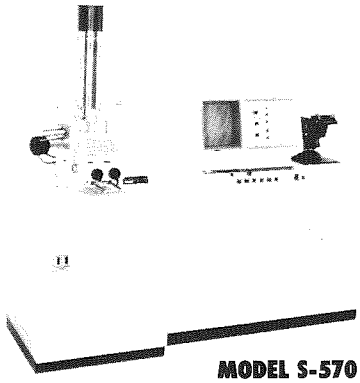

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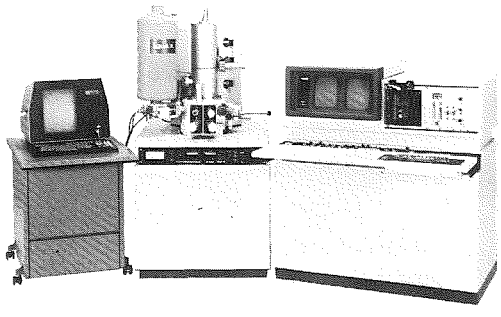

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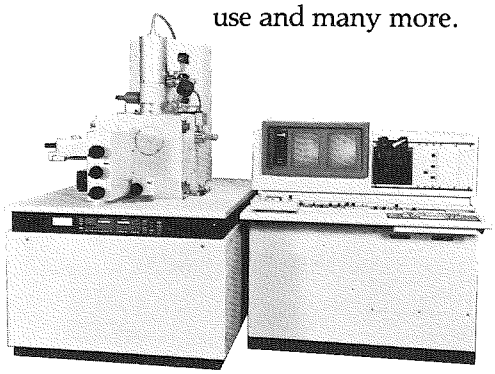

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LOCATION OF ELECTRON MICROSCOPES IN THE STATE OF TEXAS

By

Ronald W. Davis
Department of Anatomy
College of Medicine — Texas A&M University
College Station, Texas 77843

I thought it would be interesting to find out how many electron microscopes are in the state of Texas and where they are located. To do this, I contacted the major electron microscope manufacturers and asked them for information. They were very helpful, and the following information is the result of that survey. It is interesting to note that many of the companies were concerned that the microscopes not be indentified by name, as this might seem too much like a popularity contest.

The information gathered is displayed according to the type of microscope (eg. TEM, SEM, STEM) and its general location (eg. the Dallas area would include Forth Worth and other cities in that general area). The location of the microscopes was also compared to that of the TSEM membership. The results show that microscope and membership distribution are very similar. The only variation is that Austin contributes a higher percentage of microscopes (11%) than TSEM members (6%).

There were a total of 255 microscopes indentified, but there is no doubt that there are more that were not found. Some microscopes were located by using company service contract records. Others were located through computer listings. Unfortunately, many of these records did not list the older microscopes. There was no attempt to locate any RCA, Siemens or ETEC instruments.

TYPE, NUMBER, AND % OF ELECTRON MICROSCOPES IN TEXAS

Number of SEM's	143	SEM's % of Total Microscopes	56%
Number of TEM's	107	TEM's % of Total Microscopes	42%
Number of STEM's	5	STEM's % of Total Microscopes	2%
TOTAL	255		

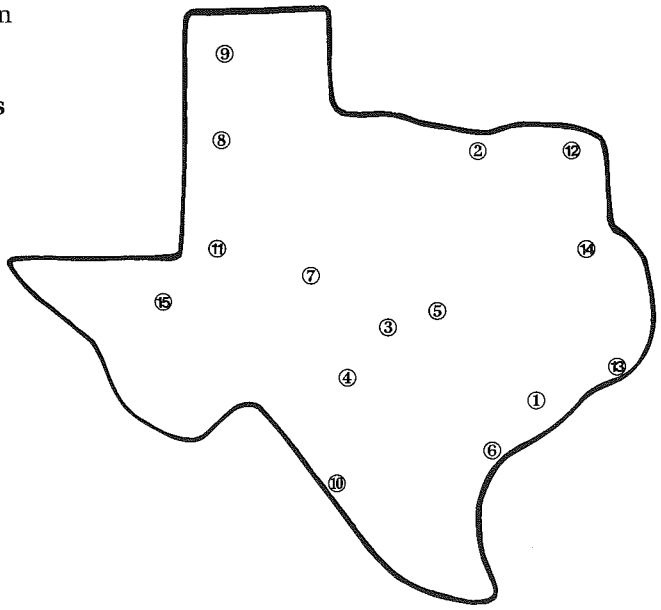
MICROSCOPES ACCORDING TO CITY*

	Number		*%*
Number of SEM's in Cities and % of Total SEM's			
Houston, Dallas, San Antonio, College Station	105	=	74%
Houston, Dallas, San Antonio, College Station, Austin	124	=	88%
Number of TEM's in Cities and % of Total TEM's			
Houston, Dallas, San Antonio, College Station	67	=	63%
Houston, Dallas, San Antonio, College Station, Austin	74		69%
Number of STEM's in Cities and % of Total STEM's			
Houston, Dallas, San Antonio, College Station	4	=	80%
Number of All 3 EM's in Cities and % of Total			
Houston, Dallas, San Antonio, College Station	176	=	70%
Houston, Dallas, San Antonio, College Station, Austin	205	=	81%

*Houston, Dallas, San Antonio and College Station were chosen because they contain 68% of the TSEM membership. Austin was added and used as an additional comparison because it contributes significantly (11%) to the number of electron microscopes but less so to the TSEM membership.

Fig. 1. Distrubution of scanning electron microscopes in the State of Texas.

Number on Map	City	Number of Microscopes
1	Houston	50
2	Dallas	45
3	Austin	22
4	San Antonio	7
5	College Station	3
6	Corpus Christi	4
7	San Angelo	3
8	Lubbock	2
9	Amarillo	1
10	Larado	1
11	Midland	1
12	Lone Star	1
13	Port Arthur	1
14	Nacogdoches	1
15	Alpine	1
TOTAL		143



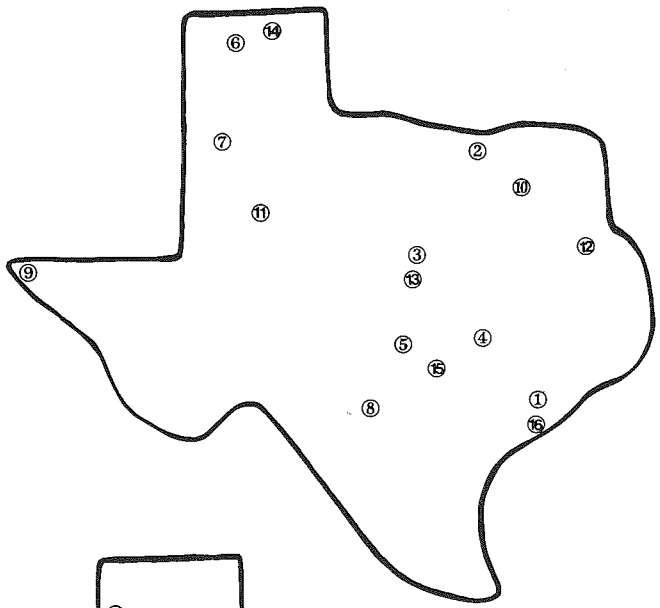


Fig. 2. Distribution of transmission electron microscopes in the State of Texas.

Number on Map	City	Number of Microscopes
1	Houston	31
2	Dallas	19
3	Waco	10
4	College Station	10
5	Austin	7
6	Amarillo	6
7	Lubbock	6
8	San Antonio	7
9	El Paso	3
10	Tyler	2
11	Abilene	1
12	Nacogdoches	1
13	Temple	1
14	Borger	1
15	Smithville	1
16	Freeport	1
TOTAL		107

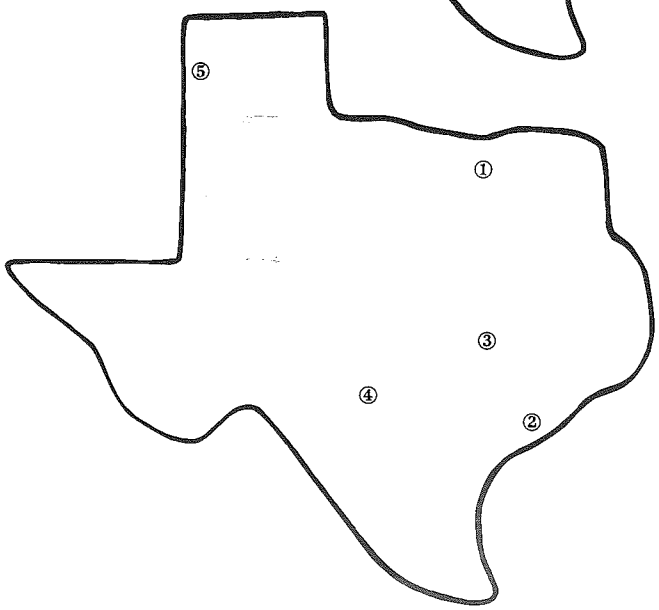


Fig. 3. Distribution of scanning transmission electron microscopes in the State of Texas.

Number on Map	City	Number of Microscopes
1	Dallas	1
2	Houston	1
3	College Station	1
4	San Antonio	1
5	Amarillo	1
TOTAL		5

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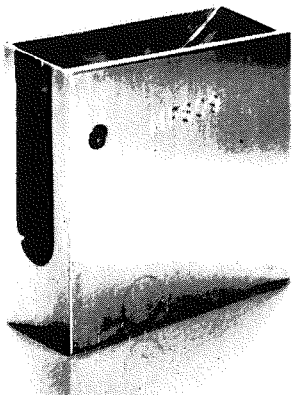

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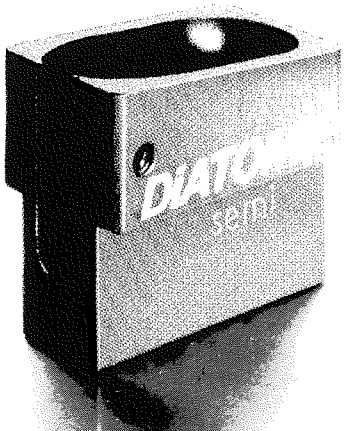

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GUIDELINES FOR SEEKING EMPLOYMENT

by Timothy Mislan
University of Oklahoma
Department of Botany and Microbiology
Norman, Oklahoma 73069

The purpose of this article is to provide a person searching for a job in the field of electron microscopy with some tips that have proven to be successful.

A concise yet informative resume is very important. It is from this resume that a potential employer will form his intitial opinion of you and your qualifications. The following should be included.

Name
Address
Telephone Number
Career objective(s)
Education
Work Experience
EMSA certification (if applicable)
Instrumentation (include all microscopes and auxilliary equipment with which you are familiar).

The resume should be confined to one page, and should be of quality print on high grade paper. A cover letter typed on the same stationery should accompany your resume. The cover letter should be of a personal nature (i.e., no form letters) and provide specific information such as various E.M. techniques mastered, exact date available, and willingness to relocate.

Once a good resume and cover letter have been written, a variety of approaches can be used to contact potential employers. Start be registering with the Statistical Office of EMSA. This can be done by contacting Dr. John H.L. Watson, EMSA Stastitical Officer, 654 Hupp Cross, Birmingham, Michigan 48010. You will be asked to complete and return a short questionnaire listing your qualifications, which will be forwarded to employers who contact the Statistical Office. The Statistical Office provides the potential employee with a listing of those employers seeking individuals with E.M. experience. Updated addenda are mailed at regular intervals. These openings are also listed in the "Positions Open" section of the *EMSA Bulletin*.

Another very successful approach is to contact the secretaries of each EMSA Local Affiliate Society, whose names and addresses are also published in the *EMSA Bulletin* (LAS News). In a cover letter, explain why you are contacting them and include your resume and a brief, single spaced advertisement which can be included in their newsletter. As long as you are willing to relocate, these officers are often aware of E.M. openings in their area, and they can put you in touch with the appropriate people.

It is of considerable help to contact friends in the field and alert them to the fact that you are looking for a new E.M. position. Often these people will know of openings via the grapevine, and they can make the initial contact for you. This holds true for sales representatives of E.M. equipment.

Letters of reference are very important once the initial contact with a potential employer has been made. Ask at least three people who are familiar with your E.M. experience to write letters which can be forwarded upon request to a potential employer.

As a final note, once you have applied for an opening by mail, follow-up phone calls are often beneficial. A phone call to a potential employer demonstrates your sincere interest, and it will give him or her a chance to become familiar with your communication skills.

Reprinted from:
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1985, page 52, 54

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A TEACHING PROGRAM FOR EM TECHNOLOGY TRAINING A "VIP"

By

Elizabeth P. Matthews, Ph.D.
Center for Electron Microscopy
San Joaquin Delta College, Stockton, California, 95207

Your EM technologist is a VIP (a very important professional). This is the individual who processes your samples, operates your electron microscopes, keeps accurate records, orders, plans, handles photographic chores and influences final project results in a most important way.

We all know how desperately the special skills of this individual are needed in electron microscopy today. Perhaps more than in any other type of technology, the EM technologist is an integral part of our professional accomplishments.

Where do we find these most valuable partners in research? Further, what educational background should they have? Indeed, these are questions which many of us have asked in our efforts to find the most qualified person for the job.

There is no major in electron microscopy at any university that I know of, only an opportunity to use electron microscopes as part of other courses, or perhaps as a graduate school project. Then how can one learn the special skills that are necessary for the professional?

There are the traditional ways. A candidate is exposed to the use of an EM in a class or two and experiences some theory as well as some "hands-on use", but because of time limits, practical experience is probably very brief at best. The student is shown a method, given help to run through a procedure and is then left to practice for a week or two. Another traditional method is to take what is known as the "short course". This is usually a one, or perhaps, two week, lecture and lab course which extends from early morning to late evening. This is the *total* immersion approach. Undoubtedly, such exposure could provide a basic framework in EM but not much depth. Finally, in another traditional way, on-the-job training might be provided to an interested person, one who we have come to call "a person off the street". Such an individual might just be looking for a job of some kind. In this case a researcher might need to spend precious hours training and fewer hours researching. Yes, most of these routes have been followed in the past. In fact, there are some outstanding EM technologists working in a number of laboratories as a result of one or more of the approaches, but I'm sure that these fine people would admit that it's a very difficult way to learn. Such traditional training methods have not provided the student with much structured learning. They are left to "teach themselves", learn from experience, and ultimately consume much more time than is necessary.

