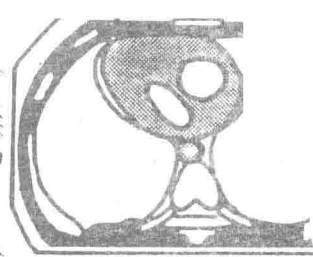
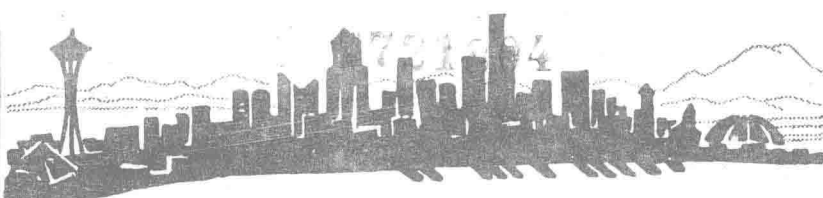
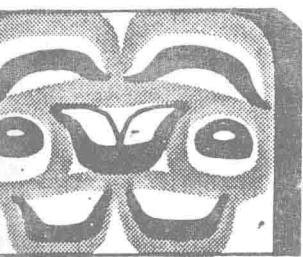


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Part 6/6

Track 5. Student Activities & Paper Competition

Track 12. Artificial Intelligence & Information

Track 13. Computers in Medicine

Track 14. Neural Networks

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TABLE OF CONTENTS

Part 6/6

THIS PART CONTAINS:

Track 5. Student Paper Competition

Track 12. Artificial Intelligence & Information Systems

Track 13. Computers in Medicine

Track 14. Neural Networks

Track 5: Student Paper Competition

Chair: Charles Lessard

Session 5.01: Student Paper Competition Regional Winners

Session Chair: Swamy Laxminarayan, New Jersey Medical School

1. **The Dynamic Breakdown of Heart Cell Membranes Exposed to Ramp Increases in Transmembrane Potential** RJ O'Neill, *Johns Hopkins University*; L Tung 1731
2. **Use of Coherence in Activation Detection During Ventricular Fibrillation** C Cabo, *Duke University*; JM Wharton, EV Simpson, RE Ideker, WM Smith 1733
3. **Interactive Video Games and Real Time Displays for the Wheelchair Aerobic Fitness Trainer** BP Flaherty, *University of Illinois at Chicago*; CJ Robinson, WE Langbein 1735
4. **Visualization of Human Brain Structure-Function Relationships** DJ Valentino, *University of California, Los Angeles*; JC Mazziotta, HK Huang 1737
5. **Automated Analysis of Dynamic Medical Image Series with A Priori Physiological Knowledge** F Frouin, *INSERM U66, Villejuif, France* 1739
6. **Spectrum Analysis of Fluctuations of RBC Velocity in Microvessels by Using Microscopic Laser Doppler Velocimetry** E Okada, *Keio University, Japan*; Y Fukuoka, J Umetani, E Sekizuka, C Oshio, H Minamitani 1741

Session 5.02: Student Paper Open Competition I

Session Chair: Francis Spelman, University of Washington

1. **Digital Demodulator for Electrical Impedance Imaging** RWM Smith, *Sheffield University, U.K.*; IL Freeston, BH Brown 1744
2. **A Rule-Based, Adaptive Window-Size Filter for the Enhancement of Subcutaneous Vascular Patterns in Thermographic Images** EK Chan, *University of Texas, Austin*; JA Pearce 1746
3. **Measurements of Argon Laser Light Attenuation in the Skin "In Vivo" Using a Unique Animal Model** ZF Gourgouliatos, *University of Texas, Austin*; S Ghaffari, AJ Welch, KR Diller, RC Straight 1749
4. **A Probabilistic Approach to the Design of Microelectrode Layouts for Neural Signal Transducers** LD Clark, Jr., *Massachusetts Institute of Technology*; DJ Edell 1751
5. **Optical Computing and Robotics in Biomedical Imaging Systems** S Ghaffari, *University of Texas, Austin*; HG Rylander, III, AJ Welch 1753
6. **Hemodynamic Model for Surgical Extracorporeal Circulation** WL Van Meurs, *INSERM U305, Toulouse, France* 1755

