CELL BIOLOGY

Chairman: RALPH JERSILD, Indiana University Medical Center

EDWARD J. HINSMAN, Purdue University, was elected chairman for 1969

ABSTRACTS

The Fine Structure of the Ventral Horn Neuron in the Calf Spinal Cord. E. J. HINSMAN and KATHLEEN MOE, Purdue University.—Tissues collected from the ventral horn of the lumbar spinal cord of gluteraldehyde perfused calves were examined. Study was directed toward the larger neurons and dendritic interrelationships. The large neurons appeared similar in appearance to large motor neurons which have been described from other species. In areas of dendritic apposition interdendritic membrane modifications were found which appeared similar to desmosomes. These consisted of the thickening of apposed dendritic membranes, an increased intercellular space and electron dense cytoplasmic condensations. Implications of their presence will be discussed.

An Intranuclear Structure in Neurons of Human Cerebral Cortex. ITARU WATANABE, SHEILA DONAHUE, and WOLFGANG ZEMAN, Indiana University Medical Center.—Examination in the electron microscope of cerebral cortical biopsy material from seven children with various intractable disorders of the central nervous system revealed occasional peculiar structures in the nuclei of nerve cells. This structure is made up of filaments approximately 100 Å in diameter. There is variation in the appearance of this structure which is probably due to the angle of the section. The filaments are seen in bundles, and forming a lattice, also as round elements in cross section between parallel filaments bearing resemblance to a corncob. The whole structure is of unknown length, the longest observed measuring more than 5μ. The greatest width observed is 1.2μ, but more often they measure only 20 to 120 mμ. They were seen in both sexes, ages from 2 to 9 years, in six different disorders, but always in nerve cells. Such structures have been observed in various vertebrates. Their significance is unknown.

Chemical Composition of Membrane Fractions Isolated from Rat Liver in Relation to Membrane Differentiation During Secretion. WAYNE YUNGHANS and D. JAMES MORRÉ, Purdue University.—The chemical compositions of Golgi apparatus, endoplasmic reticulum (ER), and plasma membrane were compared. The ratios of protein to lipid in Golgi apparatus and plasma membrane were similar but less than for ER. Phospholipids of the three membrane fractions were similar but the sterol content of Golgi apparatus was intermediate between that of ER and plasma membrane. Carbohydrate, RNA, and DNA were found in negligible amounts in the Golgi apparatus fraction indicating low contamination from glycogen, ribosomes, and nuclear material.

Disc electrophoresis patterns of structural proteins from Golgi apparatus and ER were similar but different from those of plasma mem-

brane. Structural protein of plasma membrane separated into a characteristic two-band pattern with corresponding bands being present in both Golgi apparatus and ER. The structural protein pattern from mitochondria showed several bands not present in the patterns from the other membrane fractions. These results together with electron microscopic evidence showed the Golgi apparatus to be a unique component of the endomembrane system. However, its chemical composition was intermediate between that of ER and plasma membrane and consistent with a functional role of the Golgi apparatus as a site of endomembrane differentiation during secretion. (Supported in part by grants from the NSF GB-7078 and NDEA Title IV 2599-82-11557.)

An Electron Microscopic Study of Zinc Iodide-Osmium Staining of the Golgi Apparatus of Rat Intestinal Epithelial Cells. K. M. MAK and R. A. JERSILD, Indiana University Medical Center.—A modification of the zinc iodide-osmium (ZIO) impregnation technique, used for the staining of synaptic vesicles at cholinergic junctions (Akert and Sandri, Brain Res., 7:286, 1968), has been applied to intestinal epithelial cells of the rat jejunum in an attempt to localize choline-containing phospholipids. Results showed that an electron dense reaction product was localized within the Golgi apparatus. In starved animals, one or two of the Golgi cisternae were ZIO-positive while associated vacuoles remained negative. In lecithin-fed animals, an increasing number of ZIO-positive cisternae were observed. In addition, reaction product was seen deposited around droplets, presumably of fat, accumulated in the Golgi vacuoles. Treatment of tissues in a solution containing either potassium iodide-OsO_4 (KIO), zinc sulfate-OsO_4, or OsO_4 alone failed to elicit a reaction product within the Golgi apparatus. Thus, the reaction product is specific to ZIO mixture. The affinity of ZIO for the Golgi apparatus was inhibited by pretreatment of tissues with KIO, suggesting that KIO and ZIO were reacting with the same substrate. Extraction of tissue lipids with chloroform-methanol prevented the Golgi response to ZIO. It is suggested in this study that the Golgi apparatus is a site of accumulation of phospholipids. (Supported by PHS Research Grant AM 11721-01 from National Institute of Arthritis and Metabolic Diseases.)

