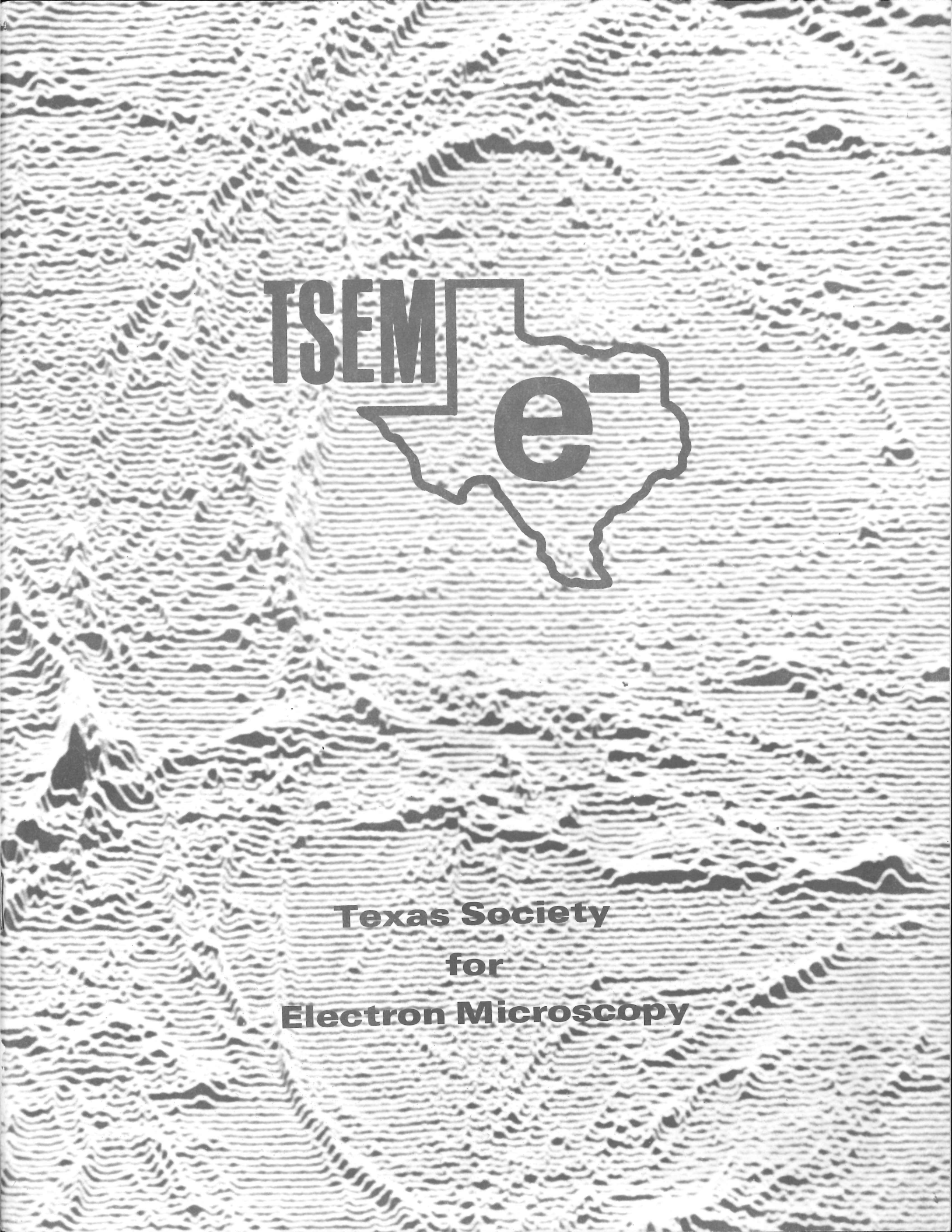
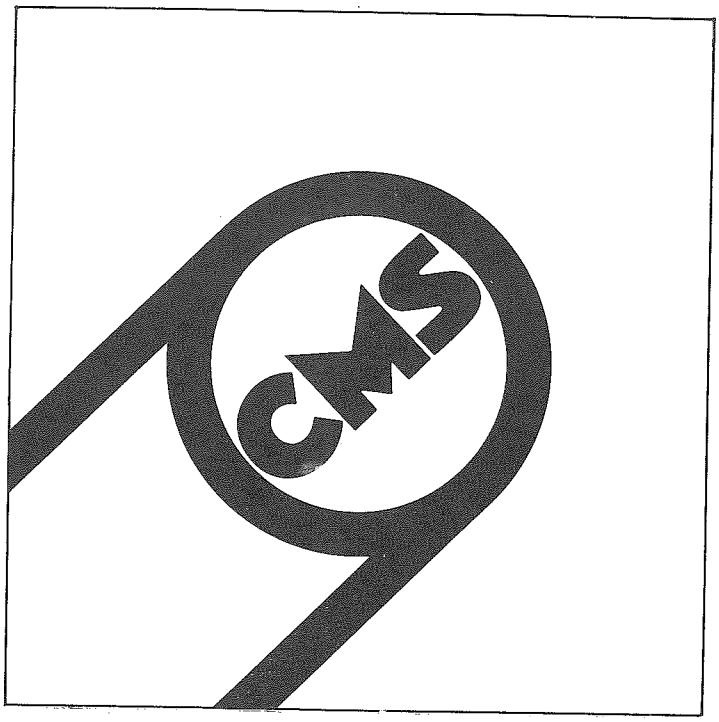


Texas Society for Electron Microscopy



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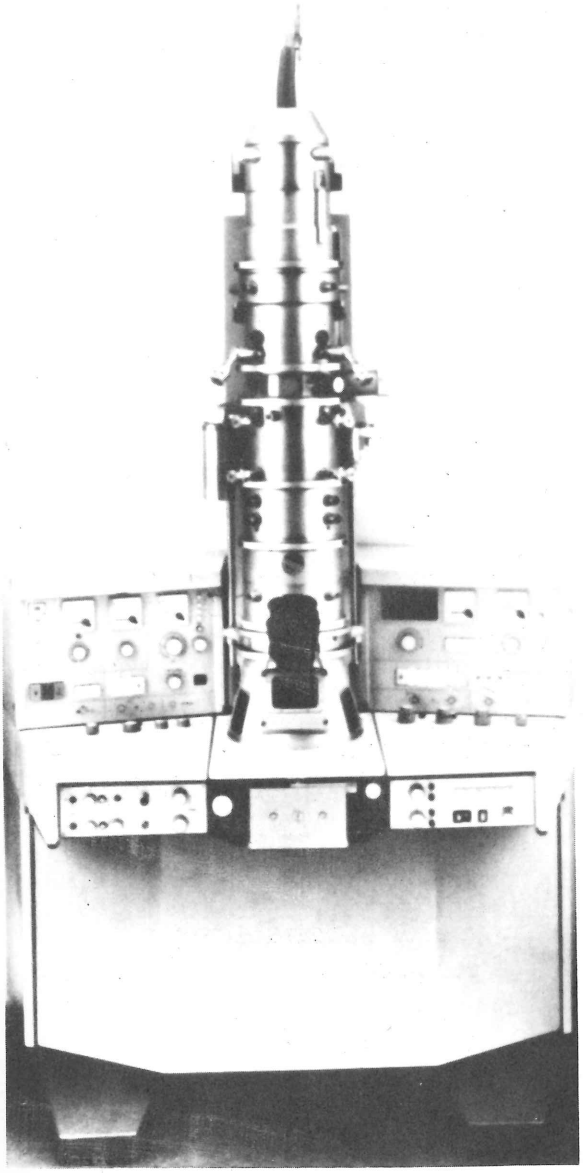

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TEXAS SOCIETY

for

ELECTRON MICROSCOPY

Volume 4 Number 2

Spring 1973

Letters and Inquiries to: Dr. C. Ward Kischer
Editor, T. S. E. M. Newsletter
Department of Anatomy
U. T. M. B.
Galveston, Texas 77550

Current Membership Strength: 285 Individuals
20 Corporations

WHITHER EDUCATION?

Part I

"There is only one thing left to say,
Up against the wall! This is a stick-up."

Mark Rudd, in a letter to
President Grayson Kirk during the
spring rebellion at Columbia in 1968.

I have asked myself many times, why should I write this, expecially since anyone with at least half a brain realizes what education is all about. It's just common sense. But, I finally decided that it should be said, and many times over. For, common sense is not always appreciated, nor practiced. There are a few activist or restless types who beat the bushes, stirring up the natives, for nefarious purposes, and who suddenly seem to vanish when the action becomes serious. True perspectives become lost for a time. And I think that, in many ways, education and educational institutions have lost their way.

One of the co-leaders, along with Rudd, also said, "If we don't have enough strength to destroy the nation, by God we can at least destroy Columbia."

What were these so-called students there for? Even if they knew, did the administration know? After the Kent State affair a high ranking KSU official stated, "The methods of these militants are extreme, but isn't it wonderful these students are committed?" YES! COMMITTED! That's what he said. Let's hear further from Mark Rudd: Almost a year after the Columbia affair, Rudd appeared on the David Susskind television show. Susskind kept asking Rudd throughout the program, ". . . but WHY did you do it?" After several attempts by Rudd to answer in Susskindese, he finally said with utter disdain, "Man, don't you understand, WE DID IT FOR THE HELL OF IT!"

And thereby hangs the tale. It is rather easy to lose sight of the real purpose of education and its institutions, especially when confronted with such activities as football frolics, spring proms, free speech movements, Give-Your-Blood-To-The-Viet-Cong club meetings, petitions for better nutrition (kill a pig for breakfast), etc.

Just what is education, and just what is the role of educational institutions in our society? It occurs to me that formal education, state sanctioned and state supported (or federally sanctioned and federally supported) must include some benefit for the state (or society), perhaps a gain of civic or social responsibility. Simply put, Aristotle said education is what makes men good (and that is good for the ociety). Josiah Royce said education is learning to use the tools which the human race over the centuries has found to be indispensable in the pursuit of truth. Webster says education is the imparting of knowledge and skill, especially through

systematic instruction or training. Well, maybe we have some idea of what education should be. But, what of the institutions established to achieve this? How is it to be done?

Recently, the New York Times sponsored a round table discussion at which it asked "What should a university be, what do you regard as its mission?" Dr. Kingman Brewster, President of Yale, answered:

> Well, I will evade the question because I think education and educational reporting suffers [sic] from excessive generalization and stereotypes. Each university should be what it is best cut out to be and I think that we spend too much time looking over our shoulders and making comparisons with everybody else. I really think the single model expectation is, as De Carlo [President of Sarah Lawrence College] has said, not only the competition of the marketplace, but its dispersion. It's a federal system. It's a dispersion in the Brandeis sense; it's a dispersion of initiative which encourages quite different styles, purposes, traditions.

My answer to that is, where was Dr. Brewster when we needed him -- at the Paris Peace Talks about five years ago? So much for Peter Palaver.

There is a basic fabric of society, part of which is its educational institutions. Those institutions train people, educate them to use tools for the good of progress of society. The trouble is our restless adversaries attempt to redefine our society every day.

What really confounds me is that in this country, more than 350 years of educational practice seems to have been forgotten. Under the guise of innovations (code word) administrators, and some activist faculty and students have promoted and instituted the following: "new" curricula, integrated courses, Open Admissions, student membership and voting privileges on faculty committees, free universities and Universities of Free Thought. Curricula, for example, are revised as though the earth had suddenly captured a new moon. Eureka! They pursue such tasks sine qua non. Much ado about nothing! But, why do they do it? Perhaps they have not had the opportunity to feel important enough. As Shakespeare said:

> . . . if a man do not erect in this age his own tomb ere he dies, he shall live no longer in monument than the bell rings and the widow weeps . . . an hour in clamour, and a quarter in rheum; therefore, it is most expedient for the wise to be the trumpet of his own virtues . . . .