Because of the setting, these educational opportunities were usually centered at universities or four year colleges, and most of the early EM professionals had at least a four year degree. But, we should ask, is that degree necessary?

Some years ago, a group of practical and theoretical scientists, including myself, got together to discuss the situation as it existed and as it influenced the education of EM technologists. Many job opportunities were available but not enough trained people to fill them. At that time, there were no planned programs, no full training facilities, only the short course type of experience. In an era of expanding molecular science, where electron microscopes were increasingly needed, a comprehensive training program for EM professionals was also needed.

To foster the growth of such a program, an advisory committee (a) was formed consisting of representatives of industry, metallurgy, medicine, and biology, who were all competent electron microscopists. Coordinating the project was Victor Remillard who ultimately established the EM Training Center here at Delta College.

We began to explore the possibilities for such a program. The curriculum needed to be centered on instrumentation and specimen preparation, but many other topics had to be included also: laboratory maintenance, care of instruments, photography, report writing, etc. It also had to be designed to provide an education for both major fields in the EM community, the biological and physical applications. A broad based curriculum was visualized which would include subjects other than EM but which were of great value to the very specialized microscopist: chemistry, physics, math, computer science, engineering.

Today, the curriculum consists of all these general courses as well as those that are specific to EM. A list of required courses is appended here (Table 1) and it should be noted that many hours of work are involved. The two year, student professional will

Table 1

Electron Microscopy (Biological) Certificate Required Program	
First Year	
Inorganic Chem.	108
Math through Trigonometry	280
Gen. Biology	144
Beginning TEM	108
Scientific Photog	90
Biological Specimen Prep.	180
Beginning SEM	90
Second Year	
Computer Science	108
Cell & Tissue Ultrastructure	36
Intro. to Physics	114
Advanced Biol. TEM	162
Adv. Biol. SEM	72
Biol. Special Projects	154
Laboratory Maintenance	90
Electron Optics & Theory	72
Other Electives Suggested:	
Complete AA Requirements	
Zoology	
Other Computer Science	
Anatomy Physiology	142
Total Class Hours =	1950
Class Time Just for EM =	1062
% of Total Class Time Just in EM =	54%

Electron Microscopy (Crystalline Materials) Certificate Required Program	
First Year	
Inorganic Chem.	108
Engineering Materials	180
Math through Trigonometry	280
Scientific Photog.	90
Beginning TEM	108
Computer Science	108
Intro. to Physical TEM	90
Beginning SEM	90
Second Year	
Electron Optics & Theory	72
Adv. Physical TEM	162
Analytical SEM	108
Laboratory Maintenance	90
Advanced Projects	108
Intro. to Physics	114
Other Electives Suggested:	
Complete AA Requirements	
Computer Sciences	
Intro to Electronics	
Engineering Drawing	256
Total Class Hours =	1964
Class Time Just for EM =	918
% of Total Class Time Just in EM =	47%

spend over 1000 hours using the EMs and learning specimen preparation. This is not only *exposure* but is also truly *immersion*.

Both program options require the completion of inorganic chemistry, math through trigonometry, basic TEM, which includes theory and operation of the microscopes, sample preparation, scientific photography and darkroom skills, computer science with programming in Basic, physics of motion and mechanics, electron optics and EM laboratory maintenance (emphasizing trouble shooting and lab safety).

In-depth study of fixation, dehydration and embedding as well as the art of ultramicrotomy is required of the biology student. In the second semester these individuals are required to produce a series of excellent electron micrographs as proof of their increasing skill with specimen preparation. The third semester biology program emphasizes the need for an EM technologist to often work independently, with a minimum of supervision. Students are assigned mini-research projects of their own. They conduct library research to establish good methods and techniques (as shown by others doing similar work) then proceed to complete the project successfully. They are graded on the quality of the scientific report and the excellence of their micrographs. Advanced courses include theory and practice of cytochemistry, cryotechniques, and, recently, immune reactions at the tissue level.

The core of the physical or crystalline materials curriculum includes properties of materials and structure of metals or ceramics, fracture studies, failure analysis, thin foils, diffraction, metallography, replication, and an emphasis on report writing.

Scanning electron microscopy is a full year course for both the biological and the physical options. Intelligent use of the SEM and parameters for its best performance are essential parts of the studies. Both options examine samples which range from insects to semi-conductor chips. Sample analysis is an important segment of the SEM courses with energy dispersive and wavelength dispersive spectroscopy included.

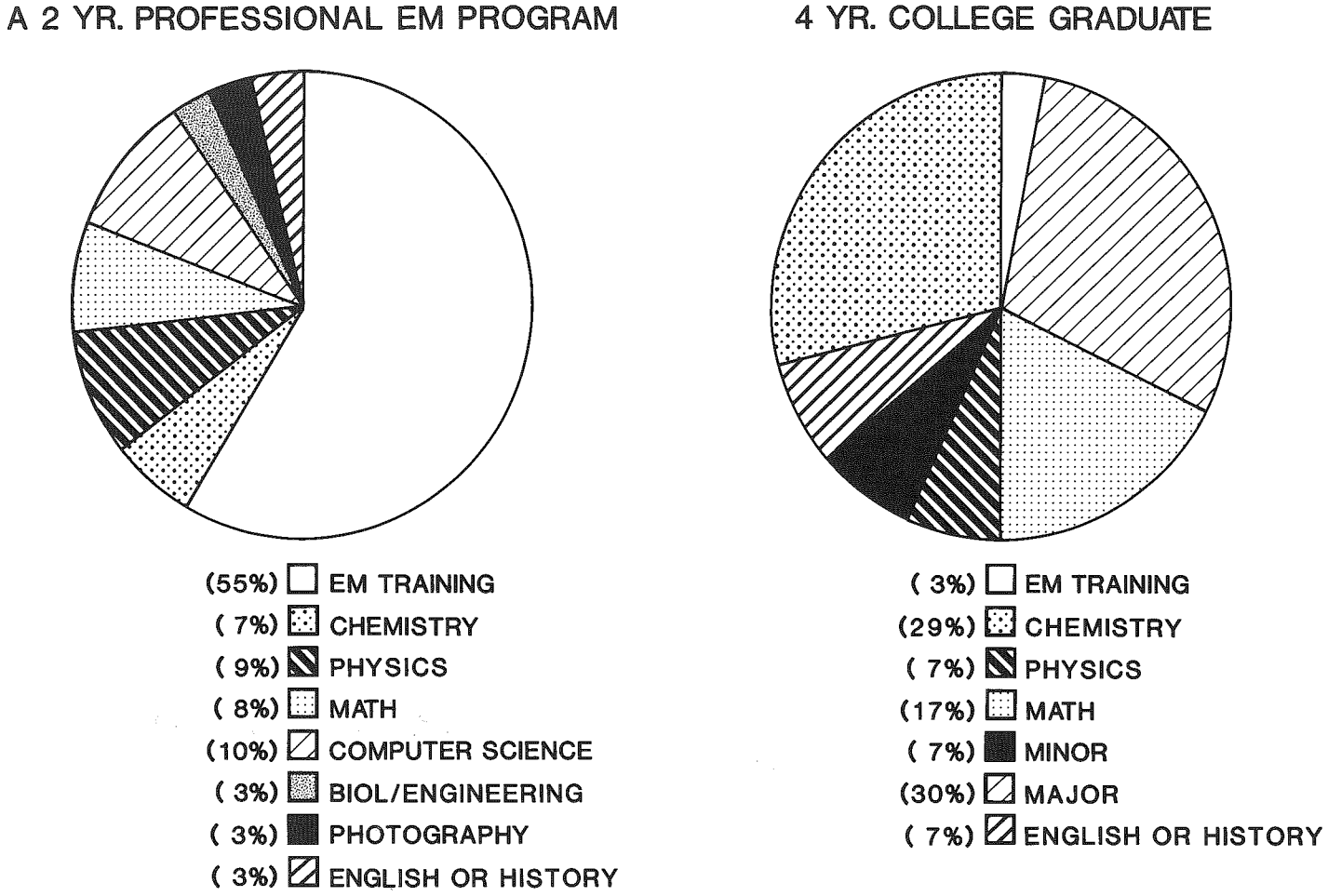
This is not an easy program. A significant drop rate testifies to the fact that only highly motivated and sincerely interested students will stick with it and finally be certificated.

The number of graduates that find jobs in the field of their choice is extremely high; 95% of those looking for EM related jobs find them very quickly. We think that these figures are a definite indication that employers like our product. The in-depth training is appreciated and the graduates are being fully utilized. As further testimony to these facts, the same universities and companies continue to call us when they have job openings in EM. Sandia Corp. has seven of our graduates. The University of California at San Diego has six while others in that same San Diego area have hired five more. The semi-conductor and integrated circuit industry has hired many of Delta's graduates and most are still employed by these companies despite the volatility of the industry. We have supplied two EM technologists to Harvard University, two to Bell Labs and other individuals to Cal Tech., University of Vermont, University of South Carolina, etc. The list is very long. One hundred and fifty of the Delta College EM graduates are now employed throughout the United States.

I was asked recently if I thought that these professionals were better at their jobs than a person with a four-year degree. In fact, a comparison study shows that the two year graduates have spent approximately 1800 hours in total class time during that two years. Over 1000(b) of those hours (55%) have been spent specifically in EM skill building. In contrast, an estimate of hours spent by bachelor degreed people performing similar tasks shows that of the 3500 hours(c) spent in total class time approximately 100 hours (3%) may involve EM (Table 2). The question of how much skill can be demonstrated by people from the two groups seem clear. If skills are the criterion, then clearly the two year professional graduate is the choice. However, there may be an employer preference for the four-year graduate on the basis of a longer and, perhaps a broader general educational background. That may be a good point indeed, but again, at least one quarter of

Table 2

HOURS SPENT ON COURSEWORK



our graduates also hold a bachelor degree.

The two full time instructors and a full time laboratory supervisor here also put in many ten to twelve hour days. The load of responsibility here is heavy on all of us but we readily admit that it is a labor of love. We are proud of this one-of-a-kind program and sincerely feel that there should be more such training facilities for EM technologists.

It has been said that the electron microscope is just one of the *tools* of a scientist. However, in our modern research laboratories there are many different kinds of electron microscopes and a great deal of skill is required in order to use these highly sophisticated instruments well. It is no longer *just* a tool called an electron microscope. There are a number of distinct types of microscopes from the ordinary TEM to the STEM, from the HVEM to the AREM. An excellent knowledge of theory and practical manipulative abilities is essential to the modern EM technologist. We feel that the type of exacting education provided by our professionaly oriented program is exactly what is needed now and for the future in electron microscopy.

FOOTNOTES:

(a) This committee consisted of Jack Mitchell and Sam DiGiallonardo from University of California, Berkeley and Lawrence Livermore, Jack Pangborn and Bob Munn from University of California at Davis, Ursula Wolff of General Electric Nuclear Research Center, Jim Herr from Dow Chemical, John Watson of the EMSA Council and myself from Berkeley and University of the Pacific.

(b) Based on a close estimate of the number of hours spent by the typical Delta EM student as noted by required courses in The Delta Digest. This Digest and the Delta College Catalogue can be obtained by writing to the same address as given for the author.

(c) Based on an estimated of hours spent on courses in a four year program leading to a degree in Physiology. This degree is considered a typical science curriculum. It is calculated from information found in the catalogue for University of California at Berkeley. Such a document is available from them upon request.

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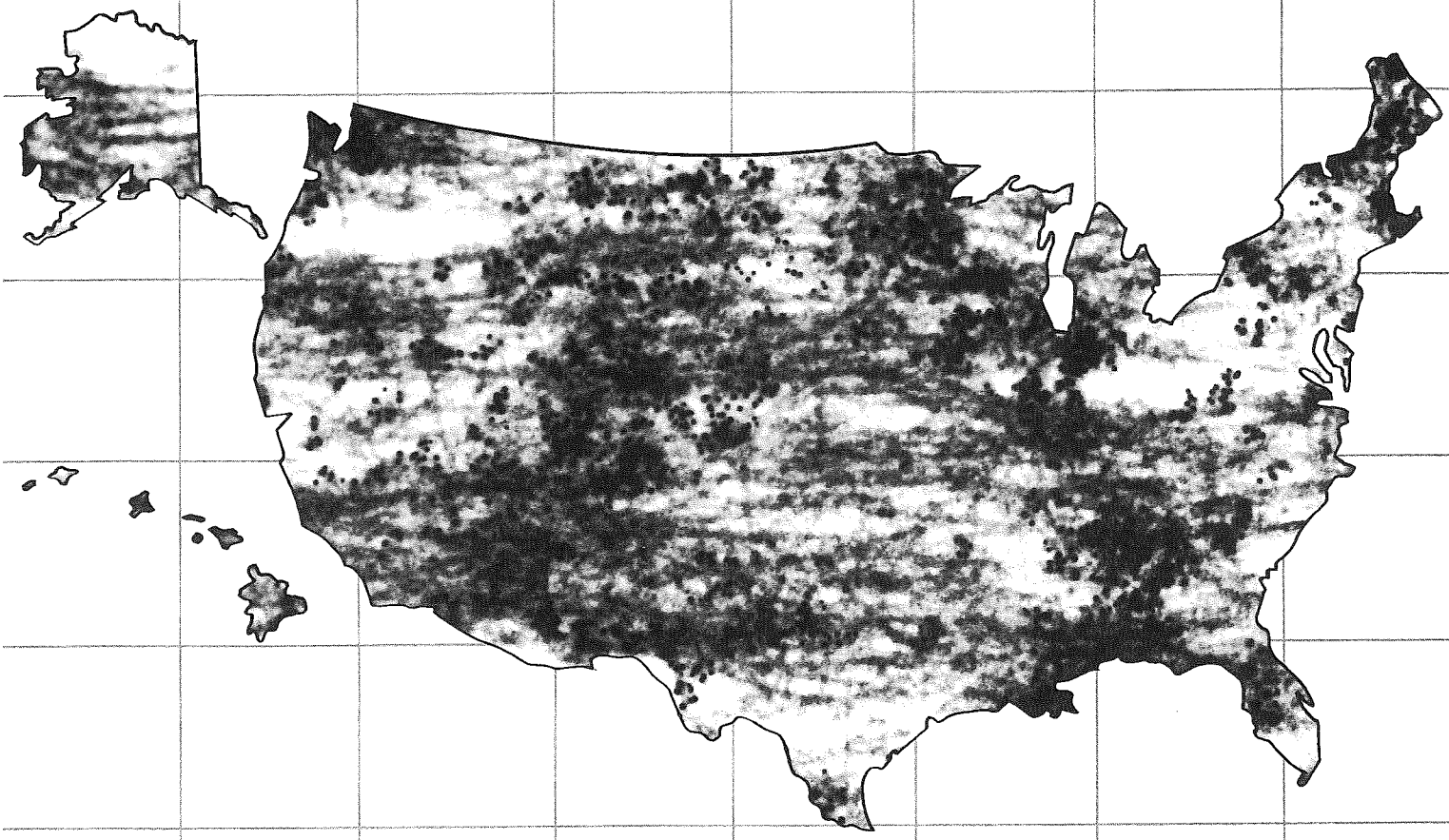

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the micrograph depicted within the USA map
Double immunogold staining of alpha-actinin and myosin in a chick heart fibroblast: myosin, labeled with 5-nm gold is confined to regions between alpha-actinin(10-nm gold).