Session 5.03: Student Paper Open Competition II

Session Chair: W. J. Tompkins, University of Wisconsin - Madison

1. **A New Ultrasonic Probe for the Local Attenuation Measurement in Biological Soft Tissues** C Martin, *INSERM U281, Lyon, France*; JY Chapelon, D Cathignol 1757
2. **Spline Generated Laplacians of Evoked Potentials** SK Law, *Tulane University*; PL Nunez, AV Nelson, KL Pilgreen 1759
3. **Measurement of Autonomic Balance in Patients with Myocardial Infarction** M Tangella, *Rutgers University*; J Li, W Craelius 1761
4. **An Adaptive Fuzzy Controller for Blood Pressure Regulation** S Isaka, *University of California, San Diego*; AV Sebald 1763
5. **Quantitative Measurements of the Three-Dimensional Anatomy of the Guinea Pig Cochlea Using Optical Sectioning** AH Voie, *University of Washington*; FA Spelman, D Sutton, DH Burns 1765
6. **A New Skin Substitute Suitable for Immediate Coverage of Severe Burns** L Shahabeddin, *Laboratoire des Substituts Cutanes, Lyon, France* 1767

Track 12: Artificial Intelligence and Information

Chair: Stanley Finkelstein

Session 12.01: Intelligent Measurement Systems

Session Chair: Ewart R. Carson, City University, London, U.K.

1. **Evaluating Intelligent Measurement Systems: A Study in Ventilator Management** ER Carson, *City University, London*; DG Cramp, R Summers 1769
2. **Statistical Analysis of the Effect of Input Errors on Expert System Advice** WA Hyman, *Texas A&M University*; DA Mitta 1771

3. **Distribution of Principal Sources of Invalid Physiologic Data** SJ Aukburg, 1773
University of Pennsylvania; PV Matsiras, D Garfinkel, ER Carson
4. **Detection of False Alarms Using an Integrated Anesthesia Monitor** MJ Navabi, 1774
University of Arizona; KC Mylrea, RC Watt
5. **RESPAID: Computer Aided Decision Support For Respiratory Data in I.C.U.** MC 1776
Chambrin, University of Lille, France; C Chopin, P Ravaux, J Mangalaboyi, P Lestavel, F Fourrier
6. **A Scheme for Updating Belief in a Hierarchy of Hypotheses and Its Application for the Interpretation of Laboratory Test Results** JJ Chelsom, 1778
City University, London; ER Carson

Session 12.02: Expert Systems in Cytology and Histology

Session Chair: Richard M. Donovan, University of California, Davis

1. **Unsupervised Conceptual Learning in a Diagnostic Expert System** PH Bartels, 1780
University of Arizona; D Thompson, JE Weber
2. **Expert System Techniques Applied to the In Situ Cytophotometric Detection of AIDS Viral Nucleic Acid** RM Donovan, 1782
University of California, Davis; L Song
3. **Guided Data Reduction for Flow Cytometry** JE Weber, 1783
University of Arizona; PH Bartels
4. **An Expert System for Cell Edge Detection Using the Heuristic Search Method** L Song, 1785
University of California, Davis; RM Donovan
5. **From Vision to Diagnosis: A Multi-Agent System in Biomedicine** C Garbay, 1787
Universite Joseph Fourier, Grenoble, France
6. **An Expert System for Banded Chromosomes Recognition** Y Lu, 1789
Southeast University, China; Y Yan