The Ultrastructural Features of Intraoral Lichen Planus, Simplex. J. B. WHITTEN, JR., Indiana University, Department of Oral Pathology, Indianapolis.—Lichen planus is a chronic, benign, dermatologic disease which is usually self-limiting. The skin lesions are elevated, scaly, biolaceous plaques which are puritic and tend to expand and coalesce forming larger lesions. The oral disease exists in several forms: namely (1) simple, (2) erosive, (3) bullous, and (4) hypertrophic. The lesions are elevated bluish white lines which cross forming beads. In other cases bluish white plaques of varying size occur without clinical identity.

Biopsies of five patients with the simple form of the intraoral disease were examined with the light and electron microscopes. The tissue for light microscopy was fixed in 10% formalin, embedded in paraffin, sectioned at about 6μ and stained with hematoxglin and eosin. The remaining tissue for electron microscopy was fixed in 4% glutaraldehyde, post

fixed in osmic tetraoxide, embedded in Epon 812, sectioned at 600 Å with glass knives and Porter-Blum ultramicrotomes, mounted on uncoated 200-mesh copper grids, and stained with lead citrate and uranyl acetate.

Three phases of lichen planus were observed on ultrastructural examination.

Stage I —Particulate accumulations were found within some of the spinous cells. The particles were composed of five (usually) electron-dense bodies 30 Å in diameter set in a much less electron dense amorphous material. Surrounding the dense bodies were clear zones 10-20 Å in thickness.

Stage II —The basal epithelial cell layers showed considerable intercellular edema with occasional interepithelial inflammatory cells usually lymphocytes. The superficial lamina propria, beneath the anchoring fibers and lamina densa, was altered. The usual collagen fibers were replaced by an amorphous material about 3μ to 5μ in thickness.

Stage III—The deep spinous and basal epithelial as well as the superficial lamina propria is degenerated and necrotic. In these areas aggregates of bacteria were often associated with the most severe degeneration. This was present even though the surface epithelium was intact.

Direct Studies of Nuclear Movements in *Schizophyllum commune*. DONALD J. NIEDERPRUEM and RALPH A. JERSILD, Indiana University Medical Center.—Past investigations employed indirect genetic techniques to quantitate nuclear migration in *Schizophyllum commune*. The current study employed living hyphae of *S. commune* and compared nuclear movements in homokaryotic mycelia, dikaryotic mycelium and an AxBmut homokaryon of this mushroom. Rates of nuclear movement were measured by phase contrast microscopy and the employment of an ocular micrometer, using hyphal apices and septa as reference points. Forward nuclear movements were observed in growing hyphal apices of homokaryotic mycelia and the dikaryon. Nuclear movements occurred within the range of hyphal growth and could account for the maintenance of centrally located nuclei. Opposed nuclear movements after mitosis greatly exceeded the rate of apical growth. Septum disruption and extremely rapid nuclear movements were recorded in an AxBmut homokaryon. Neither cytoplasmic streaming nor actively participating granules or filaments could account for any of these nuclear movements. Glutaraldehyde-fixed hyphae were examined by electron microscopy and revealed microtubular elements.

Plasma Cell Antibody Against Bovine Serum Albumin in the Rabbit Appendix as Revealed by the Fluorescent Antibody Technique. JOHN F. SCHMEDTJE, Indiana University School of Medicine.—Evidence is accumulating that heterologous serum protein can be absorbed through the normal gut epithelium of adult mammals. In the present experiment,

bovine serum albumin (BSA) was intubated into the appendiceal lumen of the adult living rabbit. Three weeks later, BSA was added to the food and water for one week. The appendix was removed, and portions were quick frozen and subsequently sectioned in a cryostat. Adjacent sections were stained with H and E. Purified BSA and goat anti-BSA conjugated with fluorescein isothiocyanate were used, according to the Coons technique, to identify any intracellular antibody against BSA.

Antibody against BSA was present in plasma cells beneath the appendiceal luminal epithelium and beneath the outer epithelium of appendiceal crypts. Antibody positive cells were not present in control rabbits that had not received the intubation of BSA.