(*Reference available on request ;* "The molecular organization of myosin in stress fibers of cultured cells", Gabriele Langanger, Marc Moeremans, Guy Daneels, Apolinary Sobieszek, Marc de Brabander and Jan de Mey)

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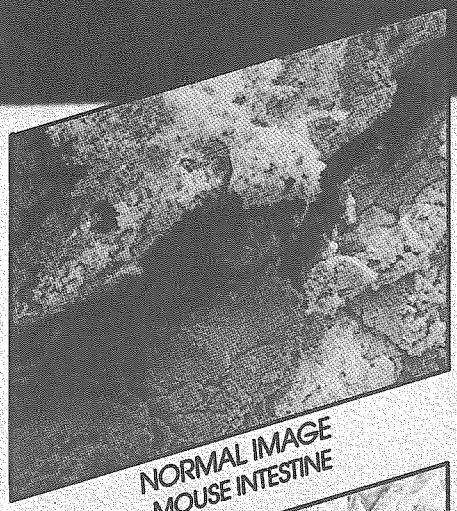

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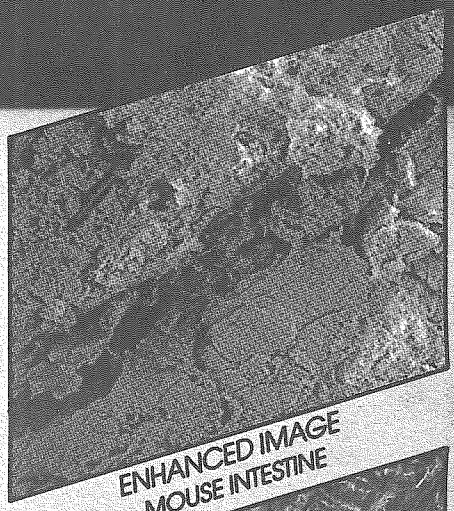

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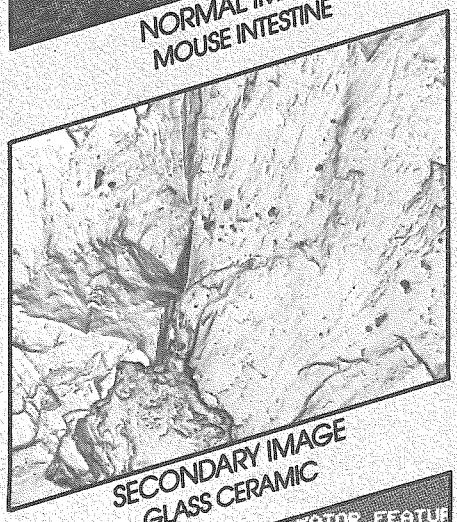

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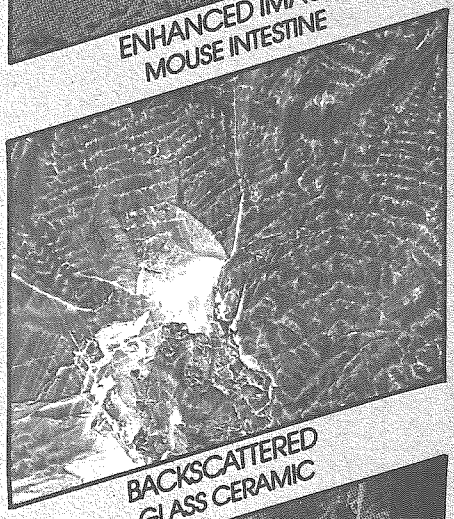

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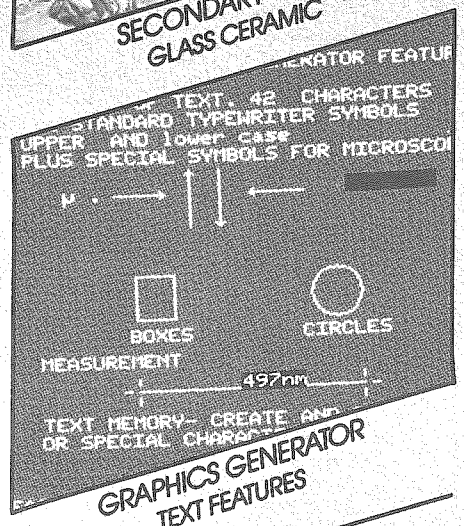

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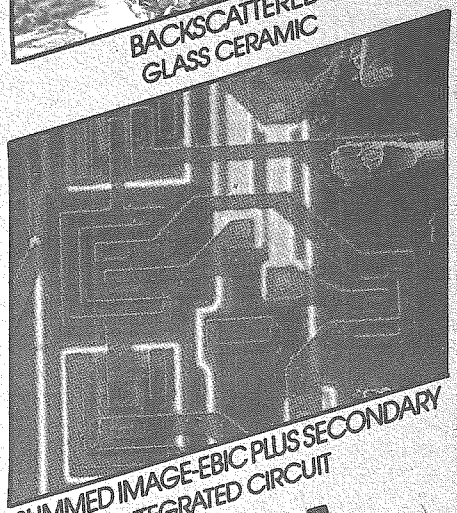

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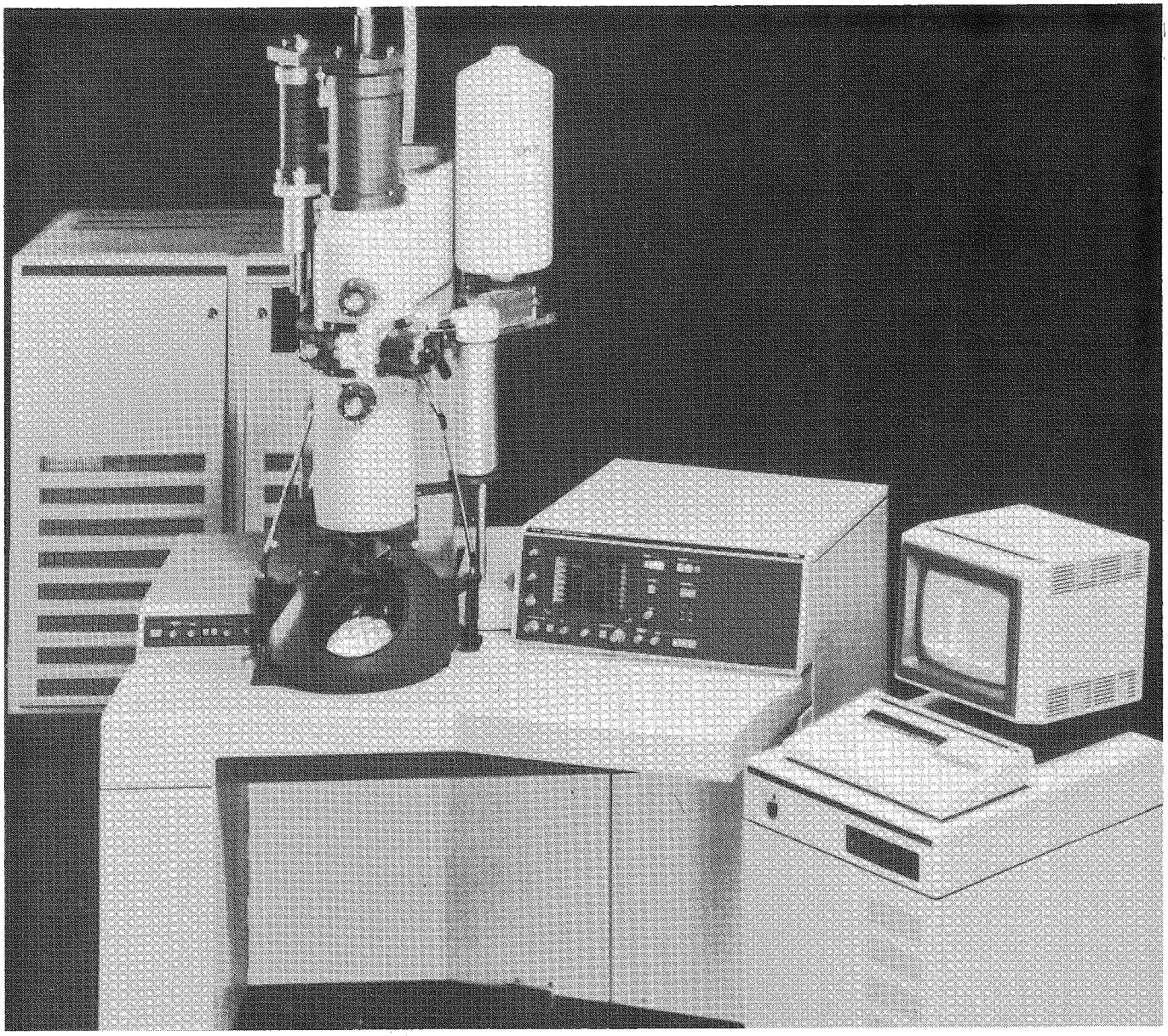

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Abstracts

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — FALL 1985

ULTRASTRUCTURE OF CHEMICALLY-INDUCED NEOPLASTIC LESIONS IN SOME SMALL FISH SPECIES. William E. Hawkins, R.M. Overstreet, and W.W. Walker. Gulf Coast Research Laboratory, Ocean Springs, Mississippi 39564

In developing a carcinogen testing system utilizing small fish, we exposed several species to the carcinogen methylazoxymethanol acetate (MAM), and to various halomethanes. Each of seven species exposed to MAM developed hepatic neoplasms and three species (Japanese medaka, guppy, and sheepshead minnow) developed neoplasms in other tissues as well. We examined hepatic and other neoplasms by EM to improve our light microscopical diagnoses and to better understand histologic progressions. Some neoplasms were prepared by conventional EM procedures and others were reprocessed from paraffin.

EM confirmed that hepatic neoplasms developed in stages that began as altered foci and progressed to hepatocellular and cholangiocellular carcinomas. Tumor cells of undifferentiated sarcomas lacked intercellular contacts as well as an external lamina. Examination of paraffin-embedded tissues reprocessed for EM provided much useful information. With this method, we examined intraocular neoplasms in medaka exposed to MAM and in a guppy exposed to halomethanes. The tumors were medulloepitheliomas and teratoid medulloepitheliomas based on cellular organization and presence of heteroplastic tissues such as striated muscle and cartilage.

These studies show that several fish species develop tumors cytologically similar to those of higher organisms and that EM is useful and convenient for tumor diagnosis in fish. (This study was supported by the National Cancer Institute, Department of Health and Human Services, Contract NO1-CP-26008, and the U.S. Army Medical Research and Development Command.

POSTER PRESENTATION — FALL 1985

MICROSCOPIC AND SUBMICROSCOPIC ANATOMY OF THE LUNG OF THE BUDGERIGAR BIRD (MELOPSITTACUS UNDULATUS).
Jerome H. Smith, (J. Meier, and E. Box)

The trachea and extrapulmonary bronchi as well as intrapulmonary secondary bronchi are lined by psuedostratified ciliated columnar epithelia comprised of basal, mucinous and ciliated cells. Parabronchi, their atria and thoracic air sacs are lined by low cuboidal to squamous cells with prominent myelinoid bodies which are secreted to form the 'surfacant' layer which covers the respiratory labyrinth as well as the parabronchi and the air sacs; 'atrial' cells appear morphologically and functionally comparable to granular (Type II) pneumocytes of mammalian lung. Holes in the floor of the atria give rise to infundibula, wedge-shaped passages into the respiratory space, which in turn branch into the air capillaries of the respiratory labyrinth. The infundibula and air capillaries are lined by squamous pneumocytes whose perikarya concentrate along the infundibula and which are overlain by the myelinoid 'surfactant' membrane. These squamous (Type I) pneumocytes share a basal lamina of the blood capillaries which they envelope. The cytoplasm of the squamous pneumocytes is markedly attenuated, often less than 100 nm., so that the air-blood barrier consists of the myelinoid 'surfactant' layer, apical and basal plasma membranes of the pneumodyte, blood capillary basal lamina and attenuated but continuous endothelia.

ULTRASTRUCTURE OF HEPATIC PEROXISOMES AND LYSOSOMES AFTER WHOLE BODY GAMMA IRRADIATION. A. Chowdhury* and E. W. Hupp, Dept. of Biology, Texas Woman's University, Denton, Texas 76204.

Male Sprague-Dawley rats were killed 2, 7 and 21 days following 7.5 Gy whole body gamma irradiation. Cytochemical localization of acid phosphatase activity in lysosomes was obtained by incubating glutaraldehyde fixed tissue in 5' cytidine monophosphate. Peroxidatic activity of catalase in peroxisomes was localized by a modified alkaline 3,3' diaminobenzidine tetrahydrochloride method. Morphometric parameters were quantitated by stereological methods. The volume, surface and numerical densities on each micrograph were estimated by counting points on a test grid superimposed on each micrograph. None of the parameters (volume, surface, and numerical densities) showed any significant differences at the 0.05 level of probability between irradiated and control. However, morphological observations of hepatic tissue from irradiated rats revealed granularity of peroxisomes, accumulation of rough endoplasmic reticulum, loss of mitochondrial cristae and presence of polyribosomes and/or glycogen at day 2. In contrast, lysosomes appeared to be normal at day 2 after irradiation. The presence of polyribosomes or glycogen and dissolution of mitochondrial cristae persisted 7 days following irradiation. Diffusion of reaction products from peroxisomes, dissolution of crystalline cores and presence of free ribosomes or polysomes were observed 21 days following irradiation.