There is nothing mystical about higher education, nothing inherently divine which guarantees changing a boy into a man, an ignorant plodder into an excellent

achiever, a follower into a leader. No innovative curriculum is going to do this. Not even a computer can be something it is not intended to be -- GIGO -- garbage in / garbage out. To coin an ancient phrase: You get out of something just what you put into it.

The student encumbers himself with the responsibility for learning. By the time he or she reaches the higher level institutions it is assumed the skills and discipline to achieve that goal have already been demonstrated. Graduate and professional schools are not the places for remedial course work.

What is the most appropriate, the most effective method of learning for the student -- or, for that matter, of teaching for the instructor? If I do not know (but I claim I do), I do know what it is not! It is not sitting in a crowd of 200 or more warm bodies who rattle paper, scratch pens, cough and whisper while trying to hear and write every word being said.

What is really effective is a personal interaction, the student with his books and library, the instructor with the student. I have thought of my undergraduate college days and asked myself what professor, if any, had a significant effect on my four year experience. I am somewhat ashamed to say, none. That is not to say the professors were not competent or interesting. As a matter of fact, the one professor who was the most popular in school, not only with the students, but also with the administration, who I thought was a "great" person, is the one I remember the least. The one whom I now appreciate the most was one selected by many students for ridicule because of his unusual mannerisms. The attitude of the administration was reflected by their refusal to promote him from assistant professor rank after more than twenty-five years of service to the university.

It was, in fact, in graduate school where the opportunity lay for a real personal interaction between teacher and student. My major professor <u>educated</u> me. I learned specific skills and along with them, examples of how to distinguish whether those skills were useful as tools for certain inquiries. It was a one-to-one situation, much like a piano teacher and student. If the latter hits a wrong key, he gets his knuckles rapped. A couple of times around and one learns to avoid the wrong keys.

Notice I said I learned examples of how to distinguish. In other words, education to me was the opportunity to claim the proof of what I was and could be.

The Coleman Report, "Equality of Educational Opportunity" (1966), has wagged tongues, and tails for that matter, when it generally concluded that scholastic achievement of students is nearly independent of quality of schools. But the message went better than that. What it really said was that the school, no matter what kind it is, is the unit of expression for the substance which makes up an individual. Simply put, it's genes that count!

My own senses tell me that education is a matter of direction and speed.

To be a good teacher you have to direct students with authority and confidence (and often direct them to the front steps of the library). You also must have the ability to detect at what speed each student moves best.

The function and responsibility of the teacher or professor, therefore, is clearly put, and set apart from that of the student. That is why I do not understand this stuff about "rapping". The students want "rap" sessions with professors. They want them to talk the language of the students. Nonsense! I believe in a generation gap. The teacher is employed to direct student traffic, not just for one or four years, but for the duration of his career. The student is a transient, temporarily taking advantage of the graces of the state and depending on the skills and knowledge of the faculty. The latter have the responsibility of maintaining their position of experience and discharging their obligations as the administration allows with the students in the most individual way possible.

John Dewey's concept of the well-rounded individual has put a blight upon the American education experience for too long. It has smothered the one really essential opportunity for each person in our society, that is, the opportunity to find one's niche. It is not important to be smarter than everyone else (we cannot be), nor to be richer (who would be poor), nor to be higher ("Let them eat cake."). It is important to be comfortable and confident in your sense of responsibility, secure in knowing that what you do you will do well, better than a great many others would do.

> Training is everything. The peach was once a bitter almond; cauliflower is nothing but cabbage with a college education.
>
> Mark Twain,
>
> in The Tragedy of Puddn' head Wilson

Ward Kischer

Editor

AU REVOIR. AUF WIEDERSEHEN. ALOHA. FAREWELL.

With this issue of the Newsletter, I am resigning as editor. I have occupied the post for four years, and I think that is long enough. It is especially so since the editor is appointed and not elected.

The Newsletter will continue, I am sure, with a new editor. I urge each and every member to support him to your utmost. You are, in effect, supporting the Society when you do this.

It has been a most rewarding experience, and much fun, really! The editorials have given me the opportunity for a little spleen-venting, some chastising, but also some praises. To all those who have contributed to the Newsletter, I thank you. I believe the most important thing the membership can do is communicate, even if it is a bit of sabre-rattling. The Society has well over 300 members, but less than one-third usually attend each meeting. How are we to know what the other two-thirds think or feel when we do not see them? Therefore, use your Newsletter!

This does not end my personal contributions to the Society. As long as I am a member I will continue to support T. S. E. M. in every way I can, whenever able.

See you at the meetings.

Ward Kischer

PRESIDENT'S MESSAGE

The Texas Society for Electron Microscopy begins its ninth year of a healthy existence thanks to the various aspects of support by the membership, individual and corporate, and by the impressive row of guest speakers high-lighting our meetings. The last one, held together with the Louisiana Society for Electron Microscopy in New Orleans, is proof that the idea of joint meetings is a good one. I would like to thank our friends in Louisiana, particularly President Robert F. Dyer and his Executive and Symposium Committees, for the organization, hosting and success of this fine meeting.

Before I leave this column to your next President, Robert A. Turner, I should point out to you the diligent efforts of the T. S. E. M. Executive Committee which worked so hard for your benefit (there was nothing left for me to do). Our Newsletter Editor, Ward Kischer, has informed me -- to my regret -- that this issue will be his swan song. Thank you, Ward, for a job well done.

Dimitrij Lang

President

TREASURER'S REPORT

A Note of Thanks

Having served as Treasurer for the Texas Society for Electron Microscopy was a rewarding experience, and it was, indeed, a personal priviledge to have had the opportunity to actively engage and participate in the affairs of a very progressive and professional society. I would like to thank my fellow officers as well as member colleagues and corporate friends with whom it has been a pleasure to work.

Joe A. Mascorro

Treasurer

TREASURER'S REPORT

TEXAS SOCIETY FOR ELECTRON MICROSCOPY

Through 31 March 1973

Total Assets:

Balance on hand	$2,420.06

Allocation of Funds:

A.	Bank Accounts:	
1.	University National Bank of Galveston	820.14
2.	1 st National Bank of Garland	150.00
B.	Savings Accounts:	
1.	U. T. M. B. Credit Union of Galveston, Savings Account Number 00996	389.92
2.	U. T. M. B. Credit Union of Galveston, Certificate of Deposit Number 9996	1,060.00
		$2,420.06

FINANCIAL REPORT

T. S. E. M. Spring Meeting

Huntsville, Texas

19-20 May 1972

Income

A.	Dues and Registration	$ 743.00
B.	Sam Houston State University: donation	250.00
	Total Income	$ 993.00

Expenses

A.	Gary Powel: music (reimbursed by AMR Corp.: Mr. Tom Baum)	50.00
B.	Woolum Florists, Huntsville, Texas	15.75
C.	Dr. Terry Hoage: registration desk supplies	32.68
D.	Jean-Paul Revel: Honorarium and expenses (donation from Sam Houston University used here)	255.00
E.	Plaque presented to Jean-Paul Revel	15.00
F.	Huntsville Holiday Inn:	
	1. Meeting rooms	62.00
	2. J.-P. Revel: food	4.60
	3. Luncheon	255.00
	Total Expenses	$ 690.03

FINANCIAL REPORT

T. S. E. M. Fall Meeting

San Antonio, Texas

27-28 October 1972

Income

A.	Registration	$ 511.00

Expenses

A.	Dr. Robert V. Blystone, Local Arrangements Chairman: for registration desk supplies, phone calls, boat rides and social hour expenses	154.33
B.	Hilton Palacio del Rio Hotel:	
	1. Luncheon plus tax and gratuity	306.00
	2. Morning coffee and danish rolls (reimbursed by Reichert: Mr. Howard Hayden)	41.41
	3. Afternoon coffee and cokes (reimbursed by AMR Corporation: Mr. Tom Baum)	40.32
	4. Social Hour: cocktails and dips	270.60
		$ 812.66

Contributions

A.	JEOL, Incorporated: Mr. Bob Steiner	383.60
B.	Reichert: Mr. Howard Hayden	see above
C.	AMR Corporation: Mr. Tom Baum	see above
D.	Newsletter add payments	
	1. JEOL, Incorporated	50.00
	2. Van Waters Rogers	50.00

FINANCIAL REPORT

Additional Expenses for the 1972-1973 Membership Year

Through 31 March 1972

A.	University of Texas Medical Branch Print Shop: Newsletter expenses and printing	$ 172.70
B.	Norm Granholm: Newsletter typing	26.25
C.	H. Fernandez-Moran: guest speaker honorarium	50.00
D.	Edward W. Dempsey: guest speaker honorarium	50.00
E.	John H. L. Watson: guest speaker honorarium	50.00
F.	Department of Anatomy, Tulane University Medical School: postage expenses and phone calls by Treasurer	28.94
G.	Margaret Hightower: for art work done on T. S. E. M. membership certificates	15.00
H.	Department of Anatomy, Tulane University Medical School: postage expenses and phone calls by Treasurer	27.27
I.	Department of Anatomy, University of Texas Medical Branch: phone calls by Newsletter Editor	40.00
J.	Department of Anatomy, Tulane University Medical School: postage, xeroxing, phone calls	20.20
		$ 480.36

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CONTRIBUTIONS OF ELECTRON MICROSCOPY TO THE STUDY OF LYMPHOCYTES

The lymphocyte has, over the past few years, attracted considerable attention resulting in a multi-faceted approach to unraveling the mysteries of its function. This rather simple appearing cell has been found to profoundly influence such areas as infection, oncology, autoimmunity and transplantation. There is considerable evidence to indicate that cells recognizable under the light microscope as lymphocytes represent a heterogeneous population of cells. This heterogeneity involves both structure and function.

Functionally, lymphocytes may be divided into two separate areas, one referred to as T-cells, the other B-cells. T-cells, or thymic-derived lymphocytes, are lymphocytes that are responsible for cellular immunity. These cells are derived from stem cells of the bone marrow which are modified by either a hormone-like thymic factor, and/or the microenvironment of the thymus and converted into an immunocompetent cell which serves as the body's primary defense against the development of neoplasia and certain infections by fungi, viruses and Gram-negetive micro-organisms. This is also the cell that causes considerable difficulty in transplantation, resulting in cellular rejection.

The B-cell is a lymphocyte derived from a bone marrow stem-cell which is also modified in some way to make it immunocompetent. These cells differ from T-cells in that when they are stimulated they develop into plasma cells and produce immunoglobulins. These immunoglobulins (G, A, M, D, and E) provide the body with specific antibody functions.

Examination of the light morphology of lymphocytes in peripheral blood, or even in lymphoid tissues, is very deceptive. With this type of examination the cells all appear very similar, generally yielding no indication of their functional capacity. For this reason considerable effort has been expended to derive techniques to distinguish between B-cells and T-cells. This would be of considerable importance in investigating the function of these two cell lines and in determining abnormalities, either quantitative or qualitative involving one or the other cell types. These methods are, as yet, not completely satisfactory, but do show considerable promise. They may be classified into several categories based on differences in characteristics of the cell line. Some of these characteristics appear to be species specific.

1. Presence of distinctive surface antigens.

In mice the theta (θ) alloantigen has been found to be a surface antigen marker for T-cells, distinguishing them from B-cells. Unfortunately this marker is limited to mice. In humans the θ antigen is not present.

2. Rosette formation.

It has been found that if sheep erythrocytes are added to a suspension of peripheral blood lymphocytes, rosettes will form. These rosettes consist of three or more sheep erythrocytes attached or stuck to the surface of a lymphocyte. These rosette forming lymphocytes represent T-cells. This phenomenon has been demonstrated to exist in man as well as in animals.

3. Presence of surface immunoglobulin.

The presence of immunoglobulin on the surface of certain peripheral blood lymphocytes has been shown in a variety of animal species, including man. That this marker distinguishes B-cells from T-cells is now widely accepted. This marker is equivalent to the θ antigen marker in the mouse which identifies the T-cells.