Session 12.03: AI-Based Augmentative Communication Systems

Session Chair: John Deller, Michigan State University

1. **AI and Augmentative Communication: Where Are We and Where to go?** BK Sy, 1791
City University of New York
2. **Use of Myoelectric Signals to Recognize Speech** MS Morse, 1793
Auburn University; SH Day, B Troull, H Morse
3. **The Structure and Function of a Speech Control Language for Text Processing and Robotic Control** DM Horowitz, 1795
Tufts University; JM Hausdorff
4. **Parsing Method for Signed Telecommunication** MB Waldron, 1798
Ohio State University; D Simon
5. **Use of Object Oriented Language in Fast Prototyping of Communicators for Disabled** 1800
J Tichon, Universite Lille, France; G Trehou, JM Toulotte
6. **Expert Systems for Guidance of Cognitively Impaired People Performing Daily Living Activities** SP Levine, 1802
University of Michigan; JE Laird, NL Kirsch

Session 12.04: Expert Systems in EEG Analysis

Session Chair: John Glover, Periklis Ktonas, University of Houston

1. **Information Processing Models for Automatic Sleep Scoring** JC Principe, 1804
University of Florida; TG Chang, SK Gala, AP Tome
2. **AI Techniques for K-Complex Detection in Human Sleep EEGs** BH Jansen, 1806
University of Houston; BM Dawant, K Meddahi, W Martens, P Griep, AC Declecrk
3. **Rule Based System for Interpretation of Evoked Potential Waveforms** JR Boston, 1808
University of Pittsburgh
4. **Spike Detection Using a Syntactic Pattern Recognition Approach** R Walters, 1810
University of Florida; JC Principe, SH Park
6. **Computer-Aided Locomotion by Implanted Electrical Stimulation (CALIES Project)** 985
P Rabischong, INSERM U103, Montpellier, France; JL Weber, E Rabischong, JP Micallef, E Peruchon, B Gilbert, JM Jullian

Session 12.05: Expert Systems in Psychiatry

Session Chair: Joseph Bronzino, Trinity College

1. **Artificial Intelligence in Psychiatry: Issues and Questions** R Morelli, Trinity 1812
College
2. **Distributed Expertise: Motivation to Explore Alternative Approaches** CH Stinson, 1814
University of California, San Francisco
3. **INTERLOCUTOR: Conferring with an Expert Diagnostic Consultant in Geriatric Psychiatry** G Werner, University of Pittsburgh; E Smith 1816
4. **An Approach to the Design of a Clinically Acceptable Expert Alert System** JW 1819
Goethe, Institute of Living, Hartford, CT;

Session 12.06: Reasoning in Clinical Expert Systems

Session Chair: Jeffrey Budd, University of Minnesota

1. **Matrix Cognition in Medical Artificial Intelligence** JH Frenster, Physicians' 1821
Educational Series, Atherton, CA
2. **Formation of Pathophysiologic Theories** LM Fu, University of Wisconsin - 1823
Milwaukee
3. **Medical Reasoning by Causal Simulation** LM Fu, University of Wisconsin - 1825
Milwaukee
4. **Causal Expert Systems Supporting Medical Decision Making and Medical Education** 1827
G Molino, Universita di Torino, Italy; L Console, P Torasso
5. **A Combined Statistical and Rule-based Classifier** D Tien, University of Sydney, 1829
Australia; P Nickolls

6. **Clinical Decision Making Based on Expert Knowledge Relational Rules** J Ruskowski, 1830
Medical Center of Postgraduate Education, Poland

Session 12.07: Knowledge Representation in Clinical Information Systems

Session Chair: Peter Kerkhof, University of Utrecht, The Netherlands

1. **Matrix Structure Representation of a Medical Knowledge Base** PLM Kerkhof, 1832
University of Utrecht, The Netherlands
2. **A Feature Selection Approach to Concept Acquisition** I Moraes, 1834
University of Toledo; KJ Cios
3. **Symbolic Processing in Intelligent Monitoring** V Moret-Bonillo, 1836
Medical College of Georgia; A Alonso-Betanzos, B Arcay-Varela
4. **Use of Objects in a Hospital Information System** JM van den Berg, 1838
Leiden State University, The Netherlands; H Berger, TD Meijler
5. **Medical Informatics at the AMA: Computer Oriented Biomedical Nomenclature** DO 1839
Schiffman, American Medical Association, Chicago
6. **Towards a Knowledge-Based System of Structural Biology** J Brinkley, 1841
University of Washington; J Prothero