PLATFORM PRESENTATION — SPRING, 1986

PRELIMINARY STUDY OF THE SEED COAT CHARACTERS OF BAPTISIA SPECIES (LEGUMINOSAE-PAPILIONOIDEAE). Louis H. Bragg, Dept. Biology, University of Texas, Arlington TX 76019.

Scanning electron microscopy was used to examine seeds from sympatric species of Baptisia (B. leucantha and B. leucophaea) to determine their seed structures and to compare the structures with previously examined paplionoid members. Both Baptisia species had a water insoluble surface exudate which was not present in other examined legumes. The testa surface patterns were different for each Baptisia species. Each species had a single palisade layer of cells which was slightly longer in B. leucophaea than in B. leucantha whereas the hour-glass sclereids of B. leucantha were slightly longer than those of B. leucophaea. Tracheid bars (a papilionoid character) were present in both species. The results of the Baptisia observations when compared with earlier observed papilionoid representatives suggest taxonomic usefulness of testa characteristics.

ULTRASTRUCTURAL OBSERVATIONS OF MEIOSIS IN THE RUST FUNGUS GYMNOSPORANGIUM CLAVIPES. J.P. Shields and C.W. Mims, Department of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

This study of Gymnosporangium clavipes was initiated in an attempt to elucidate ultrastructural features of meiosis that might be useful classifying the phylogenetic relationships of rust fungi. Thus far such data is available for only one other species belonging to this group. The results of this study should therefore provide a basis for comparison between species.

TEM was used to examine serially sectioned meiotic nuclei from flat embedded basidia. Emphasis was placed on determining the structure and behavior of the nuclear associated organelle or spindle pole body, the nature of the spindle apparatus and the behavior of the nuclear envelope and chromosomes during various stages of meiosis I and II.

THE ROLE OF ENDOPERIDIAL CALCIUM OXLALTE CRYSTALS DURING PERIDIAL DEHISCENCE IN THE EARTHSTAR GEASTRUM SACCATUM (GASTEROMYCETES). K. D. Whitney and H. J. Arnott, Department of Biology, University of Texas at Arlington, Arlington, Texas 76019.

Members of the genus Geastrum (earthstars) exhibit characteristic patterns of peridial dehiscence. At maturity the exoperidium separates from the endoperidium, and the former splits into several triangular rays which reflex away from the spore case. Recent interest has focused on the presence of calcium oxalate crystals on the endo and exoperidium of earthstars, especially Geastrum minimum. In this species abundant crystals occur on the facing surfaces of the exo and endoperidium following dehiscence, but the origin and development of these crystals is not clear, and it is not known whether these crystals play a role in peridial dehiscence. We obtained both mature and undehisced basidiocarps of another earthstar, Geastrum saccatum, and SEM examination of dehisced basidiocarps revealed only a few eroded crystals on the surface of the endoperidium, and none on the exoperidium. In immature, undehisced basidiocarps, however, numerous druse-like aggregates were found in the endoperidial layer, and these crystals appeared to originate within the walls of endoperidial hyphae. The development of these crystalline deposits in predehiscent basidiocarps, and their location along the future zone of dehiscence, suggests that these crystals play a role in peridial separation in earthstars.

SPERMIOGENESIS IN NORMAL AND MUTANT MICE STUDIED BY REAL-TIME STEREO ELECTRON MICROSCOPY, A. Cole, M. L. Meistrich and P. K. Trostle-Weige, Division of Radiotherapy, Un. of Texas System Cancer Center, Houston, TX 77030.

Germinal cells and nuclei with attached cytoskeletal elements were prepared from the testes and epididymides of normal mice and mice homozygous for the recessive azh mutation, which results in abnormal sperm heads. The material was differentially sedimented onto polystyrene coated EM grids which were carried through several treatment sequences and critical point drying. Observations and recordings utilized a real-time stereo EM system developed by A. Cole. Sperm heads, tails, acrosomes and manchettes were studied at various stages of maturation. Except for the attachment to the heads, the tail architectures were similar in normal and mutant forms. The posterior region of normal sperm heads were very flat, while that for mutuant heads were tapered cylinders. The manchette, an organized microtubular structure, which girdles the posterior region of the spermatid head, was distinctly different in normal and mutant forms. In normal spermatids, the microtubules extended outward at about a 45° angle from the long axis of the head, while in mutant spermatids, the microtubules formed a tight cylinder parallel to the long axis. Radical differences in head shapes between normal and mutant sperm could be related, in part, to the manner in which manchettes are generated and matured on the spermatids. Supported, in part by Grants HD16843 and CA06294 from the NIH.

THE RELATIONSHIP BETWEEN CHLOROPHYLL LEVELS, PHOTOSYNTHETIC RATES, AND CHLOROPLAST MORPHOLOGY IN SELECTED STRAINS OF COTTON (GOSSYPIUM HIRSUTUM). S.R. Short-Russell and J.D. Berlin, Texas Tech University, Lubbock, TX 79409.

Several strains of cotton were selected so that one group exhibited the same photosynthetic rates (mg CO_2/leaf area/time) with varying chlorophyll levels (ug/leaf area) while the second group had varying photosynthetic rates but the same cholorphyll levels. Light microscopy of transverse and paradermal sections was used to determine cell volumes and leaf thicknesses. Electron micrographs taken from transverse sections were analyzed using stereological techniques (point counting methods) to determine chloroplast size and number per cell, the amounts of starch granules and lipid bodies in the chloroplasts, and the total surface area of grana and stroma lamellae present. The relationship between chlorophyll levels, photosynthetic rates, and chloroplast morphology in these strains was determined. Supported by Cotton Incorporated and the Plant Stress Institute and the Water Resources Center at Texas Tech University.

IMMUNOELECTRON MICROSCOPY OF MACROPHAGE MARKERS IN ERYTHROBLASTIC NESTS. H.R. Higley and J.J. Trentin, Division of Experimental Biology/Surgery, Baylor College of Medicine, Houston, TX 77030

Erythroblastics Nests or islets (EN) are composed of a central monocytoid cell surrounded by a rosette of erythroid elements in various stages of differentiation. A relatively rare structure in normal mouse hemopoietic tissue, large numbers of EN form in the mouse spleen and BM in response to anemic stimuli. We have analysed the cell surface characteristics of the EN central cell by light and electron microscopic immunocytochemical techniques in an effort to understand how this complex might function in erythropoiesis. Isolated EN, and/or splenic tissue from bled, irradiated and transfused (BIT) mice and from the mutant anemic Sl/Sl^d mouse were stained with antibodies to Ia and MAC-1 antigen; both markers of the mononuclear phagocyte series. Immunofluoresence studies of acetone fixed cytospins and frozen sections revealed that only a portion of the EN population was labeled with these markers. These findings were confirmed at the ultrastructual level in paraformalin fixed samples using peroxidase-labeled antibodies. The significance of this EN heterogeneity is unknown at present. Double labeling studies are in progress to determine if the same central cell can display both markers.

PROGRESSION OF AFLATOXICOSIS IN BROILER CHICKENS: A LIGHT AND ELECTRON MICROSCOPICAL STUDY. H. H. Mollenhauer, D. E. Corrier, W. E. Huff, L. F. Kubena, R. B. Harvey, and R. E. Droleskey, Veterinary Toxicology and Entomology Research Laboratory, USDA-ARS, College Station, TX 77841.

Newly-hatched broiler chickens were placed on a diet containing 0, 1.25, 2.5, 5.0 μg aflatoxin/g feed and then assayed for clinical and pathological changes on days 3, 6, 9, 12, 15, 17, and 21. At 5.0 μg/g sample level, body weights were decreased but the relative weights of gizzard, spleen, and kidney were increased. Liver weight initially decreased and then increased. Clinically, hemoglobin levels were decreased as were serum levels of albumin, total protein, uric acid, triglycerides, cholesterol, and serum lactate dehydrogenase.

Significant changes in cell architecture were found only at the 2.5 and 5.0 μg aflatoxin/g feed treatment levels. In liver, these changes included heavy accumulations of fat in some hepatocytes and no fat in other hepatocytes, reduction in mitochondrial size, and mild infiltration of tissue with lymphocytes. In kidney, the primary change was thickening of the glomerular basement memberane. Pancreatic tissues were still being examined at the time of writing but no significant ultrastructural changes have yet been observed.

UNDIFFERENTIATED CARCINOMAS OF THE LUNG. Bruce Mackay, John M. Lukeman, and Nelson G. Ordonez, Depart. Pathology, The University of Texas M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

The majority of primary epithelial tumors of the lung can be identified by light microscopy on the basis of specific features of the cells and their architectural arrangements. Adenocarcinomas and squamous cell carcinomas vary in differentiation, and histochemical stains for mucin or electron microscopy (EM) may be necessary to establish the classification of poorly differentiated tumors. When the degree of differentiation is minimal, ultrastructural features may not clearly indicate whether a tumor is glandular or squamous, or both. Distinctive features are absent in the undifferentiated large cell carcinomas, and EM may be required to distinguish them from small undifferentiated bronchogenic carcinomas ("oat cell carcinomas"): the latter are aggressive tumors but they are uniquely responsive to chemotherapy and radiotherapy. Carcinoid tumors also enter into the differential diagnosis of undifferentiated lung carcinomas. We have examined a series of cases in order to define the range of ultrastructural features of large cell undifferentiated, small cell undifferentiated, and carcinoid tumors of the lung, and have focussed attention on neoplasms that do not readily fit into these defined categories. Our findings confirm the usefulness of EM in the diagnosis of lung tumors, but in a small percentage of cases the features are not sufficiently specific to allow subclassification.

MUSCLE FIBER MORPHOLOGY AND NUCLEI FREQUENCY FOLLOWING WEIGHT-LIFTING EXERCISE IN CATS. C. J. GIDDINGS and W. J. GONYEA, Dept. of Cell Biology and Anatomy, University of Texas Health Science Center at Dallas, Texas.

Myofiber degeneration and regeneration have been observed in response to weight-lifting exercise. In this study, myofiber morphology and muscle nuclei frequency were examined in four adult cats following prolonged weight-lifting exercise (ave 52 wks). Trained and control flexor carpi radialis muscles were fixed by vascular perfusion with glutaraldehyde. Small myofiber bundles were teased from each muscle and embeded in Epon. Myofiber morphology, cross-sectional area, and muscle nuclei frequency were evaluated in cross-sections from the mid-region of each bundle, using both light and electron microscopy. This study confirmed previously reported evidence of exercise-induced myofiber degeneration. Fiber area histograms showed that trained muscles possessed both larger and smaller fibers than were found in untrained control muscles. Trained muscles showed a greater number of more centrally located myonuclei than were found in untrained control muscles. Severely degenerative regions of myofibers appeared devoid of healthy myonuclei and satellite cells. Preliminary data from 164 myofibers from control muscle and 134 myofibers from trained muscle suggested that nuclei number/myofiber profile was greater in trained muscle (1.24) than in untrained control muscle (0.85). Satellite cell freguency showed a slight decrease in trained muscle (5.7%) when compared to control muscle (6.7%). However, a larger sample size is necessary before the significance of myonuclei and satellite cell frequency can be assessed. This study confirms that weight-lifting exercise causes myofiber degeneration and suggests that both myonuclei and satellite cell frequency are altered in response to exercise. Supported by NIH AM17615.

HEAT INDUCED MITOCHONDRIAL DAMAGE STUDIED BY REAL-TIME STEREO TEM. A. Cole, E. Armour and R. Langley, Division of Radiotherapy and Department of Hematology, Un. of Texas System Cancer Center, Houston, TX 77030.

Mitochondrial damage induced by cell hyperthermia was studied in cultures of CHO cells grown directly on polystyrene membrane coated EM grids. The cultures were heated to temperatures of 42°, 43° and 45°C for periods between 10 minutes and 6 hours, then placed in 0.5% OsO_4 which preferentially fixed membrane containing organelles. To visualize internal cellular structures, a 0.05% trypsin incubation at room temperature was used to digest part of the occluding cell proteins. This was followed by a final fixation in 0.5% glutaraldehyde and critical point drying. Total cell distributions of mitochondria were observed and recorded using a real-time stereo EM developed by A. Cole. Normal mitochondria exhibited well delineated morphologies with intact membranes and distinct christea. Mitochondria in cells treated at 43° and 45°C exhibited heat dependent damage progressing in the order: distortion and swelling, membrane and christea discontinuities, disassociation into diffuse particulates. Since the mitochondria were exposed to trypsin, the heat damage may be due, in part, to structural instabilities which increase the sensitivity of the mitochondria to trypsin digestion. Much less mitochondrial damage was observed for 42°C cell treatments. A pretreatment of 45°C for 10' at 24 hours before the assays induced a "thermal tolerance" that resulted in reduced subsequent heat damage. Supported in part by Grant CA37315 from the NIH.

UPTAKE OF [2-^{3}H]GLYCINE BY TILLANDSIA RECURVATA. J.A. Matos[1,2] and E.A. Richardson[2], [1]Dept. of Biology, Washington Univ., St. Louis, MO 63130 and [2]Dept. of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

The elaborate peltate trichomes of many bromeliads have been shown to be absorptive structures. Bromeliad trichomes which function in absorption of water and some nutrients reach their highest degree of specilization in atmospheric members of the genus Tillandsia. Young individuals of T. recurvata were immersed in a treatment solution of [2-^{3}H]glycine at 10μc/ml, 10^{-5}M $CaCl_2$ and were buffered with 10^{-5}M KH_2PO_4. Groups of young plants were allowed to absorb the treatment solution for 1, 5, 15, and 30 minutes. At the TEM level, labeled material is found associated with the cell walls of the wings of the trichomes, in vacuoles, and associated with other cellular structures. Labeled material appears to move between mesophyll cells via plasmodesmata.

EFFECTS OF MONENSIN ON GAMETOGENESIS IN ALLOMYCES MACROGYNUS. T. Sewall and J. Pommerville, Dept. of Biology, Texas A&M University, College Station, TX 77843.