4. Presence of membrane receptors for complement.

A population of lymphocytes has been found in many mammalian species, including man, which have surface receptors for modified C3 (the third component of complement). These cells appear to be B-cells. It is important to note, however, that all B-cells do not have surface receptors for complement.

5. Ultrastructural morphology.

There is considerable evidence to suggest that functional-morphological correlations for lymphocytes may exist. These correlations have been shown to exist in the avian species. In this group, two populations of large lymphocytes exist; these resemble each other very closely in all morphological parameters except that one has an aggregate ribosomal pattern and the other a dispersed ribosomal pattern. The former cell has been shown in this species to be a B-cell and the latter a T-cell. These correlations have not been confirmed in other species; however, evidence supporting this correlation has been presented.

Figure 1a: Dispersed ribosomal pattern (T-cell).

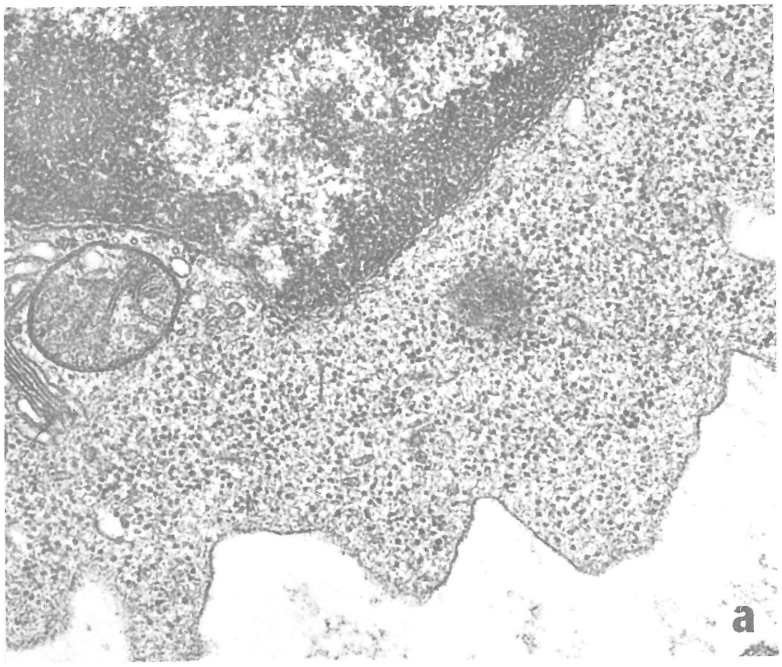
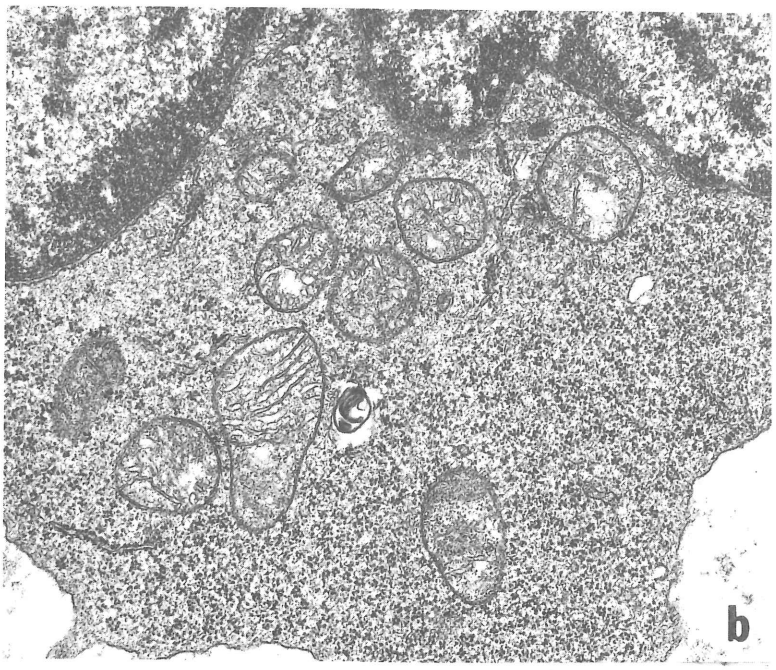


Figure 1b: Aggregate ribosomal pattern (B-cell).



In the calf these same two populations of large lymphocytes are present (Figure 1). A continuous spectrum of cells ranging from the large lymphocyte with aggregate ribosomes to a large lymphocyte with aggregate ribosomes and varying amounts of rough endoplasmic reticulum to a cell with the morphological characteristics of a plasma cell is present in thoracic duct lymph (Figures 2, 3 and 4). This spectrum of cells, with no cell intermediate between the lymphocyte with aggregate ribosomes and the lymphocyte with dispersed ribosomes, suggests that this represents the developmental sequence for this cell line. A similar picture has been seen in the thoracic duct lymph of sheep.

Figure 2: Spectrum of cells ranging from a lymphocyte with only aggregate ribosomes and only scant rough endoplasmic reticulum (a) up to a cell containing aggregate ribosomes and considerable rough endoplasmic reticulum (d).

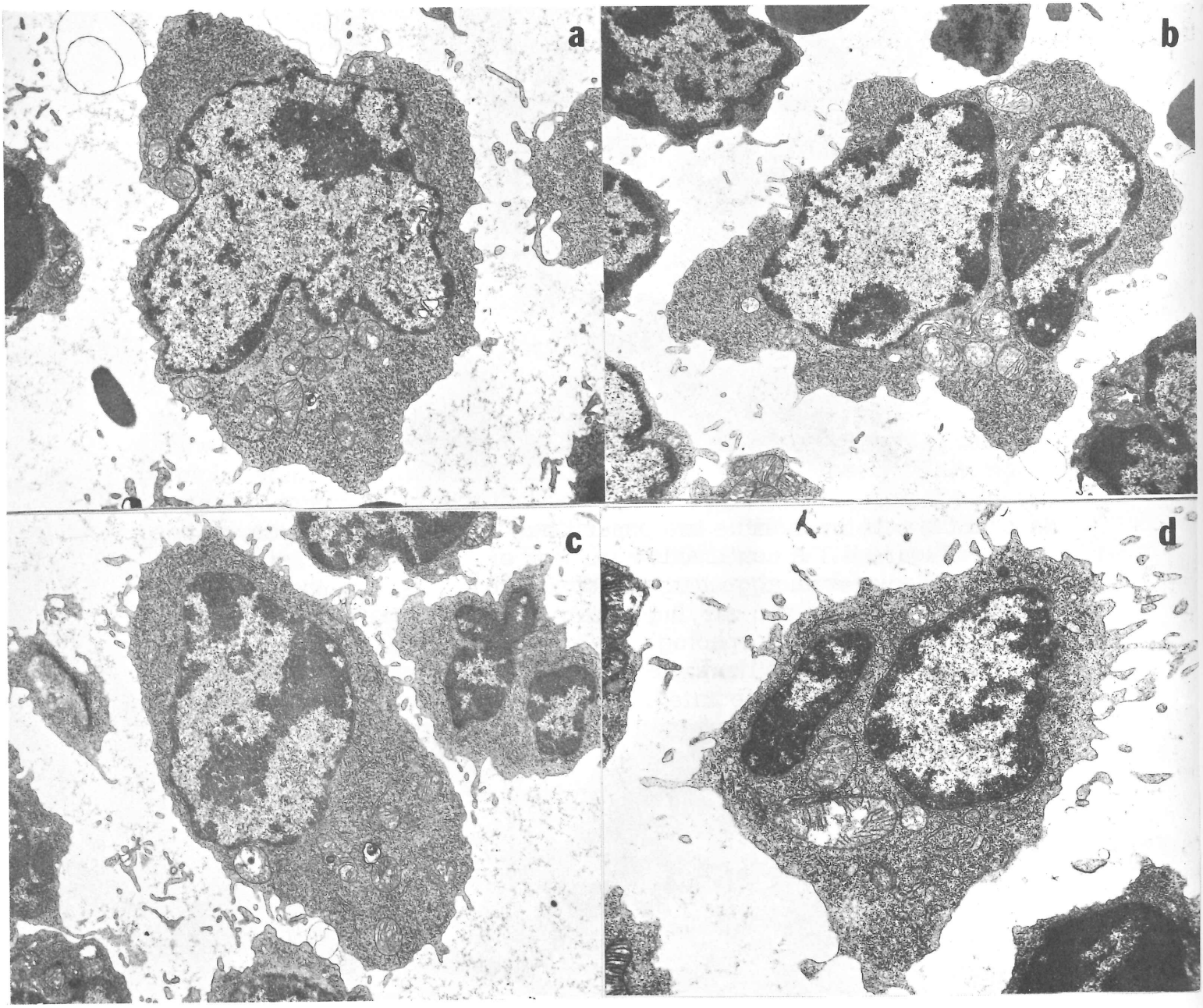


Figure 3: Immature plasma cell.

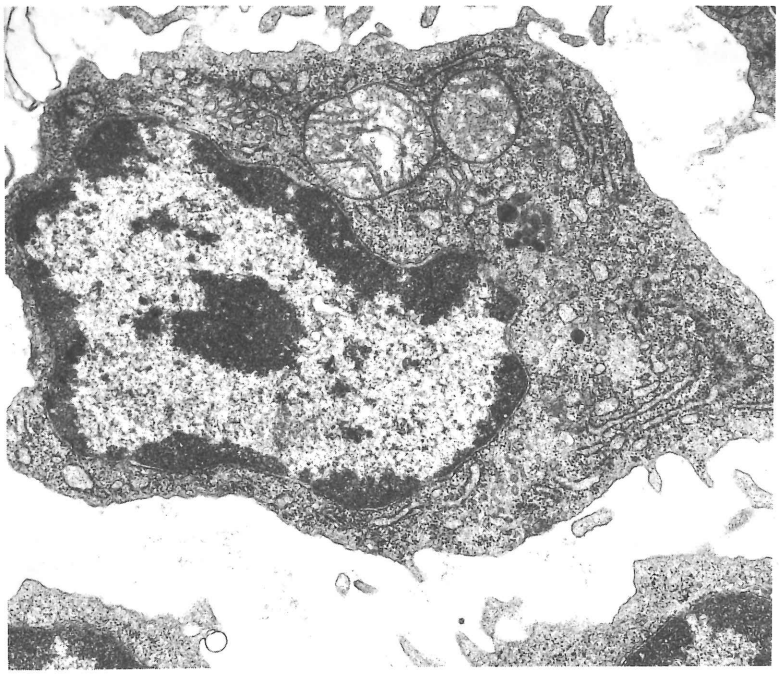
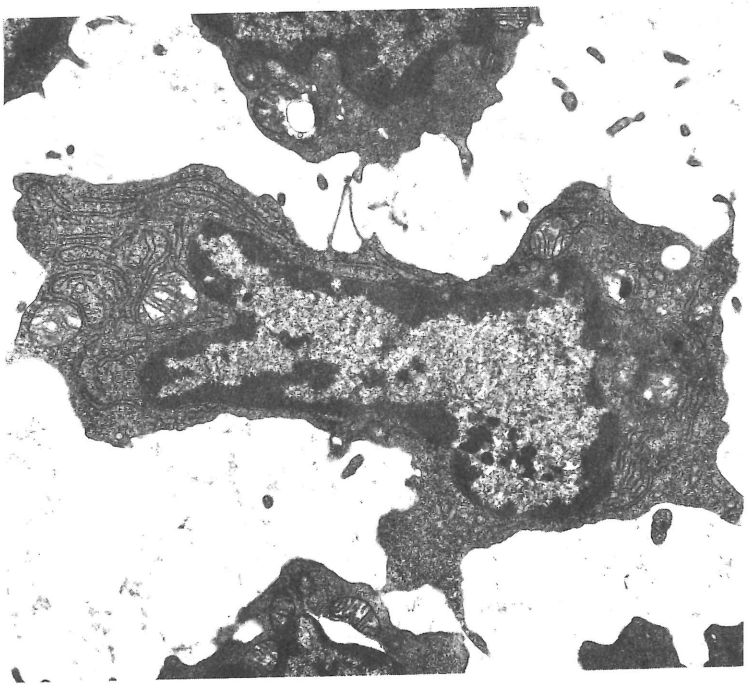


Figure 4: Mature plasma cell.