Session 12.08: Man-Machine Interfaces in Expert Systems

Session Chair: Alessandro D'Atri, Universita dell'Aquila, Italy

1. **Towards More Friendly and Powerful Medical Information Systems** A D'Atri, 1843
Universita dell'Aquila, Italy; P DiFelice
2. **GENESE: Narrowing the Gap Between Experts and Systems** JD Fekete, 1845
C2V, Paris; B Hap, R Dumeur
3. **Browsing in an Ambulatory Information System** V Curro, 1847
Universita di Roma, Italy; L Tarantino
4. **Man-Machine Interaction in Deep Diagnostic Systems** P Torasso, 1849
Universita di Torino, Italy; L Console, P Terenziani, G Molino
5. **Intelligent Databases for Medical Statistical Analysis** G Falcitelli, 1851
ISRDS/CNR, Rome, Italy; DM Pisanelli, FL Ricci
6. **Representing Image Analysis Strategies by a Hybrid Formalism** F de Rosis, 1853
Institute of Computer Science, Bari, Italy; A Giancaspro, G Pasquariello, S Pizzutilo

Session 12.09: Clinical Decision Support Systems I

Session Chair: Stanley Finkelstein, University of Minnesota

1. **ARRES: Computer Assisted Post Anesthesia Care Unit Monitoring System** PH 1855
Ketikidis, University of Pennsylvania; DS Kitz, JH Lecky, TG Mavrides, BB Matschinsky, SJ Aukburg
2. **WEANPRO: A Weaning Protocol Expert System** DA Tong, 1857
Louisiana Tech University, Ruston; EB Golden, SA Napper

3. **NST Expert: An Intelligent Program for NST Interpretation** A Alonso-Betanzos, *Medical College of Georgia*; V Moret Bonillo, LD Devoe, JR Searle, C Boveda Alvarez 1859
4. **A Knowledge Based System to Suggest Adaptive Equipment for Disabled Drivers** JC Wheeler, *Texas A&M University*; R Koppa, M McDermott, N Ellis, RD Huchingson 1861
5. **An Expert System to Aid in the Prescription of Electronic Augmentative Communication Devices** KV Bertrand, *Louisiana Tech University, Ruston*; SA Napper, PM Ezell 1863
6. **Decision Support Systems and Communication in Medicine at the University of Limburg** A Hasman, *University of Limburg, The Netherlands* 1865

Session 12.10: Clinical Decision Support Systems II

Session Chair: Masahiko Okada, Niigata University, Niigata City, Japan

1. **Implementing Cognitive Procedures in Diagnostic Processes** V Moret-Bonillo, *Medical College of Georgia*; A Alonso-Betanzos, C Hernandez-Sande 1867
2. **Problems and Solutions in the Development of an Expert System** S Shiina, *Tokyo Medical & Dental University, Japan* 1869
3. **Evaluation of a Knowledge-Based System for the Management of Fluid/Electrolyte Disorders** A Shamsolmaali, *City University, London*; PO Collinson, TG Gray, DG Cramp, ER Carson 1871
4. **Development of the Real-Time Data Check Expert System for Automatic Blood Chemistry Analyzer** K Morishita, *Hitachi Ltd., Japan*; K Nakayashiki, T Yokoyama, T Sato, K Ishida 1873
5. **COMA: A Computer Aided System for Orientation of Comatose Patients to Specialized Sections** MO Boniface, *University of Lille, France*; M Chambrin, F Fourier, C Basquin, JL Salomez, C Chopin, J Mangalaboyi, D Mathieu, P Cahen, JJ Marquis 1875
6. **EDDY: An Expert System in Dysmorphology Based on Truth-Maintenance** IP Popchev, *Institute of Industrial Cybernetics & Robotics, Bulgaria*; NP Zlatareva, LJ Sinapova 1877