The water mold, Allomyces macrogynus, does not possess a typical Golgi complex (GC) composed of stacked cisternal elements although it does contain individual cisternal elements in which GC-specific enzymes have been localized. To determine if these Golgi equivalents play a role in cytoplasmic cleavage in A. macrogynus, we used the ionophore, monensin to probe the process of gamete formation. Treatment of cells with monensin in vitro typically causes swelling and subsequent release of dilated vesicles from the trans cisternae of the GC. Induction of gametogenesis in A. macrogynus with Machlis' dilute salts (DS) solution containing a 10 uM or greater concentration of monensin increased the length of time required for gamete release and decreased the number of gametangia which released gametes. Addition of monensin before the completion of cleavage furrows inhibited release more than in those treated after cleavage furrow completion. Gametangia induced with 50 uM monensin in DS contained large vesicles filled with amorphous material and numerous small transparent vesicles. The large vesicles contained high levels of calcium and phosphorus while the smaller dilated vesicles did not. Cleavage furrows were greatly reduced but other events involving membrane formation appeared normal. Gametes released from monensin-treated gametangia frequently contained numerous nuclei, flagella, and large swollen vesicles. Monensin appeared to interfere with a portion of the endomembrane system active during cleavage furrow formation. Because of monensin's specificity in vitro, Golgi equivalents are implicated in the proper formation of the gamete plasma membrane in A. macrogynus.

BOTANICAL RESEARCH ABOARD THE SPACE SHUTTLE: THE INFLUENCE OF MICROGRAVITY ON CELL STRUCTURE. Randy Moore, Chia-Lien Wang, W. Mark Fondren, and C. Edward McClelen, Department of Biology, Baylor University, Waco, TX 76798

Roots of Zea mays seedlings grown aboard flight 61-C of the orbiter Columbia grew significantly slower than ground controls. Cells comprising the root cap of space-grown seedlings (including the putative statocytes) had a significantly different structure than those of ground controls. Specifically, the endoplasmic reticulum, nuclei, amyloplasts, and other organelles were distributed differently in cells of space-grown plants as compared to ground controls. These results indicate that gravity has a significant influence on cellular structure. These results will be discussed relative to how roots perceive and respond to gravity. This research was supported by Baylor University and the SSIP of NASA.

THE FINE STRUCTURE OF CONTINUOUSLY CULTURED BABESIA ISOLATED FROM A WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS). R. E. Droleskey, P. J. Holman,* K. A. Waldrup,* and G. G. Wagner,* U.S.D.A., Veterinary Toxicology and Entomology Research Laboratory, College Station, TX 77841 and *Dept. of Microbiology and Parasitology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843.

Red blood cells (RBC) from a white-tailed deer parasitized with a piroplasm presumed to be Babesia odocoilei (Emerson) (1) were cultured under in vitro conditions previously used for the continuous culture of bovine Babesia (2). The parasitemia rose from <0.1% on day 1 to a high of 31% on day 24. Aliquots from early and late cultures were fixed and processd for examination by both transmission and scanning electron microscopy. In addition, RBC from a Babesia-free white-tailed deer were sampled before and after 3 days of culture as controls. Examination of parasitized cultures revealed Babesia-like organisms within and frequently near the periphery of the RBC membrane. RBC from the later sampling were often found to contain more than one parasite. Parasites were frequently seen in the process of entering the RBC. Control RBC were found to be parasitized with Theileria cervi (Schaeffler) (3) which was not evident in the Babesia cultures. Details of the fine structure of the observed parasites will be presented in this report.

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(3) Schaeffler, W. F. (1961) J. Protozool. 8(Suppl):10.

DIAGNOSTIC ELECTRON MICROSCOPY IN ONCOLOGY. AN APPRAISAL OF ITS CURRENT ROLE. Mannie Steglich and Bruce Mackay, Depart. Pathology, The University of Texas M.D.Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Transmission electron microscopy (EM) can provide information about the fine structure of cells of human tumors that is useful in determining the diagnosis when it is uncertain by light microscopy. In recent years, the role of ultrastructural studies in routine tumor diagnosis has been eroded by the burgeoning of immunocytochemistry and by economic restraints. To investigate this situation and define the current role of diagnostic EM in oncology, we have reviewed the files of the diagnostic electron microscopy service at the M. D. Anderson Hospital for the past 10 years, noting the total numbers of cases submitted for electron microscopy, the numbers examined for diagnostic information, and the types of clinical problems for which electron microscopy has been requested throughout this period. The number of diagnostic requests for tumor specimens has decreased slightly while the total work load within the department has increased, but the number of EM diagnostic specimens has now stabilized, and unless there are dramatic advances in the range of applications of immunocytochemistry, it can be anticipated that EM will continue to play a significant and necessary role in tumor diagnosis, complementary to routine light microscopy and other procedures. The onus is on the surgical pathologist to be aware of the relative contributions of EM and other diagnostic methods in order to select the most appropriate procedure for a particular diagnostic problem.

MORPHOMETRIC AND ULTRASTRUCTURAL STUDY OF THE SQUIRREL MONKEY (Siamiri sciureus) VESTIBULAR NERVE. C.D. Fermin and M. Igarashi, Dept. of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas 77030.

Vestibular nerves were examined with light (LM) and electron microscopy, and computerized measures were obtained and analyzed. An average of 12,412 perikarya and 12,005 myelinated nerve fibers was obtained. The cross sectional area of 1,864 perikarya was 200-650 um^2. The cross sectional area of 1,346 nerve fibers was 3-11 um^2 for the axoplasm, and 11-12 um^2 for the myelin sheath of the same fiber. The cross-sectional area of central and peripheral fibers differed significantly ($p<0.001$). The initial segments of peripheral dendrites were usually smaller, but longer than the initial segments of the central axons. Schmidt-Lantermann incisures were more abundant in thick and heavily myelinated fibers than in thin and lightly myelinated fibers. No major ultrastructural differences were found between myelinated and unmyelinated perikarya, except at the hillock region. These data will serve to compare and evaluate modifications of vestibular nerves following surgical removal of the end organs (labyrinthectomy), one of the studies in progress in this laboratory. These studies have recently been extended to the vestibular nuclei, with particular attention to synaptic modifiability of second order vestibular neurons. (Supported by NINCDS NS-10940).

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AN EMBRYO CHICK MODEL OF INNER EAR DEVELOPMENT*. C. D. Fermin and M. Igarashi, Dept. of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas 77030.

The end organs of the vertebrate inner ear, including those of the chick embryo, develop along gradients. Developmental gradients determine progression, duration and direction of events related to the onset of function of a given structure. Studies in the chick cochlea between 5-18 days of incubation (chicks hatch in 21 days) have shown that the vertebrate cochlea may develop bidirectionally from the mid-base instead of unidirectionally from the base to the apex. More recently, we have begun studying developmental gradients of the chick vestibular system. TEM and X-ray microanalysis and ^{3}H-Thymidine studies were used to determine the synaptogensis of the afferent and efferent growth cones and the cytodifferentiation of hair cells. To our knowledge, the results of these studies provide the first demonstration of (1) gradients along the chick cochlea, (2) direct evidence of statoconia formation, and (3) possible mechanisms of calcium incorporation into the organic matrix of embryonic statoconia.
(Supported in part by grants NINCDS NS-10940, RO1 NS-22604; NASA NAG 2-342 & grant from the Deafness Research Foundation).

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*This work was initiated at Fla. Inst. Technol. under Dr. G.M. Cohen's supervision.

Tissue Surfaces and Isolated Cells from Sinoatrial Node and Atrium. L.A. Roberts and J.D. Berlin, Dept. Anatomy, Medical College of Wisconsin, Milwaukee WI 53226 and Dept. Biology, Texas Tech University, Lubbock, TX 79409.

Tissue structure of the sinoatrial node and atrium of the rabbit heart was studied using scanning electron microscopy. Samples of tissue were fixed in 2.5% glutaraldehyde, dehydrated in a series of alcohols, critical point dried in carbon dioxide using a Bomar critical point dryer and then sputter coated with 20nm gold and observed using a Hitachi 500 scanning electron microscope. Epicardial surfaces of both atrium and sinoatrial node were covered with a dense coating of microvillae. The endocardial surfaces were smooth. In the sinoatrial node region, endocardial cells were oriented longitudinally parallel to the superior-inferior vena caval axis, becoming longer near the atrial septum. Samples from each tissue were digested using 0.06% collagenase in a modified Tyrode buffer containing 180 micromolar calcium. Aliquots of digestion medium were filtered through 13mm millipore filters which were then processed in the same manner as the larger tissue samples. Digests first yielded collagen ropes and whorls of collagen weave network, then clusters of cells and finally single cells. Some cell clusters were observed with accompanying collagen ropes indicating the manner in which collagen attaches to the atrial cells. Surfaces of the cells in clusters and of single cells are covered with ridges or microplicae which probably indicate sites of cell-cell and cell-collagen attachments.

POSTER PRESENTATION — SPRING, 1986

IMPROVED TECHNIQUES FOR PROCESSING FINE NEEDLE ASPIRATIONS OF HUMAN TUMORS. M. Steglich, J.M. Bruner, N. Sneige, B. Mackay, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, 77030

Fine needle aspiration biopsy is a rapid and relatively painless technique which has proved accurate and specific in the diagnosis of both superficial and deep lesions. Its use has become widespread and continues to increase. Cells obtained by this method are rapidly fixed, stained and examined by light microscopy, and where indicated, by immunocytochemical methods. When a diagnosis cannot be made using conventional light microscopy, electron microscopy is an essential adjunct in the diagnosis. The material submitted for ultrastructural examination my consist of fewer than 50 tumor cells, often with gross contamination by blood or other cell debris. It has become imperative to develop simple techniques of tumor cell concentration, preservation and isolation that can be used in the routine processing of these specimens. We have investigated several variations of routine tissue tissue processing methods on parallel samples of fine needle aspirations from human tumors. These include the use of sucrose-buffered 2% glutaraldehyde, various tissue culture media for holding the specimen before hemolysis of red blood cells (RBC's), the use of Ficoll gradients for eliminating RBC's and low-speed centrifugation for concentration of tumor cells. Manipulation by these techniques has improved tumor cell fixation, recovery and preservation, and reduced artifacts of processing.

PLATFORM PRESENTATION — OCTOBER, 1986

SOME PROPERTIES OF THE EMBRYONIC STATOCONIAL MEMBRANE
C. D. Fermin, M. Igarashi, and T. Yoshihara. Dept. of Oto/Communicative Sciences, Baylor College of Medicine, Houston, TX 77030, U.S.A.

The chick vestibular epithelia transformed from a homogeneous layer (stage 15) of cells into a pseudo-stratified layer (stage 24). The columnal appearance near the endolymphatic lumen was evident by stage 29. Supporting cells in the macula sacculi and utriculi underwent changes that suggested a very active secretory activity (e.g. large rER cisterns filled with material similar to the primitive organic matrix). Fibrillar material of the otolithic membrane that remained attached to the supporting cells accumulated over the saccular and utricular maculae. The primitive otolithic membrane acquired stress-like lines and statoconia units emerged without a central core. As statoconia calcified, they thickened at the periphery and a core formed. Statoconial complexes from chicks injected with the isotope ^{45}Ca ($CL_2Ca.H_2O$) indicated that calcium incorporation (count per minute, CPM) was higher at 11 days ($\bar{x}$=1267) than at 8 days ($\bar{x}$=776) ($p<0.05$). DIAMOX inhibited statoconia formation and/or prevented the association between the organic matrix and calcium. Large statoconia (100-200 um diameter) were formed in embryos injected with this carbonic anhydrase inhibitor. Five major protein bands ranging between 25 and 210 K Daltons were identified. Ouabain-sensitive potassium-dependent p-nitrophenylphosphatase activity was demonstrated in the endolymphatic sac of hatching chicks.

(Supported by NASA grant NAG2-342 and NINCDS grants NS22604 and NS 10940).

IMMUNOGOLD IN PATHOLOGY. H.K. Hawkins, and L.S. Rehm, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas 77225-0269.

The immunogold technique can be readily applied to a variety of problems of interest to pathologists. It is useful not only for investigation, e.g. the dynamics of cell surface receptors, but also for retrospective studies of routinely processed clinical specimens.

Gold colloids can easily be prepared in a chosen size range by reduction of gold chloride in the presence of citrate and tannic acid. The gold sol is titrated, then poured into the protein or lectin solution, polyethylene glycol is added and the tracer is centrifuged and resuspended in PBS. The amount of protein or lectin required to coat the gold particles is determined by microtitration. All immunochemicals are dialyzed overnight against water, and passed through a micropore filter.

For research, tissue is fixed in 1% glutaraldehyde with picric acid for three hours, and embedded in LR white acrylic resin. This procedure has been useful in a clinical study of pancreatic endocrine cells in infants with hypoglycemia.

Some antigens, including polypeptide hormones and immunoglobulins, are sufficiently stable to withstand routine fixation (glutaraldehyde and osmium) and embedding. Thin sections are oxidized for one hour in saturated in sodium metaperiodate. Thus it is possible to do retrospective studies on well preserved tissue.

Alternatively, cells can be exposed to antibody without fixation, and monoclonal antibodies can be used to study surface antigens, receptors or carbohydrates. For scanning EM, the back scattered electron signal is extremely useful; all particles can be detected even in concealed locations.

THE DEVELOPMENT OF AN IN VITRO SYSTEM FOR THE STUDY OF TRACHEA EPITHELIAL CELLS. P.C. MOLLER, L.R. PARTRIDGE, V. PELLEGRINI, R.A. COX AND D.G. RITCHIE. DIV. OF CELL BIOL., UTMB, DIV. OF CELL BIOL., SHRINER'S BURNS INSTITUTE, GALVESTON, TEXAS 77550

Respiratory tract dysfunction, caused by the inhalation of noxious gases, is now recognized as a major contributing factor in burn related deaths. In vivo studies have contributed little toward understanding how these gases may affect mucus synthesis and secretion, ion transport, or regeneration of respiratory tract epithelia. We report here on the development of an in vitro system using hamster epithelium cells (HTE) in which undifferentiated basal epithelial cells can differentiate into functional ciliated and mucus cells. Hamster trachea was removed and incubated overnight at 4° C. in 0.01% pronase. The dissociated epithelial cells were then washed, resuspended to $2.5X10^7$ cells/ml in medium 199/DMEM (1:1) containing 5% FCS, gentamycin, insulin, transferrin, hydrocortisone, bovine hypothalamus extract and epidermal growth factor. The cell suspension (0.1 ml) was then seed on collagen coated Millicell-HA 0.45 micron filters and placed in 24 well culture plates containing 0.25 ml of the same medium. Under these conditions HTE cells will differentiate into functional ciliated and secretory cells in vitro in 5-7 days. After 3 days in vitro multiple centrioles were present indicating that ciliogenesis was already underway. By day 6 HTE cells appeared normal and resembled those found under in vivo conditions: pseudostratified columnar ciliated and mucus cells closely associated with squamous basal cells. These preliminary results indicate that HTE cultures can be used to study the function of respiratory tract epithelia and may represent an alternative to in vivo methods currently being used. (Supported by grant #0-18311477768 from the Shriner's Burns institute.)