In man lymphocytes of the avian bursal-derived morphology have been found to be absent from the lymphoid tissue of a patient with x-linked agammaglobulinemia (a condition characterized by a lack of immunoglobulin and an absence of B-cell function). Also in man the same cell populations described in the chicken and the calf can be recognized, suggesting the same conclusion (see below).

In normal individuals we have recognized the presence of five different cell types based upon ultrastructural morphology. By examining the peripheral blood of normal donors we have established a normal profile for these cell types as follows:

Figure 5: Small lymphocytes - 81.2 $\pm$ 3.3%

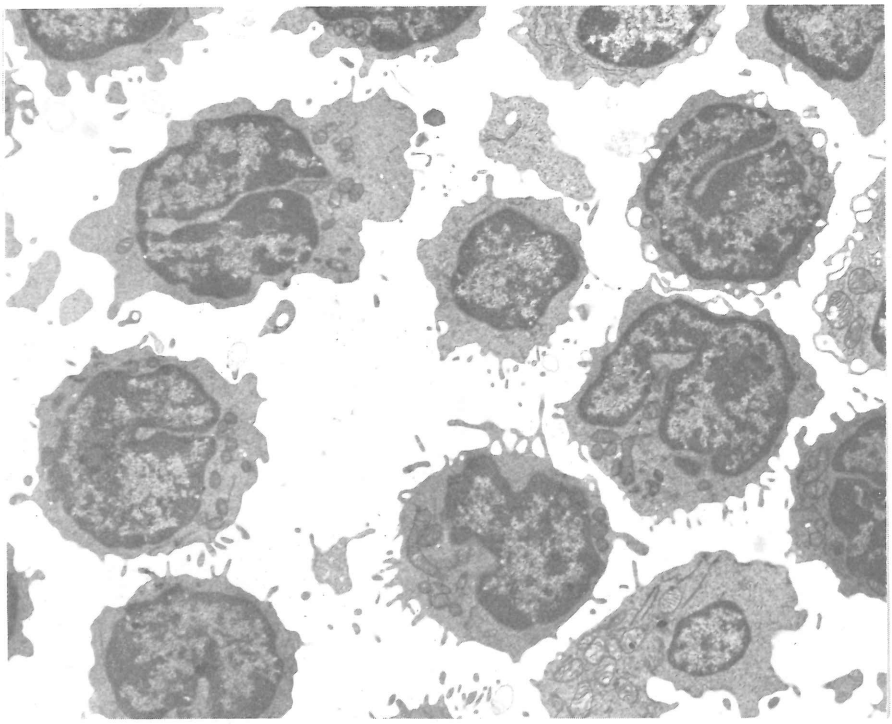


Figure 6: Large lymphocytes with dispersed ribosomal patterns 8.7 ± 2.8%.

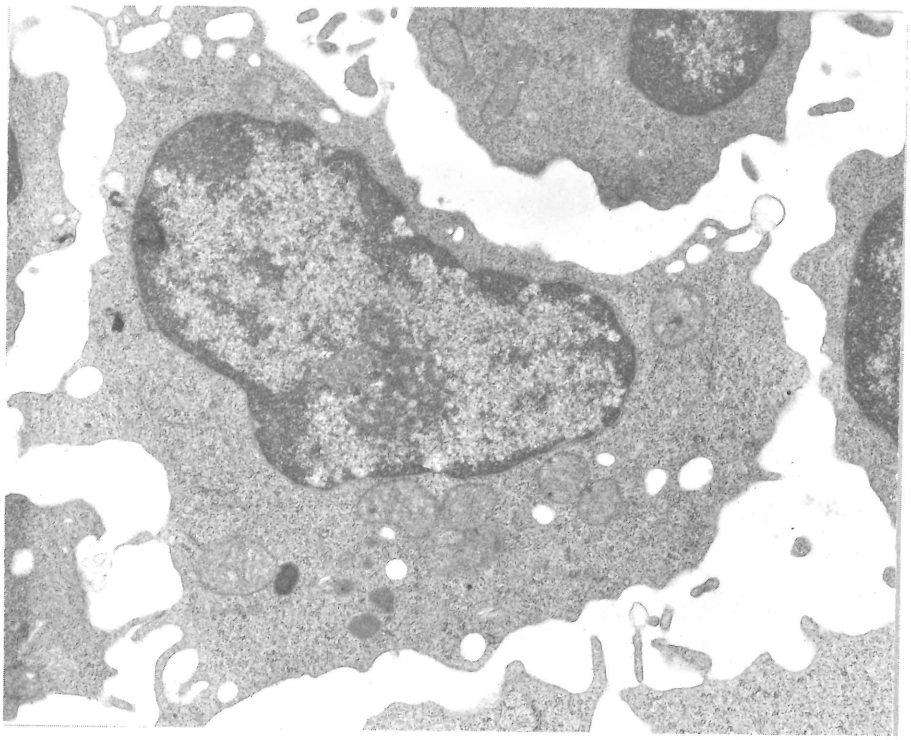


Figure 7: Large lymphocytes with aggregate ribosomal pattern and either no or only scant rough endoplasmic reticulum - 1.0 ± 0.7%

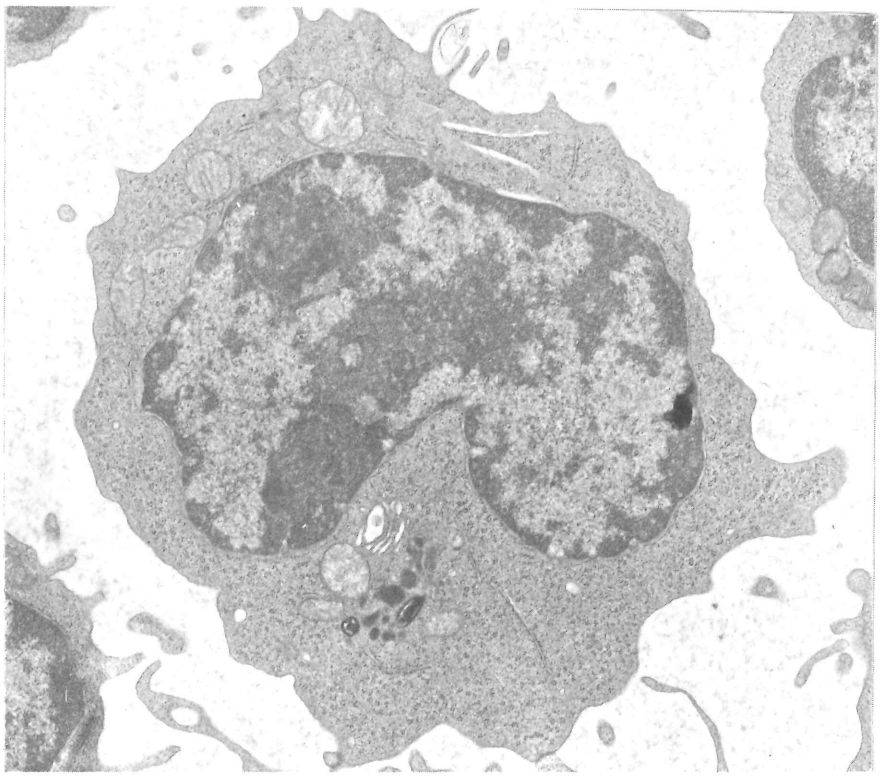


Figure 8: Large lymphocyte with aggregate ribosomes and varying amounts of rough endoplasmic reticulum - 8.6 ± 3.3%

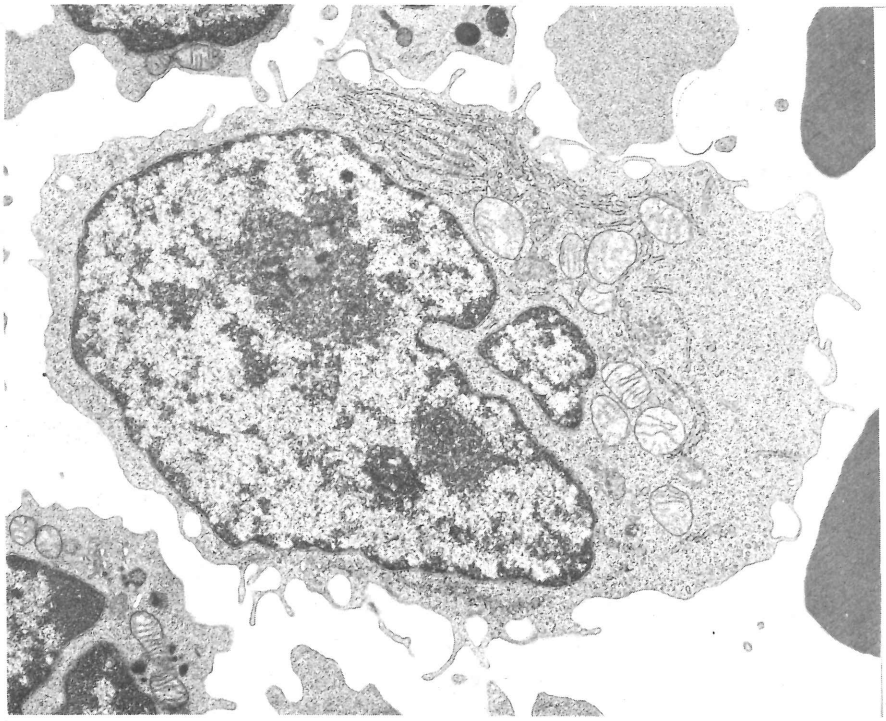
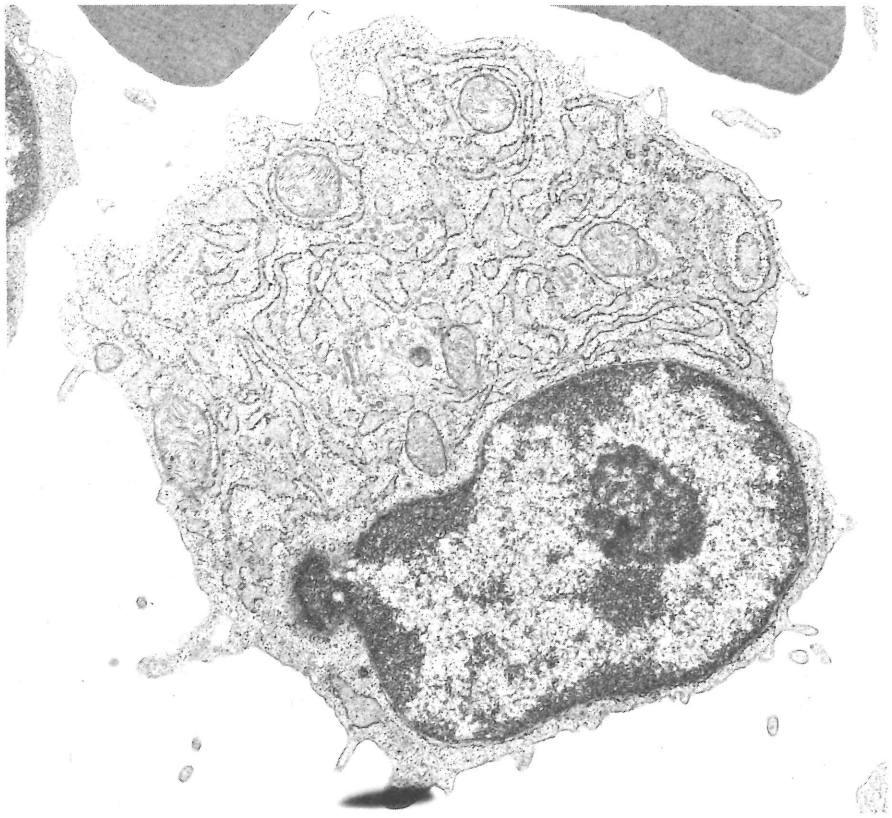


Figure 9: Mature or immature plasma cells - 0.4 ± 0.2%



The importance of recognizing these different cell types is several fold. First, it confirms the fact that the morphology of peripheral blood lymphocytes is a heterogeneous one. It also indicates that plasma cells, a cell type thought not to circulate, does circulate in small numbers. It also indicates that cells of the avian bursal-derived morphology are present in humans.