The results support the hypothesis that the intubated BSA was absorbed and acted as a sensitizing agent. The results also support the hypothesis that absorption of the oral doses occurred through the gastrointestinal tract, that these acted as challenge doses, and that this induced plasma cell antibody production in the appendix wall.

Tumor Cell Mitotic Activity in Mice Treated with Antigenic Materials. WILLIAM E. STOVALL and GORDON L. ROSENE, Ball State University.—Current interest in tumor specific immune mechanisms has prompted this pilot study to investigate such mechanisms in Strong A mice afflicted with spontaneous mammary carcinoma. The majority of previous work, excluding clinical investigations, involved transplantable tumors.

Five groups of animals were employed, consisting of one untreated control group and four receiving periodic injections of various antigenic materials. Two groups received rabbit serum. The remaining two were given tumor combined with Freunds adjuvant. Serum recipient groups received 24 injections during a 37-day period. Adjuvant recipients received 10 injections during the same period. Animals were sacrificed at 12:00 noon. Histological sections of the tumors were made and stained with H and E. Tumor cell mitotic indices were determined.

Data significance was determined using the Student t test. Mean mitotic index values from the two rabbit sera recipient groups were significantly different (5% level) from the control group. Mean mitotic indices obtained from the two Freunds adjuvant groups were not significantly different from the control group. The absence of significance in these two groups was due to extreme data variability.

It was anticipated that injected materials would evoke an autoimmune response in the recipient mouse. Literature reports suggest that spontaneous tumors are sometimes weakly antigenic. These procedures were intended to produce an enhancement of such a response. Significant decrease in tumor cell mitotic indices could indicate a decrease in tumor metabolic activity.

Microimmunoelectrophoresis of Human Blood in Regard to the Study of the Gc System. SHIRLEY FRANCES (ARCHIBALD) SMALLEY, Ball State University.—This study was conducted in order to determine if the Gc protein could be accurately and economically identified using a normal lab-

oratory technique. If the test could be adapted to a routine laboratory procedure, it would be possible to use the Gc determination in identifying the questionable parentage in a legal paternity suit. Hirschfeld *et al.* and Bearn and Cleve in previous experimental work have established the Gc inheritance line.

Also, this study was conducted on two, three generation families. One family was Negro and the other family Caucasian of Anglo-Saxon descent. The gene frequencies were determined for three generation families.

The general technique involved the collection of blood specimens for determination of serum protein. The serum was then treated by microimmunoelectrophoresis on a cellulose membrane. The Gc precipitation arcs were compared to control Gc precipitation arcs and identified for the correct Gc type.

The data collected did show that the microimmunoelectrophoresis on cellulose acetate could be used routinely to determine the Gc component of the serum. This test proved to be easy to perform, accurate, reproducible, and economical. This experiment confirmed that the Gc system was under genetic control and could be used to help establish the parentage of a child.

The verification tests by Hirschfeld could be used to eliminate any genetic variation due to rare Gc types.

The data was not conclusive in determining the gene frequencies of the Negro and Caucasian of Anglo-Saxon descent in the midwestern United States region because the number of tested individuals was too small. In calculating the data, the gene frequencies of Bearn and Cleve were used.

Quantitative Measures of In Vitro Cell Mobility by Use of a Pattern Recognition Computer. GEORGES BARSKI, JAMES W. BUTLER, and ROBERT J. THOMAS, DePauw University.—The purpose of these researches is to find a quantitative measure of cell mobility, in vitro, which takes account of the changes in the shape of the cell and of the motion of the interior parts, while ignoring the random translation and rotation of the cell as a whole; and, furthermore, to use a computer to automatically obtain such measure. The usefulness of such a measure would be in researches regarding effects on mobility of such things as temperature, different concentrations of a chemical, different chemicals, different amounts and kinds of radiation, and to compare normal with abnormal cells.

The general procedure is to take motion pictures of the cell and use the film as input to Chloe, the Argonne National Laboratory pattern recognition computer. Chloe repares a record (on magnetic tape) of the shapes it sees in each frame; this record of shapes is then analyzed by a standard general purpose computer. The program used for this analysis is one which is also used for automatic karyotyping of chromosomes; it computes the area, centers of mass, moment of inertia, and other higher moments of the shapes (in combinations which are invariant

under translation and rotation in the plane). Another program then analyzes the changes in these quantities from frame to frame and obtains a measure of the motion of the cell. It is in this latter area that most of the research has been and is being carried out, as there are many ways to combine and use the information available on the moments.