PRELIMINARY OBSERVATIONS ON THE SEED ANATOMY OF Citrullus lanatus (WATERMELON). J. Shelton[2], A. Neumann[1] and R. Martyn[2], [1]Department of Biology and [2]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

As part of a continuing investigation of Fusarium infection of watermelon crops, we conducted a review of seed anatomy in selected cultivars of Citrullus lanatus (Thumb) Matsum & Nalcai (family Cucurbitaceae). The laterally compressed seeds are located in the "fleshy" mesocarp tissues of the mature fruit. Each mature seed is composed of a dicotyledonous embryo encased in a multilayer testa. The testae of the cultivars examined are apprently composed of a heavily sclerotized outer palisade layer (which often develops fissures upon drying), followed by a thick parenchymatous layer and finally by a thin parenchyma layer surrounding, but not adnate, to the embryo. A single vascular bundle enters the funiculus and can be traced along one lateral (flattened) side of the inner-most testa layer from the micropylar end to the chalazal end of the seed and eventually becomes nondecerniable as it loops back to the micropylar end of the seed along the other lateral wall.

DEVELOPMENT OF THE EQUINE EMBRYONIC SUB-ZONAL LAYER
J.M. Wilson, T. Caceci and K.F. Neck, Departments of Animal Science and Veterinary Anatomy, Texas Agricultural Experiment Station, College Station, Texas 77843

The equine embryo appears to be unique among the domestic species in that a sub-zonal layer (capsule) develops on the interior surface of the zona pellucida after the embryo enters the uterus at approximately day (d) 6 post-ovulation. This capsule remains intact until at least d 20 and may aid in the migration (up to d 16 post-ovulation) of the early equine embryo.

The embryos in this study were recovered non-surgically d 6 to 8 post-ovulation and were prepared for scanning and transmission electron microscopy by standard techniques.

The capsule appears to develop from a flocculent material which is observable between the trophoblast cells and the zona pellucida. This material appears to form fibrils prior to being deposited on the inner surface of the zona pellucida. The deposition process must occur rapidly once the embryo has entered the uterus. The capsule is not evident when the embryo first enters the uterus, but has developed sufficiently by d 6 to allow for the zona pellucida to be shed. The source of the material which forms the capsule has not been elucidated, nor has it been chemically defined. However, large vacuoles which contain a material similar to that seen in the perivitelline space are evident in the trophoblast cells of the early blastocysts. It has been suggested that the material which forms the capsule is an orderly glycocalyx deposited by the trophoblast cells to allow the embryo to expand rapidly (4 mm by d 10) and continue to migrate throughout the uterus. This migration may be necessary for maternal recognition.

HEPATOPANCREAS OF THE PACIFIC WHITE SHRIMP PENAEUS VANNAMEI. T. Caceci, D. H. Lewis, K.F. Neck, and R. F. Sis, Coll. Vet. Medicine, Texas A&M University, College Station, TX 77843

The Pacific white shrimp, a hardy west-coast species of considerable commercial value, is the subject of studies of the feasibility of large-scale shrimp farming in Texas. The hepatopancreas of the decapod Crustacea serves many of the functions which in mammals are divided between pancreas and liver, and is vital to maintaining health and normal growth rate in culture. Structural studies of this organ in this species have not previously been done. The hepatopancreas of P. vannamei takes the form of blind tubules opening off the digestive tract, formed of a simple columnar epithelium which shows evidence of at least two, and possibly four cell types. One type has abundant profiles of what appears to be agranular endoplasmic reticulum, or perhaps enormously ramified Golgi apparatus. A second type shows immense vacuoles filled with flocculent material, which appear to arise from the coalescence of smaller similar vacuoles. Most, but not all, cells show microvilli, but in some cases these become irregular and anastomotic, and may be are absent. The tubules occasionally show scanty strands of muscle between them, which may aid in expulsion of the contents. The epithelium of the tubules appears to be involved in holocrine secretion, with necrosis and destruction of cells a normal event; it is presumably repaired cyclically by differentiation of younger cells. Cells in all stages can be observed in sections, but no localization of stages to different parts of the tubules could be assigned. The hepatopancreas in other Penaeid shrimp is known to be the site of digestive enzyme synthesis, and is presumed to be so in P. vannamei, but nothing corresponding to mammalian zymogen granules was seen.

EXTRACELLULAR ORIGIN OF LIPID DEPOSITS IN THE EARLY ATHEROSCLEROTIC CORE REGION SUGGESTED BY ELECTRON MICROSCOPY. Thomas A. Schifani, Thomas M.A. Bocan, and John R. Guyton, Dept of Medicine, Baylor College of Medicine, Houston, Texas 77030

The formation of the core region of small, raised, atherosclerotic lesions has been examined by electron microscopy. In addition to routine fixation and staining procedures, an osmium-thiocarbohydrazide-osmium (OTO) staining sequence was used to enhance visualization of extracellular lipid droplets. An abbreviated dehydration was employed to prevent excessive leaching of the lipid droplets from the tissue. These extracellular lipid droplets account for approximately 40% of the lipid-rich core volume. The lipid droplets were often found distinctly associated with elastin and/or collagen. Crystalline clefts suggesting cholesterol monohydrate were also observed. Upon stereologic analysis of the lipid-rich core components, marked reductions in the volume fractions of cells, reticular ground substance and basement membrane were observed, while the extent of extracellular lipid increased 7-10 fold. Monocyte-macrophage foam cells were prominent above the core region in the lesion cap, but were seldom found within the core region. Cellular lipid droplets were much larger (profile-diameters six-fold higher) than extracellular droplets. With these data as well as transitional morphologic features at the boundaries of the core region, it is suggested that 1) the abundant extracellular lipid does not derive from cell necrosis, and 2) lipid deposition in association with extracellular matrix constituents is an early event in the development of the lipid-rich core.

SATELLITE CELL FREQUENCY AND MYOFIBER MORPHOLOGY FOLLOWING WEIGHT-LIFTING EXERCISE IN CATS. C.J. Giddings and W.J. Gonyea, Dept. of Cell Biology and Anatomy, University of Texas Health Science Center at Dallas, Texas.

Myofiber satellite cells participate in muscle regeneration following injury. The role that satellite cells play in the degeneration/regeneration response to weight-lifting exercise has not been determined. In this study, myofiber morphology and satellite cell frequency were examined in four adult cats following prolonged weight-lifting exercise (ave 52 wks). Trained and control flexor carpi radialis muscles were fixed by vascular perfusion with glutaraldehyde. Myofiber morphology, cross-sectional area, and satellite cell frequency were evaluated in cross-sections from the mid-region of small fiber bundles, using both light and electron microscopy. This study confirmed previously reported evidence of exercise-induced myofiber degeneration. Trained muscles possessed both larger and smaller fibers than were found in untrained control muscles. Severely degenerative regions of myofibers appeared devoid of healthy myonuclei, although small mononucleated cells were occassionally observed within the basal lamina. Data from 371 myofibers from control muscle and 467 myofibers from trained muscle suggest that myonuclei number/myofiber profile was greater in trained muscle (1.08) than in control muscle (0.89). Satellite cell frequency showed a slight decrease in trained muscle (4.2%) when compared to control muscle (6.8%). This study confirms that weight-lifting exercise causes myofiber degeneration and suggests that both myonuclei and satellite cell frequency are altered in response to exercise. Supported by NIH AM17615.

THE INFLUENCE OF MICROGRAVITY ON CELLULAR DIFFERENTIATION IN ROOT CAPS OF CORN. Randy Moore, Mark Fondren, Eddie McClelen, and Chia-Lien Wang, Biology Department, Baylor University, Waco, TX 76798

We launched imbibed seeds of corn into outer space aboard the space shuttle **Columbia** to determine the influence of microgravity on cellular differentiation in root caps. Microgravity tended to 1) increase the relative volumes of hyaloplasm and lipid bodies, 2) decrease the relative volumes of plastids, mitochondria, dictyosomes, and the vacuome, and 3) exert no influence on the relative volume of nuclei in cells comprising the cap. The reduced allocation of dictyosomal volume in peripheral cells of flight-grown seedlings correlated positively with their secretion of significantly less mucilage than peripheral cells of Earth-grown seedlings. These results indicate that 1) microgravity alters the patterns of cellular differentiation and structures of all cell types comprising the root cap, and 2) the influence of microgravity on cellular differentiation in root caps of corn is organelle specific. This research was supported by a grant from NASA.

THE INFLUENCE OF MICROGRAVITY ON FORMATION OF AMYLOPLASTS IN COLUMELLA CELLS OF CORN. Randy Moore, Mark Fondren, & Colin Koon, Biology Department, Baylor University, Waco, TX 76798

Columella (i.e., putative graviperceptive) cells of corn seedlings grown inthe microgravity of outer space allocate significantly less volume to putative statoliths (amyloplasts) than do columella cells of Earth-grown seedlings. Amyloplasts of flight-grown seedlings are significantly smaller than those of ground controls, as is the average volume of individual starch grains. Similarly, the relative volume of starch in amyloplasts in columella cells of flight-grown seedlings is significantly less than that of Earth-grown controls. Microgravity does not significantly alter the volume of columella cells, the average number of amyloplasts per columella cell, or the number of starch grains per amyloplast. This research was supported by a grant from NASA.

ULTRASTRUCTURAL FEATURES OF A HUMAN THYROTROPIC PITUITARY ADENOMA. J.F. Greene Jr, MD, R.A. Turner, MS, J.A. Jackson, MD, Departments of Pathology and Endocrinology, Scott & White Clinic, Temple, Texas 76508.

Thyrotropin-secreting pituitary adenomas are rare (0.2% to 1% of adenomas). The first case documented by radioimmunologic, histologic, and immunocytochemical studies was published in 1976. Since then few adenomas have been reported with a detailed description of ultrastructural features. We report the electron microscopic studies of a thyrotropic pituitary microadenoma in a patient who presented with hyperthyroidism and autonomous hypersecretion of thyrotropin and alpha-subunit. Ultrastructural findings were those of a monomorphous tumor with long cytoplasmic processes. The nuclei were mostly uniform but a few were large and pleomorphic. Golgi-complexes were extensively developed. The size of secretory granules in most cells fell within the range of 100 nm to 300 nm.

EXTRACELLULAR ORIGIN OF LIPID DEPOSITS IN THE EARLY ATHEROSCLEROTIC CORE REGION SUGGESTED BY ELECTRON MICROSCOPY. Thomas A. Schifani, Thomas M.A. Bocan, and John R. Guyton, Dept of Medicine, Baylor College of Medicine, Houston, Texas 77030

The formation of the core region of small, raised, atherosclerotic lesions has been examined by electron microscopy. In addition to routine fixation and staining procedures, an osmium-thiocarbohydrazide-osmium (OTO) staining sequence was used to enhance visualization of extracellular lipid droplets. An abbreviated dehydration was employed to prevent excessive leaching of the lipid droplets from the tissue. These extracellular lipid droplets account for approximately 40% of the lipid-rich core volume. The lipid droplets were often found distinctly associated with elastin and/or collagen. Crystalline clefts suggesting cholesterol monohydrate were also observed. Upon stereologic analysis of the lipid-rich core components, marked reductions in the volume fractions of cells, reticular ground substance and basement membrane were observed, while the extent of extracellular lipid increased 7-10 fold. Monocyte-macrophage foam cells were prominent above the core region in the lesion cap, but were seldom found within the core region. Cellular lipid droplets were much larger (profile-diameters six-fold higher) than extracellular droplets. With these data as well as transitional morphologic features at the boundaries of the core region, it is suggested that 1) the abundant extracellular lipid does not derive from cell necrosis, and 2) lipid deposition in association with extracellular matrix constituents is an early event in the development of the lipid-rich core.

BABESIA BIGEMINA AND PARASITIZED RBC ULTRASTRUCTURE IN VITRO. R. E. Droleskey, P. J. Holman,* and G. G. Wagner,* U.S.D.A., Veterinary Toxicology and Entomology Research Laboratory, College Station, TX 77841 and *Dept. of Vet Microbiology and Parasitology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843.

Babesia bigemina parasitized red blood cells (RBC) isolated from a B. bigemina infected splenectomized calf were cultured under in vitro microaerophilic conditions used for the continuous culture of bovine babesia. The inital parasitemia of B. bigemina, when isolated from the infected calf, was 3.5%. After establishment, the cultures were routinely subcultured at a 1:5 split ratio every 48 h. After culturing for 10 weeks samples from a culture with a parasitemia of 3% were fixed and processed for examination using transmission electron microscopy. Examination of the RBC revealed B. bigemina merozoites with normal appearing ultrastructure located within RBC and some free in the surrounding medium. In addition, parasitized RBC possessed knob-like projections on their outer membranes. These projections were also present on the inner membrane of vacuoles located within parasitized RBC. This report will detail the fine structure of the observed B. bigemina and RBC membrane projections.

EFFECT OF WATER STRESS ON MESOPHYLL CELLS OF SORGHUM LEAVES. Jerry Berlin, Glenn Hoskins, Jill Tatum, Department of Biological Sciences, Dan Krieg, Department of Plant and Soil Sciences, Texas Tech University, Lubbock, TX 79409.

Stereological techniques were used to evaluate the effect of water stress on sorghum leaves. Field-grown leaves of Sorghum bicolor were collected the first of August, 1985 from irrigated and nonirrigated plants. The samples were initially fixed in glutaraldehyde and routinely processed for light and electron microscopic examination. Electron micrographs were obtained of mesophyll cells from three sites within the leaves: 1) adjacent to the upper epidermis overlying a vein, 2) in the middle of the leaf between veins, and 3) adjacent to the lower epidermis underneath a vein. Collectively, the mesophyll cells from the dryland plants contained a higher fractional volume of chloroplasts and lower fractional volume of mitochondria, peroxisomes, and vacuoles compared to their irrigated counterparts. Within both irrigated and dryland plants, the fractional volumes of chloroplasts and mitochondria in mesophyll cells were greater at the lower sites within the leaf. Mesophyll cells nearer the leaf surfaces were more highly vacuolated than those cells in the middle of the leaves. Peroxisomes in mesophyll cells appeared to be uniformly distributed throughout the leaves. Supported in part by the Water Resources Center at Texas Tech University.