In examining large numbers of cells from patients with various types of lymphocyte abnormality, a continuous spectrum of cells may be seen ranging from the large lymphocyte with aggregate ribosomes to cells with more and more rough endoplasmic reticulum. This spectrum ends with a cell which can be classified as a plasma cell. This suggests, even though it does not prove, that this is the maturational sequence for this cell population. In examining these cells, one does not see a cell type intermediate between the large lymphocytes with dispersed ribosomes and the large lymphocytes with aggregate ribosomes, suggesting that this former cell is not part of the sequence.

In following individuals serially over a period of time changes in the above normal profile have been recognized in association with mild and, at times, sub-clinical viral infections. This suggests that a peripheral blood lymphocyte differential done by electron microscopy might serve as an early indicator for certain types of clinical problems in much the same way as a leukocyte differential done by light microscopy in the clinical laboratory is used to indicate other types of clinical problems.

Examination of profiles of this type in patients with severe congenital or acquired immunological deficiencies may help to unravel some of the mysteries related to these problems.

It appears at this time that the electron microscopic evaluation of lymphocytes may offer an easy, accurate, quantitative method for distinguishing between T-cells and B-cells at an ultrastructural level.

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Assistant Professor
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Internal Medicine

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Asking price is about 10,000.

AREA NEWS

College Station

TEXAS A & M UNIVERSITY: Recent publications include:

Thurston, E. L., J. R. Scott, and G. L. Schroeter. 1973. A low-cost, one-piece nitrogen burst processing rack for 3-1/4 x 4 and 4 x 5 inch TEM and SEM negatives. J. Microscopy (in press).

Tomas, R. N. and E. R. Cox. 1973. Observations on the symbiosis of Peridinium balticum and its intracellular alga. I. Ultrastructure. J. Phycol. (in press).

Tomas, R. N., E. R. Cox and K. A. Steidiger. 1973. Peridinum balticum, an unusual dinoflagellate with a mesocaryotic and an eucaryotic nucleus. J. Phycol. (in press).

Dallas

SOUTHERN METHODIST UNIVERSITY: Robert Specian, Lindy Anderson, and Jaswant Gidda are new members of TSEM from the Department of Biology. The department has recently acquired an ISI-Mini-SEM for graduate student projects. Dr. John Ubelaker has been awarded a Texas Utility Company contract: "Impact Study" -- Lake Granville. The contract is for 1973 and 1974, and is funded at $106,000. Dr. Eugene Copeland, Tulane University, presented a seminar entitled: "Salt secretion and water transport in epithelia".

Recent publications:

John E. Ubelaker, V. F. Allison, Robert Specian. 1973. Surface topography of Hymenolepis diminutia by scanning electron microscopy. J. Parasitology (in press).

Robert Specian, Venita F. Allison, J. E. Ubelaker, and J. H. Martin. Preparation of amyl acetate and acetone labile eggs from parasitic nematodes for scanning electron microscopy. 31st Ann. Proc. Electron Micros. Soc. Amer. (in press).

V. F. Allison and R. S. Sohal. 1973. Neuron-glia relationship in the brain of the housefly, Musca domestica. 31st Ann. Proc. Electron Micros. Soc. Amer. (in press).

Venita F. Allison, R. W. Webster, Jr., John E. Ubelaker, and Jeanne M. Riddle. 1973. Redescription of Porrocaccum sulcatum (Rudolphi, 1819) from the sea turtle Chelone mydas. Trans. Amer. Micros. Soc. 92(2):291-297.

El Paso

THE UNIVERSITY OF TEXAS AT EL PASO: Department of Biology:

Recent publication:

Duke, Eleanor L., Joyce Lewin and B. E. F. Reiman. 1973. Light and electron microscope studies of diatom species belonging to the genus Chaetoceros Ehrenberg. I. Chaetoceros septentrionales Oestrup. Phycologia (in press).

Fort Sam Houston

U.S.A. INSTITUTE OF SURGICAL RESEARCH has recently acquired a Bomar critical point dryer.

Recent publication:

Paulette C. Langlinais and Daniel W. McKeel. Massive accumulation of alveolar lattice formations and lamellar bodies in experimental phycomycotic pneumonitis. Texas Reports on Biology and Medicine (summer issue).

Houston

M. D. ANDERSON HOSPITAL: Mr. Douglas C. Hixson, Department of Virology, has been awarded a Rosalie B. Hite Fellowship to conduct research involving ultrastructural, histochemical, and biochemical studies on the surface coats of normal cells, malignant cells, and associated virus particles.

Visitors giving seminars:

Dr. David H. Gillespie, Senior Investigator, Litton Bionetics,

Bethesda, Maryland, presented a seminar September 20 titled: "Molecular hybridization experiments with nucleic acids from RNA tumor viruses".

Dr. Mrs. K. J. Ranadive, Head, Department of Tumor Biology, Tata Memorial Centre, Cancer Research Institute, Bombay, India, was visiting professor in the Department of Virology from 18 September 1972, to 27 November 1972. On 5 October 1972, Dr. Mrs. Ranadive presented a seminar titled: "Experimental studies on murine and human tumors".

Professor Dr. Richard Bootsma, Chairman, Department of Cell Biology and Genetics Medische, Medische Faculteir, Rotterdam, presented a seminar 9 October 1972 entitled: "Cell hybridization in the study of gene linkage and complementation in man".

Dr. Arthur S. Levine, Senior Investigator, Hematology and Supportive Care Branch, N. C. I., Bethesda, Maryland, presented a seminar 12 January 1973 titled: "Genetic analysis of the DNA tumor virus SV 40 using Ad 2 - SV 40 hybrid viruses".

Dr. Royce Lockhart, Supervisor of Virology Research, Du Pont, Wilmington, Delaware, gave a seminar 8 February 1973 titled: "The comparative molecular biology of rhino viruses".

Dr. J. Michael Bishop, Associate Professor, Department of Microbiology, University of California, San Francisco, California, gave a seminar 22 February 1973 titled: "Presence and expression of RNA tumor virus genes in normal and transformed cells: detection by molecular hybridization".

Recent publications:

L. Dmochowski and E. S. Priori. 1972. Present status of studies on an RNA (ESP-1) virus isolated from human lymphoma. Przeglad Lekarski 29(6):647-651.

L. Dmochowski, E. S. Priori, W. C. Williams, B. Myers, and J. M. Bowen. 1972. Comparative studies on breast cancer in animals and man. Inserm, Fundamental Research on Mammary Tumours, Grenoble, France, 12-17 June 1972, pages 351-360.

T. Shigematsu and L. Dmochowski. 1973. Studies on the acid mucopolysaccharide coat of viruses and transformed cells. Cancer 31(1): 165-174.

Other: Dr. J. M. Bowen has been elected president of the Southwest Section of the American Association for Cancer Research for 1973.

New Orleans

TULANE MEDICAL SCHOOL: Recent visitors to the Department of Anatomy include:

Dr. Milton W. Brightman, Head, Section on Neurocytology, Lab. Neuropathology and Neuroanatomical Sciences, N. I. N. D. S., Bethesda, Maryland, who gave a seminar titled: "Induced leaks in the blood-brain barrier to proteins".

Dr. George Wald, The Biological Laboratories, Harvard University, who gave a seminar titled: "Acupuncture Analgesia".

Dr. Henry de F. Webster, Head, Section on Cellular Neuropathology, Laboratory of Neurophatology and Neuroanatomical Sciences, N. I. N. D. S., Bethesda, Maryland, who seminar was titled: "Axons, Schwann Cells, and myelin formation".

Dr. Jean-Paul Revel, Division of Biology, California Institute of Technology, Pasadena, California, who spoke on "Cell surface topology".

Dr. Thomas N. James, Director, Cardiovascular Research and Training Center, The University of Alabama, Birmingham, Alabama, whose seminar title was: "The sinus node".

New grants approved and funded in the Anatomy Department since January 1973 include:

Robert D. Yates, Professor and Chairman, "Ultrastructure -- Carotid Body -- Paraganglia" through N. I. H.

Jeffery P. Ellison, Associate Professor: Established Investigatorship Cardiac Innervation: A Morphological Study, through the American Heart Association, and "Reflexogenic Zones of the Heart: A Morphologic Study", through N. I. H.

Paul M. Heidger, Associate Professor: "Vasectomy: Medical and Laboratory Investigation", through N. I. H. and "Vasectomy: Ultrastructural and Cytochemical Effects", also through N. I. H.

C. Allan Roberts, Assistant Professor, a Schleider Foundation award for "Epilepsy -- Possible Cause and a New Potential Therapeutic Agent.

Kenneth H. Jones, Instructor, "Immunologic Resistance to the Ehrlich Ascites Tumor in Mice", through the American Cancer Society.

Recent equipment acquisitions include a Siemens 101 EM with scanner, the MT2B ultramicrotome, and a Durst Enlarger.

San Antonio

SOUTHWEST FOUNDATION: New member of the Microbiology and Infectious Diseases group is Mr. G. C. Smith III.

Recent publications include:

Kalter, S. S., R. J. Helmke, M. Panigel, R. L. Heberling, P. J. Felsburg, and L. R. Axelrod. 1973. Observations of apparent C-type particles in baboon (Papio cynocephalus) placentas. Science 179:1332-1333.

Kalter, S. S., R. J. Helmke, R. L. Heberling, M. Panigel, A. K. Fowler, J. E. Strickland, and A. Hellman. C-type particles in normal human placentas. J. Natl. Cancer Inst. (in press).

Temple

SCOTT AND WHITE CLINIC: Recent publication:

James C. Stinson, Albert Leibovitz, G. V. Brindley, Jr., Ronald H. Hayward, and William B. McCombs III. Filamentous particles in human alveolar cell carcinomas: Electron microscopy studies of six cases [preliminary report].

Waco

BAYLOR UNIVERSITY: The Department of Biology has acquired a Philips EM201 with a portion of the $72,000 Baylor University awarded Harley W. Reno and the Biology Department to establish an EM laboratory in Biology for all natural sciences.

Recent Publication:

Reno, H. W., F. R. Gehlbach, R. A. Turner. 1973. Skin and æstivational coon of the aquatic amphibian Siren intermedia Le Conte. Copeia 4:625-631.

T. S. E. M. PLACEMENT SERVICE

Positions Available

Electron Microscopist / Research Associate: B. S. or M. S. Basic research lab in membrane biology. High resolution transmission EM, freeze-etch EM, and scanning EM. Interest in development of techniques and applications desirable. Experience in immunotechniques helpful. Send resume to: Dr. C. R. Hackenbrock, Department of Cell Biology, U. T. Helath Science Center, Southwestern Medical School, 5323 Harry Hines Blvd., Dallas, Texas 75235. An equal opportunity employer.

Summer Students: One, maybe two, must be skilled in tissue processing, section cutting, and EM. Apply to: Dr. C. Ward Kischer, Department of Anatomy, University of Texas Medical Branch, Galveston, Texas 77550.

Positions Wanted

EM Technician: Male. M. S. degree in Biology, Trinity University. Experienced in all aspects of transmission electron microscopy. Desires position in Houston, San Antonio, or Dallas area. Available after 1 March 1973. Ask for reference #100.