Track 13: Computers in Medicine

Chair: Swamy Laxminarayan

Session 13.01: Physiological Data Characterization

Session Chair: Robert Peura, Worcester Polytechnic Institute

1. **New Mathematical Tool for Testing Functional Homology Between Protein Sequences** D Pantic, *Centar za Multidisciplinarne Studije, Yugoslavia*; S Kun, Z Bozovic, Z Stojiljkovic, M Trajkovic 1879
2. **Dimensional Analysis of the Electroencephalogram During General Anesthesia** RC Watt, *University of Arizona*; KC Ehlers, PJ Scipione, ES Maslana, SR Hameroff 1881

3. **Efficient Generation of Clinical EEG Contour Maps** TM Mohansingh, *Case Western Reserve University*; TF Collura 1883
4. **Fractal Number and Spectral Skewness: Two Features for the Pattern Classification of Motor Unit Action Potentials** AF Kohn, *University of Sao Paulo, Brazil* 1885
5. **A Method of Spectra-Analysis for Abnormal Voice Signals** G Kang, *Huazhong University of Science & Technology, China*; J Lin, Q Yang, G Cai 1887
6. **A Practical Technique for Measuring Peripheral Nerve DCV** ZL Kovacs, *University of Sao Paulo, Brazil* 1889

Session 13.02: Biomedical Supercomputing - Molecular Dynamics

Session Chair: Janardan S. Yadav, NJ Med School; T P Singh, All India Inst of Medical Sciences

1. **Designing of Structures of Peptides and Proteins** HC Patel, *All India Institute of Medical Sciences, India*; P Narula, TP Singh 1890
2. **Analysis and Prospective Design for the Treatment of Cancer and AIDS** FH Hausbeer, *University of Texas Health Science Center at San Antonio* 1892
3. **Protein Dynamics by CRAY Supercomputer: Gating of a Ca^{++} Channel in a Unique 19kD Hydrophobic Phosphoprotein, Amelogenin from Bovine Tooth Enamel** V Renugopalakrishnan, *Harvard Medical School*; M Prabhakaran, SG Huang, E Strawich, J Glimcher 1895
4. **Molecular Modeling in Protein Design and Engineering** FA Momany, *Polygen Corp., Waltham, MA* 1897
5. **Molecular Modeling of Oligosaccharides in Biomolecular Engineering** M Dauchez, *INSERM U279, Lille, France* 1899
6. **Conformational Structure of the Immunologically Active Pentasaccharide of Forssman Antigen** PNS Yadav, *Banaras Hindu University, India*; DK Rai, JS Yadav 1900

Session 13.03: Biomedical Supercomputing - Drug Design

Session Chair: V. Renugopalakrishnan, Harvard Medical School

1. **Supercomputers in Drug Design** JS Yadav, *University of Medicine & Dentistry of New Jersey*; S Laxminarayan, L Michelson 1901
2. **Using Theoretical Descriptors in Quantitative Structure Activity Relationships** GR Famini, *US Army Development and Engineering Center, Aberdeen Proving Grounds*; LY Wilson 1903
3. **The Role of Shape Analysis in Drug Design** PG Mezey, *University of Saskatchewan, Canada* 1905
4. **Quantitative Measures of Molecular Similarity** GA Arteca, *University of Saskatchewan, Canada*; PG Mezey 1907
5. **Crystallography of Carbohydrates** MK Strumpel, *Free University of Berlin, FRG*; P Luger 1909