CYTOCHEMICAL LOCALIZATION OF Na-K-ATPase IN THE INNER EAR. T. Yoshihara, C. D. Fermin, S. Usami, M. Igarashi. Dept. Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, TX. 77030

To determine the localization of Na-K-ATPase activity in the inner ear, K-p-nitrophenylphosphatase activity (K-NPPase) was studied ultracytochemically. Chicks (Gallus domesticus) and squirrel monkeys (Saimiri sciureus) were used for this study. To demonstrate K-NPPase activity, we applied the one-step lead citrate method developed by Mayahara et al (1980).

It is well known that the cochlear endolymph in birds and mammals shows the ionic composition of high K ions and low Na ions, which is essential to the auditory transduction process. Biochemically, the tegmentum vascularis (TV) and the stria vascularis (SV) are known to contain large quantities of Na-K-ATPase. Also, the ultrastructures of the TV, the SV and the vestibular dark cell (VDC) resemble those of the various ion-transporting epithelia such as the renal tubule cell or salivary duct cell. The present study revealed that Na-K-ATPase activity is localized in the dark cell of the TV, the marginal cell of the SV and the VDC. Reaction products were restricted to the cytoplasmic side of the basolaterally folded plasma membranes of these cells. The reaction was markedly reduced when the specimens were incubated in a medium containing ouabain or a K-eliminated medium by replacing K ions with Na ions.

It can be concluded that the dark cell of the chick TV, and the marginal cell of the SV and the VDC of the squirrel monkey play an essential role in the maintenance of the ionic micro-homeostasis of the endolymph.

PLATFORM PRESENTATION — FALL 1986

A survey of the silicon deposits in the leaves of Selaginella emmeliana using TEM, SEM and EDX analysis. R.W. Davis. Medical Anatomy, Texas A&M University, College Station, Texas.

Silicon (Si) deposits have been previously demonstrated in the large ventral leaves of Selaginella emmeliana using SEM and EDX analysis. The current study was begun to more specifically determine the nature of these deposits, using TEM, SEM and EDX analysis, in both the large ventral and smaller dorsal leaves.

Si was found to be present in epidermal projections called cuticular warts, some stomatal guard cells, and restricting or occluding some stomata. The presence of Si in the surface walls of epidermal cells was found to be zonal with a lower density in epidermal cells associated with a central strip on the abaxial side, containing stomata. Additional Si was found in cell walls separating contiguous epidermal and mesophyll cells, in the lumina and adhering to the inside surfaces of xylem vessels, and in spaces sometimes created where two or more cells are joined. Si was also found adhering to the air space surface of many mesophyll cell walls.

The results of this study agree with some of the conclusions previously described (e.g., Si in cuticular warts, some stomata, and zonal distribution) but do not corroborate others (e.g., Si primarily in abaxial cells and stomata only on abaxial surface). Si containing stomata appear to result from its accumulation within the guard cell walls, lumina, and apertures. This may be associated with passive transpiration and/or cell death. The reason for the large accumulation of Si in the cuticular warts is unclear as is the zonal distribution within the epidermis. Other patterns of Si deposition can possibly be explained by passive transpiration.

POSTER PRESENTATION — FALL 1986

ULTRASTRUCTURAL AND IMMUNOLOGICAL CHARACTERIZATION OF ISOLATED PICK FIBRILS FROM PICK DISEASE. K.M. Hammon, C.L. White, III, D.R. Sparkman, Departments of Internal Medicine, Pathology, and Neurology, University of Texas Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas, 75235.

Pick disease (PD) is a rare form of progressive degenerative dementia which resembles Alzheimer disease (AD) clinically, but is distinctively different in microscopic pathology. We have recently reported the first purification of the straight 15 nm fibrils (PF) that occur intraneuronally and give rise to a characteristic cytoplasmic inclusion known as the Pick body (PB). The PF have been examined ultrastructurally by negative staining and appear to be composed of straight and twisted forms with intermediate forms. The Pick fibrils (PF) were found to be insoluble in boiling SDS and variety of chaotropic salts, but were solubilized in concentrated formic acid. It was found that monospecific antibodies to the paired helical filament (PHF) of AD immunohistochemically labeled the PB. We have analyzed purified PF by double-diffusion gels, Western blotting, and colloidal gold labeling. This work was supported in part by a grant from Mrs. Dorothy Kirsten French of the John Douglas French Foundation for Alzheimer's Disease.

THE INFLUENCE OF MICROGRAVITY ON STRUCTURE OF COLUMELLA CELLS IN ZEA MAYS. Randy Moore, Mark Fondren, Eddie McClelen, & Chia-Lien Wang, Biology Department, Baylor University, Waco, TX 76798

We launched imbibed seeds and seedlings of Zea mays into outer space aboard the space shuttle **Columbia** to determine the influence of microgravity on the structure of columella cells of roots. In Earth-grown controls, the endoplasmic reticulum (ER) was localized primarily along the periphery of the cells, and amyloplasts sedimented in response to gravity to the lower sides of the cells. Seeds germinated on Earth and subsequently launched into space had a distribution of ER in columella cells similar to that of Earth-grown controls, but amyloplasts were distributed throughout the cells. Seeds germinated in outer space were characterized by the presence of spherical and elipsoidal masses of ER and randomly distributed amyloplasts. These results indicate that 1) columella cells can maintain their characteristic distribution of ER in microgravity only if they are exposed previously to gravity, and 2) gravity is necessary to distribute the ER in columella cells of this cultivar of corn. This research was supported by a grant from the National Aeronautics & Space Administration.

DEVELOPMENT OF IN VITRO SKIN MODEL. Cheryl Lamke-Seymour and Dr. Frederick Grinnell, Dept. Cell Biology and Anatomy, University of Texas Health Science Center, Dallas TX 75235

Freshly isolated keratinocytes from the skin of a rabbit's ear were placed on the surface of hydrated collagen gels that had been reorganized by human foreskin fibroblasts. The epidermal cells were organized into three distinct layers as seen by transmission electron microscopy. A well defined basement membrane was not observed. Indirect immunofluorescent studies of frozen sections showed bullous pemphigoid, pemphigus vulgaris antigens, and keratin present in the epidermal cell layers.

Other studies used a composite skin graft as the substratum for freshly isolated human keratinocytes. A composite skin graft was prepared from frozen, split-thickness allograft skin. After incubation in growth medium the epidermis could be removed from the dermis using forceps. The basement membrane region of the dermal portion of the allograft as well as the topographical features remained intact. The dissociated epidermal cells incubated on the separated dermal allograft reformed a highly differenciated tissue within one week as evidence by TEM. The reconstituted tissue appeared similar to normal skin in many respects. (Research supported by a grant from the NIH GM31321.)

DIFFERENTIATION AND MATURATION OF HUMAN MONOCYTES TREATED WITH PHORBOL ESTERS: MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATION.
C. Bucana, J. Williard, Y. Keisari.
Department of Cell Biology, University of Texas System Cancer Center, Houston, Texas 77030.

Phorbol esters are known to induce differentiation of myeloid cells to mature macrophages. The purpose of this study was to determine the effect of various doses of 12-O-tetradecanoyl-phorbol-1-acetate (TPA) on the morphology, function, and biochemical properties of normal human blood monocytes. Enriched suspensions of monocytes were obtained using Ficoll Hypaque gradient and the suspension was plated on 24-well tissue culture dishes in the absence or presence of various concentrations of TPA. Samples were removed at different times, washed with phosphate buffered saline, and examined for adherence. Parallel samples were processed for transmission and scanning electron microscopy, phagocytosis, tumor cell cytotoxicity, oxidative burst, and B-galactosidase assays. TPA-treated monocytes were more pleomorphic than the untreatd monocytes. Preliminary results showed that 1 ng/ml TPA enhanced adherence of human monocytes to plastic dishes 2 to 5-fold but higher doses of TPA (>4 ng/ml) were toxic to human monocytes. Increased adherence was associated with a decrease in the ability of monocytes to phagocytose either latex beads or lectin-coated erythrocytes and to respond in an oxidative burst. TPA-treated monocytes showed recovery of oxidative burst functions within 1-4 weeks post- treatment. The TPA treatment of adherent monocytes seem to result in more viable and functional culture for extended periods of time.

LOCALIZATION OF ACID PHOSPHATASES IN SOYBEAN PROTOPLASTS, R.D. Record, G.R. Aliaga, and L.R. Griffing, Dept. Biology, Texas A&M University, College Station, TX 77843.

A β-glycerophosphate substrate and a variation of the Gormori lead procedure were used for the histiochemical localization of acid phosphatases in soybean (Glycine max L.) protoplasts. This procedure was post-fixed by osmium. Optimal incubation conditions (temperature and time) for the substrate mixture were used. The protoplasts were made from a cell suspension culture. Both LR White and Araldite have been used as embedding material. Electron dense precipitate (reaction product) was seen in Golgi membranes, along the endoplasmic reticulum, in plastids, and in the cytoplasm, possibly associated with ribosomes. No reaction product was seen in the vacuoles, in contrast to previous results with other lytic enzymes. Further studies will be done to show sorting at the Golgi membranes of the lytic enzymes, like acid phosphatases, in relation to the final internal (the vacuole) or external (the extracellular media or cell wall) target.

THE ELECTRON MICROSCOPE AS A DIAGNOSTIC TOOL FOR SKELETAL DISEASE. J.E. Bilbao, M.D., R.A. Turner, R.T. King, and J.C. Stinson, M.D.

Striated muscle has a limited number of reaction patterns but these alterations and alterations in adjacent structures are of help in diagnosis.
Inflammatory: Fiber alterations, inflammation and viral-like inclusions.
Dystrophic: Duchenne's dystrophy showing fibrosis, degeneration and phagocytosis.
Neurogenic: Atrophic fibers are present but some architectural features remain.
Core Disease: The central core shown here shows disruption of normal architecture and a loss of mitochondria.
Mitochondrial disease: Mitochondrial abnormalities are of limited diagnostic value but are interesting biochemically and morphologically.
Metabolic: A case of carnitine deficiency with increase lipid and mitochondrial aggregates.
Congenital: A heterogenous group. Nemaline myopathy is the example shown here.
Miscellaneous: Variations seen in muscle diseases but without known significance are shown.

AN ELECTRON MICROSCOPIC ANALYSIS OF RESIDUAL ASBESTOS REMAINING IN PREPARATIVE VIALS FOLLOWING BLEACH DIGESTION
C. J. Corn, M. G. Williams, R. F. Dodson, Department of Cell Biology and Environmental Sciences, The University of Texas Health Center at Tyler, P.O. Box 2003, Tyler, Texas 75710

A question that concerns investigators using digestion techniques for the study of asbestos load in tissue is, "What percentage of the total fibers remains adhered to the walls of the preparative vials after digestion?" A test series was established which enabled us to define this residual fiber content following several variations in the rinsing procedure. The test material for all studies was a digested piece of lung tissue from a former asbestos worker. The digestion procedure used was based on the bleach digestion technique presented by Smith and Naylor (1). This tissue contained $3x10^8$ fibers and $8x10^5$ ferruginous bodies per gram and had a fiber per ferruginous body ration of 37.5. Aliquots (1.5ml) of the digest were used to create reproducible standards. These were placed in clean polystyrene blood dilution vials that were previously cut to fit in an AMRAY 1000A scanning microscope interfaced to a Tracor Northern 1710 x-ray energy dispersive analysis system. The aliquots, each containing $8.4x10^6$ fibers and $6.8x10^4$ ferruginous bodies, were allowed to remain in the vials for 10 min before rinsing was initiated. The variables studied were: (1) duration of digestion (30 min or 24 hrs) and (2) type of rinsing solution used (distilled water or additional bleach followed by distilled water). The maximum errors encountered were 0.5% for fibers and 0.01% for ferruginous bodies and both of these occurred after 24 hrs of digestion.

M. J. Smith and B. Naylor, Am. J. Clin. Pathol. 58(1972)250.

IMAGING AND ANALYTICAL CAPABILITIES OF A TEM WITH AN INTEGRAL IMAGING ELECTRON ENERGY SPECTROMETER.
W.I. Miller, III; Carl Zeiss, Inc., Thornwood, NY 10594.

Recently an electron microscope with an electron energy spectrometer integrated into its imaging path was introduced. This instrument allows very precise selection of both the energy level and the energy band width of the transmitted electrons. This capacity gives the microscope some unique or greatly improved imaging and analytical capabilities.

Biological material and embedding media often have similar chemical composition and thus exhibit poor contrast in unstained or lightly stained sections. By appropriate selection of the energy of the electrons excellent structure-specific contrast can easily be achieved. As section thickness increases so does the chromatic aberration of the image. Resolution is partially related to the ratio of the energy lost to the accelerating voltage. The traditional approach to resolution in thick sections has been to increase the accelerating voltage. Now, by limiting the band width of the electron energy loss we do not have to resort to high voltage to get good resolution with thick sections.

From the analytical standpoint by selecting the energy of the imaging electrons we can provide an undistorted, high resolution elemental image. By adding an electron detector below the spectrometer electron energy loss spectra are easily obtained. The spectrometer has a resolution of 1.5eV and a jump ratio at the carbon K edge of greater than 20. Iron spectra have been recorded from a single ferritin molecule and with appropriate image processing as little as 10E-21 grams can be imaged.