Research Associate: Male. Ph. D. degree in plant pathology, Pennsylvania State University, 1972. Experienced in all aspects of transmission electron microscopy. Good training in biochemistry. Numerous publications in plant pathology, electron microscopy. Languages: Chinese, Japanese, English. Ask for reference #101.

Research Technician: Female. B. A. Biology. Excellent grades. Undergraduate courses in electron microscopy. Desires beginning position in biological EM lab in Houston, Dallas, or San Antonio areas. Available 1 June 1973. Ask for reference #103.

Summer Employment: Graduate student, Eastern New Mexico University. Ten years experience in routine maintenance, operation, and specimen preparation for EM. Available 1 June 1973. Contact Mr. V. K. Berry, P. O. Box 3904, Eastern New Mexico University, Portales, New Mexico 88130.

Abstracts of Papers Presented

at the

Spring 1973 Meeting of the

TEXAS SOCIETY FOR ELECTRON MICROSCOPY

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25-26 May 1973

An Ultrastructural - Histochemical Study of a "Virus-like" Particle in Cardiochiles nigreceps (Hymenoptera).

J. R. Scott, S. B. Vinson* and E. L. Thurston

Department of Biology, Electron Microscopy Center, *Department of Entomology, Texas A&M University, College Station, Texas 77843

Globular proteins and virus-like particles have been reported to exist in the calyx fluid of several hymenoptern species. In Cardiochiles nigriceps, similar particles were observed in the calyx fluid and it is speculated that such particles act as the supressor of the immune response in the natural host.

The genital tract was examined with the transmission electron microscope and large numbers of these particles were observed in the nuclei of the cells composing the neck region of the genital tract. These oblong particles, measuring 650-1300 Å in diameter, exhibit an electron dense central core and differential histochemical extraction coupled with controls, indicates the core substance to be DNA.

A Comparison of Drying Techniques used for the Preparation of Tissue Culture Cells for Observation with the SEM.

Jack D. Austen and S. Mc Connell

Department of Veterinary Microbiology, Texas A&M University, College Station, Texas 77843

A study was conducted to evaluate fixation procedures used in scanning electron microscopy for the examination of cells in culture. Both virus infected and non-infected monolayers of cells grown on cover-slips were evaluated using critical point and air-dried fixation.

The method of fixation demonstrably influenced the cellular morphology observed in both the infected and non-infected cell sheets. Air-dried preparations remained more firmly attached and the cytoplasm was seen as a flattened confluent sheet and the nucleus as an "egg-yolk-like" structure containing a variable number of smaller raised areas corresponding to the nucleoli. The cell thickness was slightly reduced and a decreased number of microtubules were observed.

Cells prepared by the critical point method of drying appeared to be more loosely attached, more fragile and easily disturbed. There was a higher incidence of microtubule formation and cytoplasmic streaming was observed at the site of cellular attachment.

A Scanning Electron Microscopy Study of Chemoreceptors and Tactile Receptors of Cardiochiles nigriceps (Hymenoptera)

W. N. Norton, S. B. Vinson* and E L. Thurston

Department of Entomology, Department of Biology, Electron Microscopy Center

Texas A&M University, College Station, Texas 77843

Antennal flagellar chemoreceptors of parasitic Hymenoptera function in such various capacities as courtship, host detection and selection. Chemoreceptors have been traditionally classified according to the thickness of their cuticular wall as determined by the uptake of dye and monitored by light microscopy.

Scanning electron microscopy of C. nigriceps flagellar sense receptors has provided knowledge concerning their classification, morphology, abundance and distribution. Two distinct categories of chemoreceptors, thin and thick-walled and tactile receptors are found on the antenna of C. nigricep.

Three morphologically distinct types of thin-walled chemoreceptors are present. One occurs on the lateral-ventral portion of the female flagellum or that area of the flagellum which comes in contact with the substrate and is most abundant on the apical segment. They occur also on the anterior portion of all subsequent flagellar segments. This type is believed to function in the location of the host organism Heliothis virescens for potential parasitism by the female and the absence of such receptors on the male flagellum supports this concept. A second type is found on both the male and female flagellum. These inclined, non-striated chemoreceptors are slightly curved and relatively small in size. They are very abundant on all sides of the male flagellum, whereas they are restricted to the lateral regions of the female flagellum. A third type, placodea sensilla, are laterally grooved, elongated, and slightly elevated above the surface. They are present in both sexes and are distributed along the entire length of the flagellum.

Tactile receptors are the most numerous type of sensilla present on the antennal flagellum of both sexes and characterized by being inclined, slightly curved and apically pointed.

CHANGES IN THE FINE STRUCTURE AND TEMPLATE ACTIVITY OF CHROMATIN FOLLOWING SEQUENTIAL EXTRACTION OF PROTEIN FRACTIONS.

W. A. Pavlat, K. M. Knieriem*, J. R. Jeter*, C. Schneidler*, and I. L. Cameron. Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

The effect of sequentially removing saline soluble proteins (.14 M NaCl), histones and other acid soluble proteins (.25 N HCl), acidic nuclear proteins (buffered phenol, pH 8.4) and residual nuclear proteins (pronase) was studied in the avian erythrocyte nucleus and the nucleus of Tetrahymena. In isolated nuclei areas of condensed chromatin and pale areas were apparent. Removal of NaCl soluble proteins resulted in a more uniform distribution of chromatin within the nuclei. The removal of histones caused the reappearance of pale staining areas with distinct boundaries. In addition, the diameter of chromatin fibrils decreased from approximately 205 ± 25 Å to 85 ± 17 Å in the avian erythrocyte. Removal of acidic nuclear proteins made the fibrillar nature of the chromatin even more apparent. Fibrils as small as 40 Å could now be seen in the Tetrahymena nuclei. Treatment with pronase resulted in a decreased amount of electron dense nuclear material and also a further decrease in fibril diameter in both Tetrahymena and the chick erythrocytes. The amount of protein removed by each extraction step was determined in the case of Tetrahymena nuclei and the ability of the material remaining to act as a template for RNA synthesis was determined by radioautography in the erythrocyte nuclei. Removal of histone and acidic proteins activated the template capacity of previously inactive chromatin. (Supported by Grant GB51580 from NSF and R-A-33 from Morrison Trust).

ULTRASTRUCTURAL EXAMINATION OF PHAGOCYTOSIS OF A BLUE-GREEN ALGA BY A CHRYSOMONAD ALGA. Garry T. Cole, Michael J. Wynne*, Department of Botany, University of Texas, Austin, Texas 78712 and Bernard L. Prows*, Dow Chemical Company, Freeport, Texas.

The recent discovery that Ochromonas danica has a voracious appetite for the toxin-producing, blue-green alga Microcystis aeruginosa, provided the potential for developing a mechanism of biological control which may be useful in freshwater lakes. We have correlated our light microscope observations of living material with freeze-etch and thin-section studies of fixed cells. Freeze-etching has been particularly helpful in demonstrating membrane associations within phagocytotic cells which are not preserved to the same degree using thin-section techniques. The ultrastructure of O. danica at various stages of engulfment and digestion of the blue-green cells has shown the high activity of the Golgi complex, accumulation of vesicles around the digestive vacuoles and progressive breakdown of vacuolar contents. Cytochemical analyses, using acid phosphatase, have demonstrated localization of hydrolases in specific regions of the Golgi, in vesicles which are identified as lysosomes, and associated with digestive products within the vacuoles. A diagrammatic interpretation of the details of phagocytosis is presented in summary.

ULTRASTRUCTURAL DIFFERENCES BETWEEN LINT AND FUZZ FIBERS IN COTTON*. Melvin W. Watson and Jerry D. Berlin. Department of Biology, Texas Tech University, Lubbock, Texas 79409.

Cotton seeds produce two types of fibers - 1. Lint fibers of commercial value that are easily removed from the seed during ginning; and - 2. Fuzz fibers which lack commercial value and remain on the seed during ginning. Certain species and varieties produce lint fibers only, fuzz fibers only, or both lint and fuzz fibers. Those cotton varieties which produce both fiber types often differ in the distribution of the two types of fibers. An example of a cotton producing only fuzz fibers is *Gossypium tomentosum*, in which the fibers have a very thick secondary cell wall with numerous electron dense striations and a central lumen containing electron dense pigment. An example of a cotton producing only lint fibers is Mexican Acala - although its lint is too sparse and short to be of commercial significance. The fibers of the latter variety are easily removed from the seed, have a thinner wall with no electron dense striations, and very little electron dense pigment within the central lumen. Florida Green Seed and Higgan Botham are examples of cottons in which the lint and fuzz fibers are each differently pigmented - Florida Green Seed having white lint and green fuzz and Higgan Botham having light brown lint and darker brown fuzz. The lint fibers on the last two varieties and also on Dunn 56C and Greg 35 structurally resemble the fibers of Mexican Acala. Similarly the fuzz fibers of the latter varieties structurally resemble the fibers of *Gossypium tomentosum*.

*This study was supported by the U.S. Department of Agriculture with funds made available through Cotton Incorporated (Project No. 71-522).

AN ULTRASTRUCTURAL DESCRIPTION OF

COTTON FIBER DEVELOPMENT *

Dr. Jerry D. Berlin
Department of Biology
Texas Tech University
Lubbock, Texas 79409

The cotton fiber is a single cell that arises from the outer epidermal layer of the cottonseed. The initiation of fiber elongation begins on the day of anthesis and the elongation stage continues for 15-20 days. Subsequent to the cessation of fiber elongation, secondary cell wall formation occurs leading to increased fiber thickness. Electron microscopic examination of the above events has shown fiber development to be a programmed process beginning at 20-30 days preanthesis and concluding some 60 days postanthesis. The initiation of fiber elongation is preceded by a variety of subcellular alterations including nucleolar segregation and cytoplasmic dispersal of polyphenolic compounds. Fiber elongation per se involves the deposition of primary cell wall via a secretory mechanism involving the dictyosomes and a protein synthetic mechanism sufficient to supply the proteins required for the expanding plasma membrane and tonoplast. During the elongation phase, the ribosomes are mainly monosomic whereas they assume a polysomal configuration during later development. Fiber thickening, characterized by the formation of secondary cell wall, appears to result from the action of extracellular, i.e., just outside the plasma membrane, cellulose synthetase.

* This study was supported by the U.S. Department of Agriculture with funds made available through Cotton Incorporated (Project No. 71-522).

UNTRASTRUCTURE OF LYMPHOCYTES FROM A CHILD WITH SEVERE COMBINED IMMUNODEFICIENCY.

Harold Jordan, M.S.[1], B. Sue Criswell*, Ph.D.[2], Mary Ann South*, M.D.[2], and John R. Montgomery*, M.D.[2]. Northrop Services, Inc.[1], and Baylor College of Medicine[2].

Lymphocytes from a male infant delivered by C-section and placed into a germfree environment have been examined by electron microscopy (EM). The child has a sex-linked severe combined immunodeficiency. At monthly intervals for 9 months, 1 ml of whole blood from the subject was obtained and the buffy coat fixed for 1 hour in 3% glutaraldehyde in 0.1M PBS (pH 7.3), post-fixed for 1 hour in 1% osmic tetroxide in 0.1M PBS, and embedded in epon-araldite. Sections were cut on an ultramicrotome, stained with lead citrate and uranyl acetate, and the lymphocytes examined at 80KV. The cells were atypical, having a very sparse cytoplasm with little rough endoplasmic reticulum (ER) but abundant smooth ER. The nuclear membrane was pulled away from the nuclear space, and no evidence of nuclear pores or aggregated ribosomes were found. Mitochondria were intact. Repeated injections of KLH and typhoid vaccine, and skin grafting during the 9-month period yielded no significant observable change in the fine structure of the subject's lymphocytes. At 11 months, the subject was given transfer factor, a substance reported to stimulate uncommitted T (thymic) lymphocytes. Following repeated injections of this material, the original cell type was still present but a new type of lymphocyte was also observed by EM examination. The new cell type is smaller than normal lymphocytes, has a more dense cytoplasm, some aggregated ribosomes, detectible amounts of rough ER and more intact nuclear membranes. This new type could represent a small population of uncommitted T cells responding to the stimulant (transfer factor). (Supported by NASA Contract NAS 9-1300, NASA grant NGR 44-003-044, and CRC grant FR-00188 and RCDA K4-AI-23820.)