6. **Parallel Processing and Computational Chemistry** P Weiner, *Alliant Computer Systems, Littleton, MA* 1911

Session 13.05: Medical Databases

Session Chair: O. Prakash, Erasmus University, The Netherlands

1. **A Computerized Approach to Injury Description** G Reddy, *University of New Mexico*; D Fletcher, T Osler 1912
2. **The MeDEA Project: Supporting Coding of Medical Documents** A Rossi-Mori, *ITBM CNR, Rome, Italy*; DM Pisanelli, P Chiappetta, M Riccardi 1914
3. **An Electronic System for Integrated Management of Patients' Data and Radiological Images** A Agnifili, *Universita dell L'Aquila, Italy*; G Di Stefano, P Pavone, C Buoni, R Passariello 1916
4. **Relational Database: A Radiation Therapy Machine Control Software Development Tool** J Jacky, *University of Washington*; R Risler, S Brossard, I Kalet 1918
5. **Personal Computer Analysis of Risk Factors in Kidney Transplantation Programmes** A Buscaroli, *Universita di Bologna, Italy*; B Stagni, A Temi, S Stefoni 1920

Session 13.06: Visualization & 3-D Modeling

Session Chair: Shiva Ayyadurai, Massachusetts Institute of Technology

1. **Computer Visualization Techniques Applied to Vestibular Research** MD Ross, *NASA Ames Research Center*; G Meyer, L Cutler, T Lam, D Mugler 1922
2. **Automatic Identification of Tissue Interfaces** H Rusinek, *New York University* 1924
3. **The Analytical Descriptors of Three-Dimensional Geometry of Human Organs** G Tritto, *Clinique Hartmann, Paris*; MC Tritto, G Pirlo 1926
4. **Display of EEG Chaotic Dynamics** FS Yu, *University of Florida*; J Principe, S Reid 1928
5. **Active Volumetric Compositing to Display Fuzzy Structures from Multiscale Volume Data** C Barillot, *Pontchaillou Hospital, Rennes, France* 1930
6. **Modeling Human Organs with Free-Form Surfaces** J Sequeira, *IBM Paris Scientific Center, France* 1932

Session 13.07: Computer-Aided Medical Procedures

Session Chair: Y. C. Pao, University of Nebraska, Lincoln

1. **Computer-Assisted Stereotaxic Surgery** CF Walker, *Tulane University*; U Taneja 1934
2. **A Three-Dimensional Locating Pointer for Stereotactic Neurosurgery** RL Galloway, *Vanderbilt University*; CA Edwards, GL Haden, RJ Maciunas 1935
3. **A Computer Assisted Stereotactic Approach for Multibeam Radiation Therapy Planning** F Fresne, *Pontchaillou Hospital, Rennes, France*; B Gibaud, C Barillot, C Toumoulin, D Lemoine, JP Manens, JM Scarabin 1936
4. **A Review of CAD/CAM Procedures for the Production of Custom Made Artificial Hip Joints** GR Harvey, *Queen's University of Belfast, U.K.*; RAH Harvey, DRH Harvey 1938

5. **Bi-Cubic Spline Fitting and Display of Tooth External Surface and Canal** YC Pao, 1940
University of Nebraska, Lincoln; PY Qin, QS Yuan
6. **Flow Dynamics of Different By-Pass Configurations: A Numerical Approach** R 1942
Pietrabissa, Politecnico di Milano, Italy; F Inzoli, R Fumero