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A DISCUSSION OF RECENT DEVELOPMENTS IN SEM ELECTRON OPTICS
S.S.Spiers, International Scientific Instruments, Milpitas, CA 95035

There are three basic parameters which limit the performance of an electron lens.Spherical aberration, Chromatic aberration and Astigmatism.
Spherical and chromatic aberration cannot be corrected, however, their effects are reduced by using a high strength lens combined with specific pole piece geometries. It is also important to have high stability lens current and voltage supplies.
Astimatism may be corrected by using either the vector principle to orient a magnetic field at right angles to the residual astigmatism or the use of seperate controls to change the magnitude and azimuth of the correcting field.
An additional problem associated with a magnetic lens is the inability to contain the field within the lens. Magnetic field leakage has been reduced to a minimum using an asymetrical pin hole lens design.
There are some disadvantages to this design which come principally from the diameter of the lower segment.This often prohibits large diameter samples from being tilted to high angles at short working distances.
The need for a more conically shaped lens has become apparant so that large diameter samples could be tilted to high angles at a short working distance. However increasing the cone angle of the lower pole piece segment creates other problems.
The continual need to image at lower and lower accellerating voltages, at high resolution, has been responsible for the development of a symetrical wide bore lens which facilitates through the lens signal detection. This technique increases signal detection efficiency which is particularly important at low voltage. It also enhances true surface imaging.
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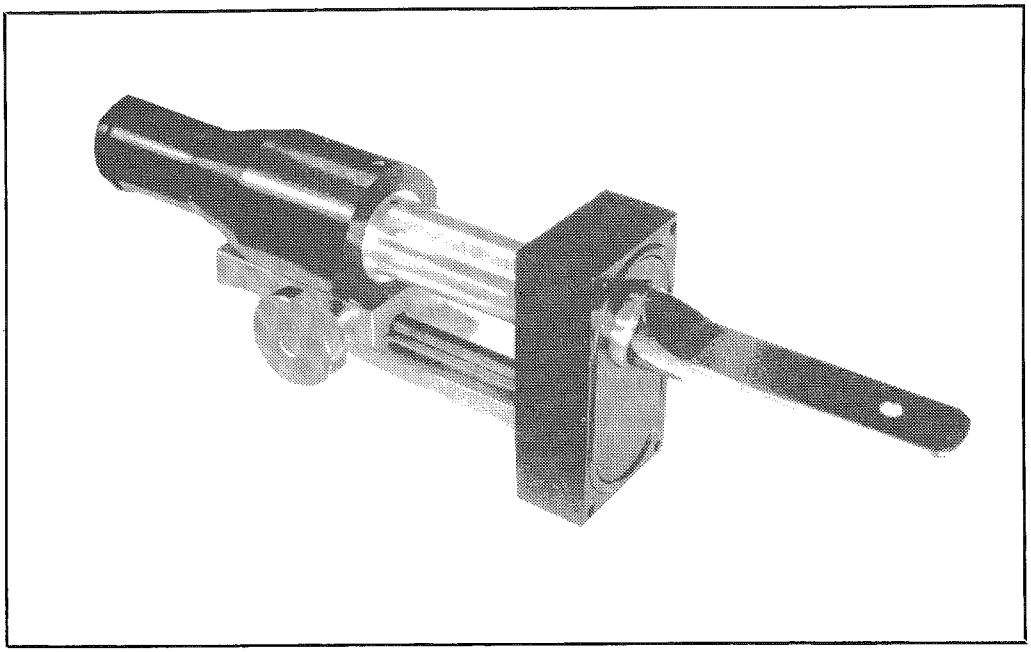

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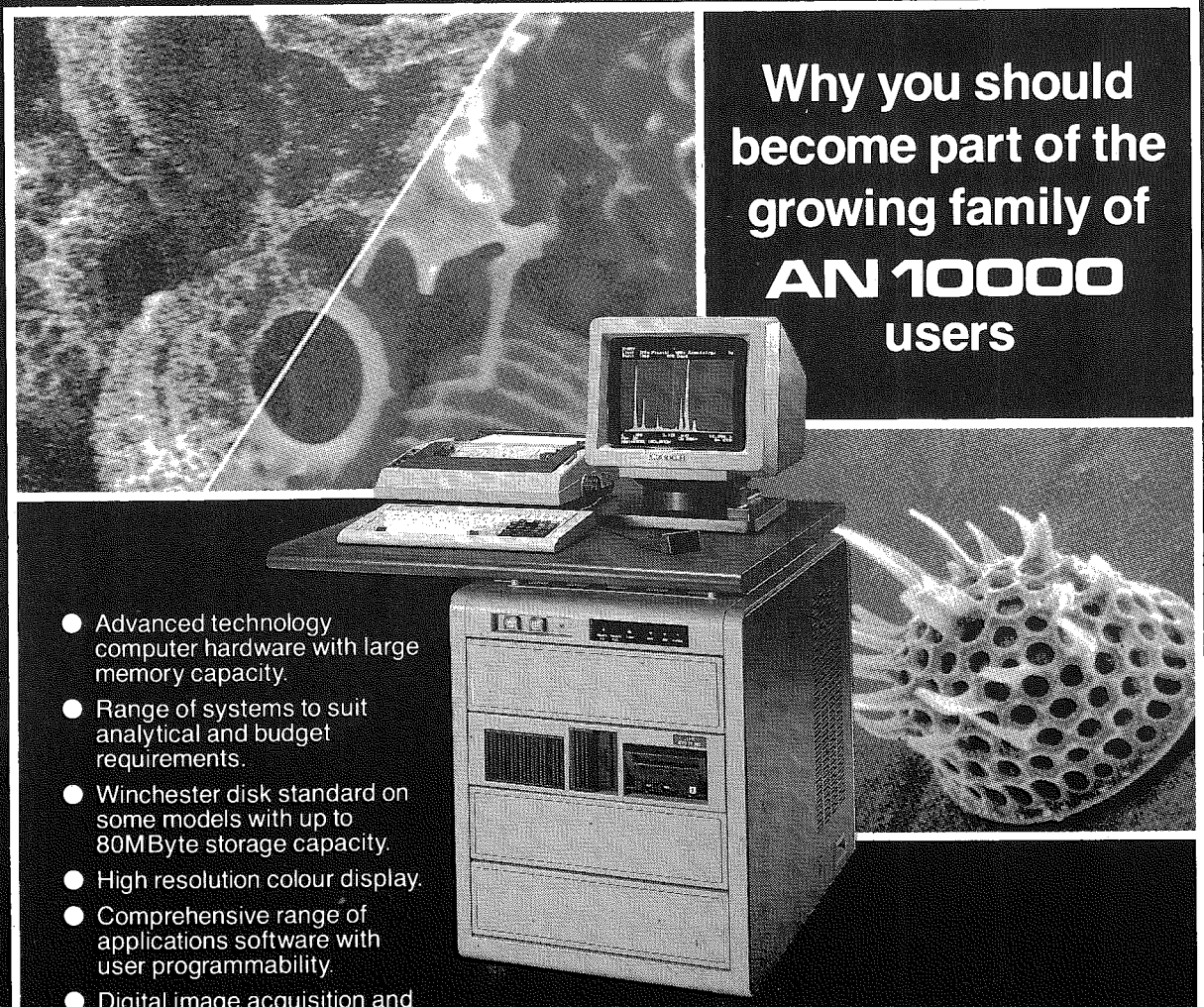

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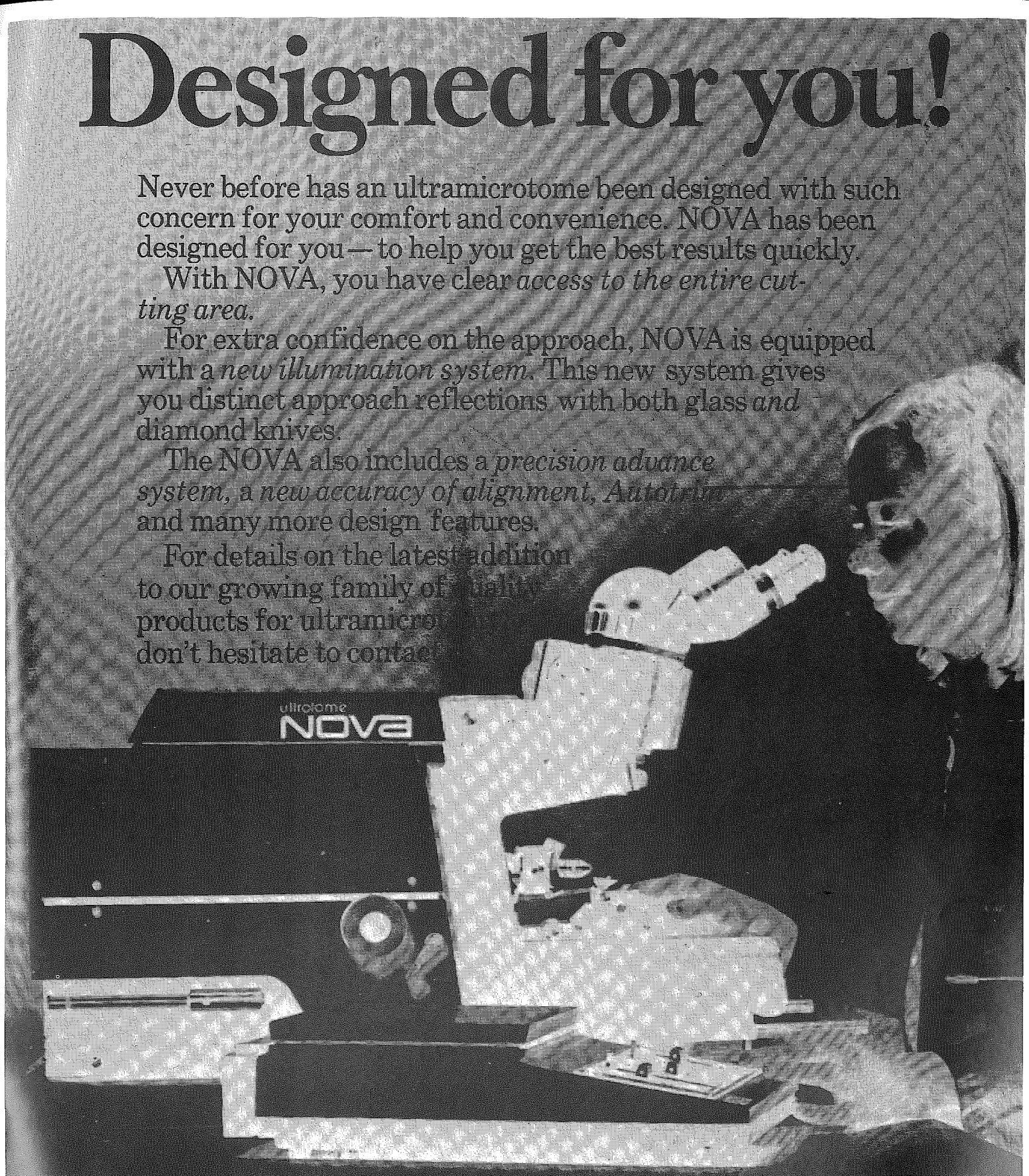

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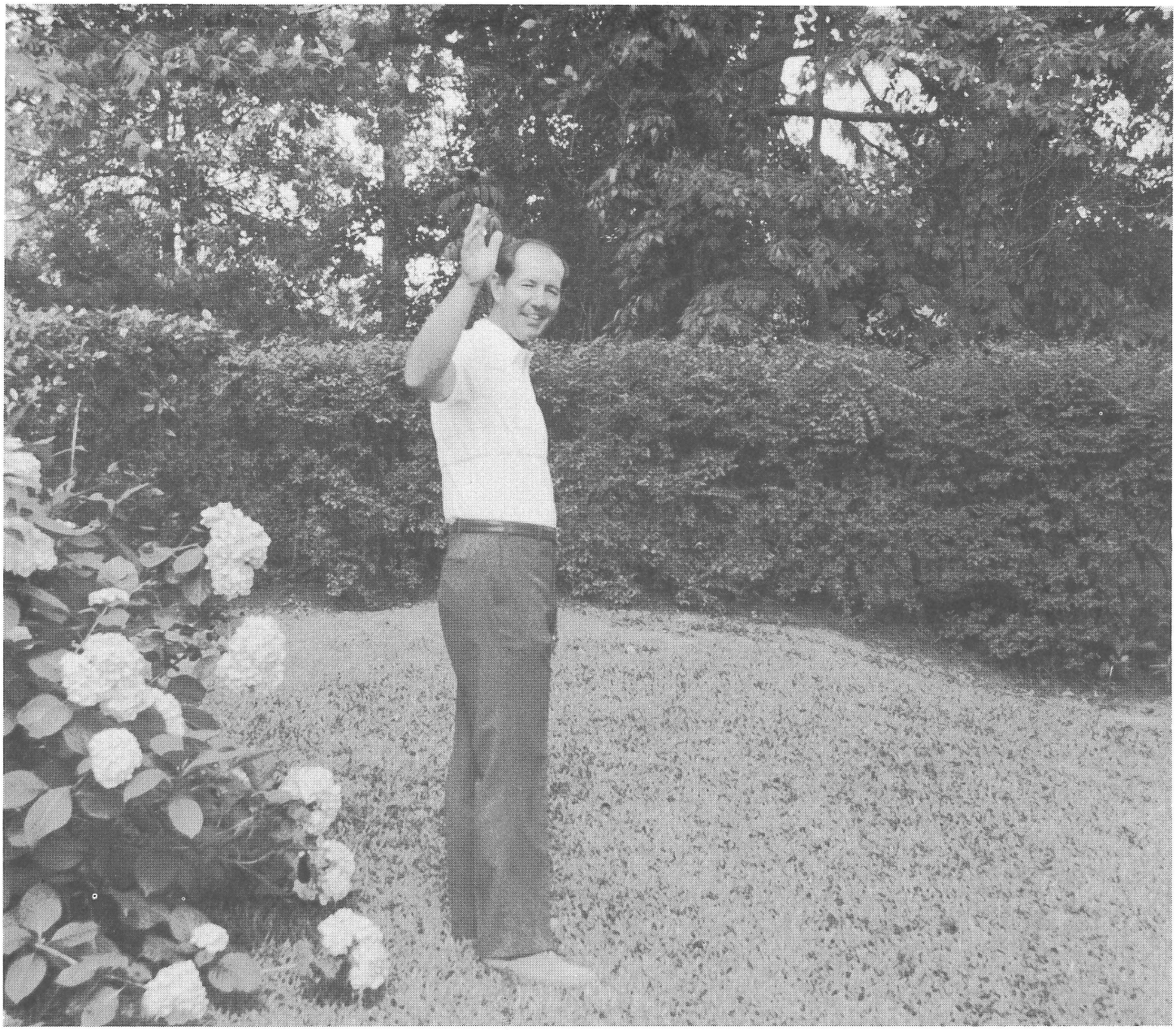

Good-bye and Good Luck to Dr. Charles Mims from all the TSEM Members.

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