Dark Field Electron Microscopy Measurement

of Mammalian DNA Mass

Edgar Durbin, Jr. and Arthur Cole
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A measurement of the mass of Chinese hamster ovary DNA molecules is being made at M. D. Anderson Hospital in Houston using dark field electron microscopy. The DNA samples will be prepared by lysis of CHO cells and sedimentation of DNA directly onto thin carbon foils supported by electron microscope grids mounted on the bottom of special 1" long centrifuge tubes. These tubes contain shutters which permit exposure of one grid at a time during various periods of the centrifuge run. The selectively sedimented molecules deposited on the grids will be observed with a Hitachi HU-11A electron microscope modified in our laboratory to permit dark field microscopy with an electron beam of variable tilt. The mass of the object imaged in the micrograph will be determined from its total optical density. A density - to-mass calibration will be made using micrographs of bacteriophage and bacteriophage DNA of known molecular weight. An alternate calculation of sample mass will be made using scattering theory and the measurement of absolute scattering intensity. The possibility of radiation damage to the unstained sample and consequent loss of mass will be investigated theoretically and experimentally.

ADAPTATION AND APPLICATION OF A TRANSMISSION ELECTRON MICROSCOPE AS AN ELECTRON MICROPROBE FOR X-RAY FLUORESCENCE. S. Robinson and P. M. Corry, Department of Physics, M. D. Anderson Hospital, 6723 Bertner Avenue, Houston, Texas 77025.

An Hitachi transmission electron microscope has been modified to allow the measurement of the fluorescent characteristic x-rays generated by the electron passing through the sample. The primary features of the system are: (1) use of a high resolution (<200 eV) lithium drifted silicon X-ray detector. This detector permits the measurement of characteristic x-rays with energies as low as 400 eV. (2) The use of the transmission electron optics for precise localization of the electron beam spot (0.2μ). This permits measurements of x-ray fluorescence at the cellular and subcellular level for ions such as Na, Ca, Mg, Fe, S, P and K. (3) Rapid real time data acquisition and analysis using a 4096 channel analyzer, coupled with a bidirectional high speed data acquisition and control line to a Nova 1200 computer (12K, 256K fixed head disk, 9 track tape unit.) This system acquires and stores a 1024 channel spectrum in approximately 700 milliseconds. Subsequent to spectrum stripping and analysis, the spectra are plotted on an incremental plotter a few seconds after acquisition. These features are accomplished by lowering the accelerating voltage of the electron microscope to 25 KeV and raising the sample above its normal position to the top of the objective lens. The detector is mounted approximately 40 millimeters from the sample in a specially constructed housing whose vacuum is isolated from that of the microscope by a formvar foil. This system has been used for the measurement of the effects of radiation on ion distributions at the cellular and subcellular level.

A METHOD FOR EMBEDDING MAMMALIAN CELLS GROWN ON CELLOPHANE IN MICROTITER PLATES

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A variety of methods are available for embedding cell monolayers but all have certain drawbacks. Many require extreme heat or cold to separate embedded cells from growth substrate and some call for face-on sectioning of monolayers. Recent methods which utilize cells grown on plastic membranes are complicated by uneven cell growth, poor sectioning properties, and loss of transparency for light microscopy.

In the present study many of the above problems are minimized by embedding tissue culture cells following growth on cellophane (1) in microtiter plates (2). Cellophane discs, cut with a $\frac{1}{4}$" paper punch from moist cellophane are sterilized (overnight or longer) in ethanol, washed in sterile phosphate buffer, and placed in wells of microtiter plates (Falcon 3040). Wells are filled with media and a drop of suspension containing about 10^6 cells/ml is added. After incubation, monolayers of cells are fixed and embedded in the usual manner. Thin sections mounted on filmed grids, are stained and examined in the electron microscope.

This method has been widely applied in study of morphological and histochemical aspects of several cell lines. Cells grown on cellophane have been labeled by the con-A-horseradish peroxidase method (3). Cellophane is easily sectioned and cells, readily visualized in an inverted microscope, display uniform growth and normal morphology. Examples are presented.

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ULTRASTRUCTURE OF MALIGNANT LYMPHOMAS.

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Malignant lymphomas are classified as lymphocytic, histiocytic, mixed and reticulum cell types, implying that they are composed of histogenetically unrelated cells. Sporadic ultrastructural studies using confusing terminologies have further complicated what may be a simple problem of semantics. In an effort to clarify the pathogenesis of the malignant lymphomas, material from 60 patients has been studied using light and electron microscopy. Normal human lymph nodes were used as controls. Only morphologic criteria were used to evaluate the observations.

In normal lymph nodes, the germinal center is composed of a spectrum of cells including cleaved and non-cleaved large follicular center cells (LFCC) and small follicular center cells (SFCC), together with mature lymphocytes. Intermediate forms can be recognized. Reticular cells, macrophages and plasma cells are also present. The same cells are seen in the perifollicular and interfollicular zones but in different proportions.

Malignant lymphomas are composed of morphologically similar cells, and it is the predominance of a single cell type that determines the histologic classification of a particular lymphoma. Lymphomas originating in the follicular centers will remain nodular for an indefinite period before becoming diffuse. Lymphomas presenting a diffuse pattern either originate in the interfollicular tissues or are composed of follicular center cells manifesting a highly infiltrative potential. The findings indicate that malignant lymphomas are derived from the same family of cells, and the morphologic varieties within the group reflect the fact that the majority of the cells in any one tumor are at a particular stage of maturation.

ELECTRON MICROSCOPY OF HUMAN BREAST CARCINOMA CELLS IN TISSUE CULTURE.

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Reports in the literature of attempts to establish cell lines of significant duration from human breast carcinomas make it clear that this is a difficult and rarely successful undertaking. The present report will summarize findings of a collaborative effort to establish breast cancer cell lines from solid tumors and from pleural effusions, and of light and electron microscopic studies performed to examine the morphology of the growing cells. Specimens of freshly excised solid tumors are prepared for electron microscopy and cultivation in various media. Cell monolayers on Falcon-plastic flasks and on Teflon-coated coverslips are then embedded in Epon. Detailed photographic documentation of the living cultures, the embedded monolayers, and the fine structure of the growing cells as revealed by electron microscopy, is obtained and correlated with the morphology of the solid tumors. Successful maintenance of epithelial cell growth for a period of several months has been achieved. Similar studies have been performed on sequential specimens of pleural effusions from patients with breast carcinoma. The use of pleural effusions offers technical advantages over the solid tumors and permits correlation of morphology with response to therapy, but the heterogeneous cell population present in the aspirates creates the necessity for careful morphological identification of tumor cells, mesothelial cells and reticuloendothelial cells, and this can only be achieved by electron microscopy.

THE PROLIFERATIVE HISTIOCYTOSES: LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS. D. Crisp*, B. Mackay, S. Masse*, and J. L. Smith*.

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Six cases falling within the spectrum of disorders known as the proliferative histiocytoses (histiocytosis X) have been studied by light and electron microscopy, and the morphologic findings have been correlated with the clinical course in each case. Two were localized lesions (eosinophilic granulomas), and three were disseminated processes in which the rapidity and mode of extension varied considerably. Solid tumor specimens were obtained for histological studies from each of these patients. The sixth case was a malignant histiocytosis, and the material for electron microscopy was taken from a bone marrow aspirate.

The proliferating cell in these conditions bears some morphologic resemblance to a histiocyte, but lysosomes are few and inconspicuous and the cytoplasm typically contains small rod-shaped bodies (Langerhans granules). The number of granules in a cell varies considerably. Frequently the granules are attached to the cell membrane, suggesting that they are derived from it. They may also be numerous in the vicinity of the golgi complex where contiguity with a membranous sac confers a distinctive racquet-shaped profile. Similar granules occur in dendritic cells within normal epidermis, and while the function of these cells is not known, the possibility exists that they might influence the rate of epidermal cell turnover. Despite the morphologic resemblance, cells of the proliferative histiocytoses are probably not directly related to the epidermal cells since the latter appear to be of ectodermal origin. Comparisons with the fine structure of cells of the malignant lymphomas indicate that the histiocytoses are a separate entity.

ULTRASTRUCTURE OF MALIGNANT TUMORS OF THE LUNG. M. Mandavia*, B. Mackay, and C. F. Mountain*. Departments of Pathology and Surgery, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025.

Cancer of the lung is the most common malignant neoplasm in males, and the incidence continues to increase. The prognosis is absymally poor, and only 5% of the patients survive for five years following diagnosis. Improvements in diagnosis and therapy are obviously essential, and for the evolution of effective modalities of therapy, accurate morphologic diagnosis is necessary. The onus thus passes to the pathologist who must determine whether a tumor is primary within the lung or metastatic. If it is primary, he must offer as accurate a classification as possible from the morphology of the tumor. Currently, the classifications used for malignant tumors of the lung reflect our poor understanding of the origin of the different pulmonary neoplasms. In an attempt to achieve a better understanding of the histogenesis of lung carcinomas, and to provide a sound histological basis for their classification a series of 50 primary malignant tumors of the lung has been studied with light and electron microscopy. The ultrastructural observations reveal definite and often striking differences among the cell types present in these tumors, thus providing criteria that are useful in classifying the tumors where this cannot be done by conventional light microscopy. Comparison of the fine structure of the tumor cells with that of cells present in the normal human lung has also been carried out. This has proved instructive in offering insight into the genesis of the lung tumors.

THE FINE STRUCTURE OF AN ADAMANTINOMA OF THE TIBIA. Jorge Valenzuela* and Bruce Mackay. Department of Pathology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025.

Adamantinoma of the long bones is an uncommon malignant neoplasm that possesses a particular predilection for the tibia. In histological sections, the tumor can resemble basal and squamous cell carcinomas, or adenocarcinomas, or it may exhibit a sarcomatous pattern suggesting angioblastic or synovial origin. This polymorphic composition has prompted the numerous suggestions which have been advanced in attempts to explain the nature of the tumor, but the tissue of origin remains obscure. The tumor derives its name from an apparent resemblance to neoplasms of the enamel organ.

The opportunity arose recently to study a case of this unusual tumor, and in addition to conventional light microscopy, tissue was prepared for electron microscopy. Light microscopic sections showed a poorly organized arrangement of the tumor cells into clusters surrounded by a collagenous stroma. The appearance of the tumor cells suggested possible mesenchymal origin, but the electron microscopic studies clearly established the epithelial nature of the tumor. Numerous complex desmosomes united adjacent tumor cells, and extensive bundles of tonofilaments extended throughout the cytoplasm of the cells. At the periphery of the groups of tumor cells, a basal lamina could frequently be demonstrated at the interface between the cells and the surrounding stroma. Occasional cells possessed irregular microvillous-like projections at free surfaces, but these were not a feature of the tumor. The fine structural studies thus confirmed the epithelial nature of the tumor; prototypic tissue and peculiar predilection for the tibia remain enigmas.

EFFECTS OF COLCEMID AND LUMI-COLCEMID ON SPERMATOGENESIS IN CHINESE HAMSTERS.

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Several microtubular systems are involved in the proliferation and morphogenesis of mammalian germ cells. In previous reports, we have described the effects of various microtubule inhibitors such as Colcemid, the vinca alkaloids, and cold shock on mammalian spermatogenesis. Chinese hamsters receiving daily injections of Colcemid at doses of 50 to 100 μg/ml displayed extensive degeneration of germinal epithelium and tubule atrophy resulting in complete sterility within 35 to 40 days. Upon cessation of Colcemid injections, complete renewal of the germinal epithelium and resumption of fertility was apparent within 75 to 90 days. In order to identify microtubules as the target for Colcemid-induced sterility, identical experiments have been carried out using UV inactivated Colcemid or Lumi-Colcemid (Aronson and Inoué, J. Cell Biol. 45:470, 1970). Lumi-Colcemid is not effective as a mitotic inhibitor and is apparently incapable of binding to microtubule protein. In our system, loss of antimitotic activity was assayed by measuring the mitotic index of cultured Chinese hamster fibroblasts treated with unirradiated Colcemid or Lumi-Colcemid for 3 hrs. Lumi-Colcemid at a concentration of 75 μg/ml was injected daily into each scrotal sac (0.5 cc per sac). Control animals received daily injections of either physiological saline or Colcemid at equivalent doses. Animals receiving continuous injections of Lumi-Colcemid displayed no changes in the histological appearance of the seminiferous epithelium. Ultrastructural studies indicated no apparent damage to any of the microtubular systems of the seminiferous epithelium. These results support our contention that Colcemid-induced sterility results from disruption of microtubular systems in the cells of the seminiferous tubules. (Supported by NIH Contract No. 69-2139 from the Contraceptive Development Branch of NICHD.)

SERTOLI CELL ULTRASTRUCTURE FROM HYPOPHYSECTOMIZED RATS

J. N. Lindsey*[1], E. Steinberger*[2], and B. R. Brinkley[1]

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Light microscopic preparations of testes from hypophysectomized rats show atrophic changes in the Sertoli cytoplasm and displacement of the nucleus toward the tubule lumen from its normal basal position. Nitrofuran inhibits germ cell production but does not disturb androgen production or Sertoli cell morphology.

The above observations have led us to believe that changes observed in the Sertoli cells of hypophysectomized rats are under hormonal control. Electron microscopic and histochemical studies were undertaken to determine the effect of hypophysectomy on Sertoli cell structure and function. Sixty to seventy day old male rats were hypophysectomized and their testes removed at time intervals of 4, 10, 20, and 40 days.

By the 20th day following hypophysectomy the germ cells are lost in selected areas of the seminiferous tubules which contain practically all Sertoli cells bordering one upon the other. In contrast to the normal morphology of the Sertoli cell, the polygonal profiles of these cells surround a reduced cytoplasm practically filled with pleomorphic nuclei. On many Sertoli cell borders the Sertoli-Sertoli specialized junctions form. Nuclear profiles are of the parallel type which predominate in Sertoli cells of stages IX - XIV and I - IV in the seminiferous cycles. Characteristics of the nucleolus and satellite karyosomes are similar to those from normal histological sections.

Qualitative estimations indicate a reduced number of mitochondria in the Sertoli cytoplasm of the hypophysectomized rat. In addition, configurational changes may be found in the cristae of these mitochondria.

THE ULTRASTRUCTURE OF PHEOCHROMOCYTOMA

R. B. Marshall and E. O. Mueller, University of Texas Medical Branch, Galveston, Texas

Five tumors of the adrenal medullary cells were studied by electron microscopy. The tissue was obtained at surgery, fixed in 2.5% glutaraldehyde with phosphate buffer, processed and stained routinely. Three patients had a unilateral tumor and the morphology was very similar to normal human adrenal medullary cells. There was moderate variation between individual cells and different tumors. Two patients had involvement of both adrenals. In one patient adrenal vein biochemical assay showed the presence of norepinephrine only with complete absence of epinephrine. The second patient did not have these assays done. The granules observed in the latter patients were uniformly smaller with a "normal" sized limiting membrane. Fixation and possible stimulation of the tumor may account for this variation but it is still suggested that the small granule size may relate to the characteristic appearance of norepinephrine with electron microscopy. The occurrence of these tumors at puberty and the bilateral involvement suggests a failure of methylation of norepinephrine with formation of epinephrine a physiologically more critical neuroamine in the human adult.

Effect of Puromycin on Ultrastructure of Hepatocytes and Spermatogenic Cells of Chinese Hamster.

A. Ubukata and J. P. Chang. Division of Cell Biology, University of Texas Medical Branch, Galveston, Texas 77550.

Various doses of puromycin were injected into testis or peritoneal cavity of Chinese hamsters. In either case, the drug administration produced some ultrastructural changes in the hepatocytes. The Golgi apparatus became somewhat disorganized with dilated cisternae, increased vacuolization, and a loss of dense protein material in the saccules. ER appeared dilated and the outer layer of the nuclear membrane became extended, although the nucleus maintained its normal appearance.

After intratesticular injection in seminiferous tubules, the spermatocytes were severely injured, as evidenced by degeneration and alteration of the Golgi apparatus, vacuolization of cytoplasm, and occasional pyknotic nuclei. Spermatogonia and Sertoli cells were affected to a much lesser degree and spermatids were the least affected.

The data indicated that the antibiotic affected the membrane system of the cells. It is entirely possible that the changes in hepatocytes produced from intratesticular injection were due to leakage from injection. (Supported by a Research Contract No. 69-2139, from NIH)

Dynamic Bias of Integrated Circuits

- D. Lloyd Crosthwait and Frank W. Ivy, Texas Instruments

Increased device complexity, sensitivity to electron beam bombardment and extensive use of oxides overcoating interconnect metallization have made voltage contrast in the SEM difficult to obtain. Some of the considerations of electron beam/integrated circuit interaction phenomena will be discussed and examples of charge minimization techniques will be presented. Dynamic bias of integrated circuits by synchronous testing will also be presented in a ten minute video tape.

Title: ULTRASTRUCTURE OF DARK CELLS - Susan Miner, M. D., Baylor Medical

Electron micrographs of 400 cases filed in the Department of Pathology, at Baylor University Medical Center were examined for electron dense cells. Most of the normal tissues contain osmophilic cells as part of the fibrovascular tissue, bone and cartilage. They were abundant in the squamous epithelium and only seen occasionally in the liver, pancreas and brain. Approximately 200 carcinomas, 120 sarcomas and 40 benign lesions were reviewed. Carcinomas showed dark cells more frequently than sarcomas. Undifferentiated neoplasms showed dark cells rarely. Benign lesions were mostly of epithelial origin and contained numerous dark cells. Further investigation is needed to classify dark cells.

A brief review of the literature is presented to emphasize the importance of dark cells in physiologic and neoplastic conditions.

EXPERIENCES WITH X-RAY MICROANALYSES IN THE SEM. Gary G. Paulson and Ray E. Ferrell*. Materials Evaluation Laboratory, Inc., 8000 GSRI Avenue, Baton Rouge, Louisiana 70808

The SEM with an accessory X-ray microanalysis system (energy dispersive) is a versatile analytical device. In this short review, we would like to highlight our experiences with the combination instrument and illustrate its usefulness as well as limitations, with special emphasis on the options available to the analyst.

The SEM-EDS can be used as a macro or microprobe for the characterization of particulate matter by changing the raster scan or selecting the "spot mode". In air pollution studies, the chemical composition of the total sample or individual particles can be determined and supplement particle size and morphological analyses. X-ray distribution maps can yield quantitative microanalyses of minerals or plot sulphur and phosphorous concentrations in rice grains. Line scan profiling can demonstrate dramatically the changes in the composition of growth bands on ferromanganese nodules or slag inclusions within stainless steel weldments.

Sensitivity and spatial resolution of qualitative techniques and the precision and accuracy of quantitative methods are influenced by the data collection and analysis routines. Specimen position, choice of coating materials, take-off angle, surface roughness, and beam current modify the peak-to-background ratio and the limits of detectability. The precision of quantitative methods is controlled in part by counting statistics. Empirical or theoretical methods of data reduction may produce different accuracies. The net result is a "tailored" routine which achieves efficiently the goals of the analyst.

HISTOCHEMICAL EXAMINATION OF BOVINE AND OVINE PINEAL GLAND. Andrzej Lukaszyk* and Russel J. Reiter* (Introduced by E. G. Rennels). Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

Previous histochemical examinations of pineal gland were concerned primarily with its sympathetic nervous control and indoleamine activity. However, in several species two or three types of parenchymal cells can be distinguished electron microscopically, and moreover, both indoleamines and biologically active polypeptides have been extracted from bovine pineals. Besides, an intense dipeptidase activity has been demonstrated in the gland. This presumed heterogeneity of pinealocytes and the possibility that the gland secretes active polypeptides led us to select the histochemical methods for demonstration of APUD series cells. Bovine and ovine pineal glands were used for study. Reactions were performed in fresh frozen sections and from material fixed in formol-calcium, glutaraldehyde and glutaraldehyde-Bouin fluid. Especially in sheep pineal gland, staining among pinealocytes was markedly different in sections incubated for α-glycerophosphate, succinate and NADH tetrazolium salts oxidoreductases and unspecific esterases. In sheep some pinealocytes were positive for pseudocholinesterase but acetyl-cholinesterase and cholinesterase were associated with nerve fibers as estimated using BW284C51 and iso-OMPA. In cow pineals this reaction was less pronounced. In both species, alkaline phosphatase reaction occurred in blood vessel walls, and the distribution pattern for acid phosphatase resembled that for unspecific esterases. The most important finding concerns the specific substances detected intra- and extracellularly in cow pineals with azure A, toluidine blue or safranin after hydrolysis of glutaraldehyde-Bouin fluid fixed sections in 0.2N HCl at 60 C during 1 to 1.5h. This reaction is selective for some polypeptidic hormones. The substances were present in selective parenchymal cells, which were obviously not mast cells. Besides, the substances were found in intracellular spaces as small granules or large "lakes", and within blood vessel walls. (Supported by USPHS grant, HD-06523).

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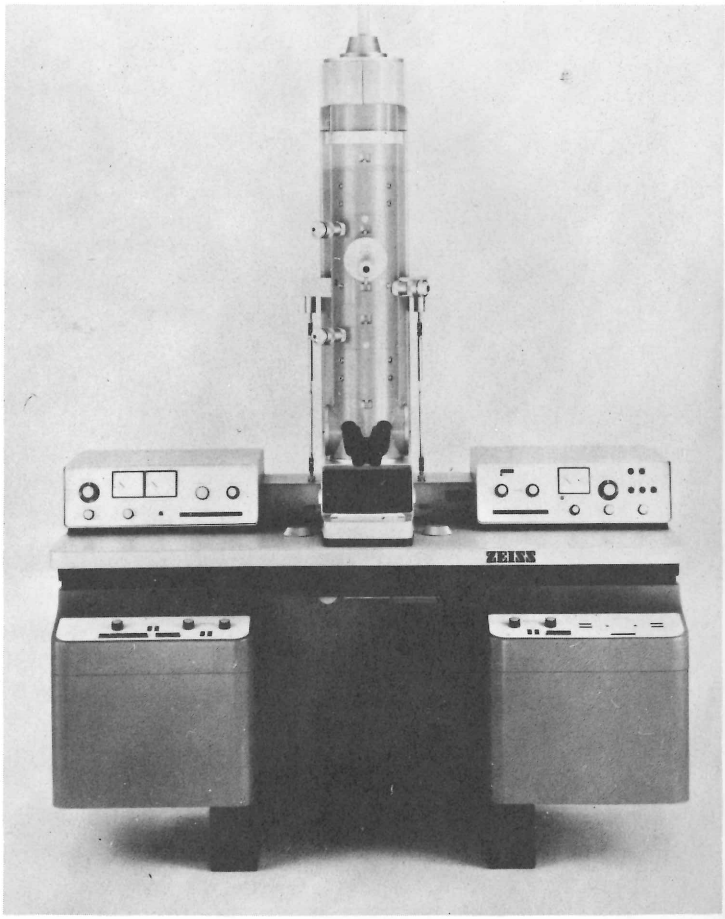

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