Session 13.08: Computer Models of Physiological Systems

Session Chair: Amit Chatterjea, Indiana Univ-Purdue University, Ft Wayne, IN

1. **Computer Modelisation of Myocardial Depolarization. Application to Fibrillation** AL 1944
Bardou, INSERM U256, Hopital Broussais, Paris; P Auger, A Coulombe, MC Govaere, JM Chesnais, D Von Euw
2. **Optimal Design of Corneal Refractive Surgery** MR Bryant, *University of* 1946
Wisconsin-Madison; SA Velinsky
3. **Cardiac Dysrhythmias — A Phenomenon of Chaos** B Valiquette, *Ecole* 1948
Polytechnique de Montreal; G Lambert Torres, D Mukhedkar
4. **Soft Tissues' Expanders: Computer-Assisted Simulation of Elastic Mesh Deformation** 1951
G Tritto, Clinique Hartmann, Paris; G Pirlo, MC Tritto
5. **A Comparison of Mathematical Models of Nasal Pressure-Flow Data** RE Frye, 304
University of Pennsylvania; A Mester, DA Deems, RL Doty
6. **Human Body Motion Image Analysis System** GA Rong, *Tsinghua University,* 1953
China; JW Huang

Session 13.09: Data Acquisition & Simulation Systems

Session Chair: F. Pinciroli, Politecnico di Milano, Italy

1. **High Speed PET Data Acquisition with a Standard Mini Computer** TK Lewellen, 1955
University of Washington; CP Anson, SD Vannoy, RS Miyaoka
2. **A Desktop Computer Software Package for Display and Analysis of Multidimensional** 1957
Medical Images BJ Erickson, *Mayo Clinic; RA Robb*
3. **The EKG Challenger Revisited** SK Ananthraman, *New Jersey Institute of* 1959
Technology; SS Reisman, KJ Friedman
4. **Teaching Dynamic Electrocardiography by Means of Environmental Simulation:** F 1961
Pinciroli, Politecnico di Milano, Italy; V Castelli, G Mosca
5. **A Generalized ECG Simulator: An Educational Tool** I Sadighi, *Montana State* 1963
University; M Kejariwal
6. **A Simulator of Therapies for Education in Diabetes** EJ Gomez-Aguilera, 1965
Universidad Politecnica de Madrid, Spain; MT Arredondo, JL Zoreda, F del Pozo

Session 13.10: Computer-Based Medical Systems

Session Chair: J. Kampmann, Medizinische Hochschule, Hannover, FRG

1. **An Integrated Real-Time Computerized Hearing Testing System** L Shao, 1967
University of Michigan; BE Pfingst

2. **A Workstation for Quantitative Analysis of Magnetic Resonance (MR) Cardiac Images** TG Kubit, *Medical College of Ohio*; JW Klingler, LT Andrews, RF Leighton, J Zeiss 1969
3. **Quantitative Analysis of Joint Images from Patients with Rheumatoid Arthritis** RM Valente, *Mayo Clinic*; HS Luthra, RA Robb 1971
4. **A Device to Measure Inhalation with a Powder Inhaler** PO Fagerstrom, *R&D Electronics, Lund, Sweden*; T Wendel, L Berggren, G Nilsson 1973
5. **Performance Optimization for Real-Time Computers in Medical Applications** EA Schroepel, *MODCOMP, Fort Lauderdale, FL* 1975
6. **Implications of Surgical Simulation Technology Upon the Training of Arthroscopic Surgeons** JS Hersh, *Rensselaer Polytechnic Institute* 1977

Session 13.11: Microcomputer Applications

Session Chair: Swamy Laxminarayan, New Jersey Medical School

1. **An Efficient Algorithm for Estimating Circulatory Mechanical Parameters by Microcomputers** G Avanzolini, *University of Bologna, Italy*; A Cappello, G Gnudi 1979
2. **A Computer Based System for Monitoring Heat and Fluid Balance in Severely Burned Patients** JC Ferguson, *University of Aberdeen, U.K.*; CJ Martin, C Rayner, JR Mallard 1982
3. **Patient Care Workstation for Realtime Monitoring** O Prakash, *Erasmus University, The Netherlands* 1984
4. **Quantification of Body Tremor** HG McAllister, *University of Ulster, U.K.*; PJ McCullagh, J Kelly 1985
5. **A Microcomputer-Based Modular Data Acquisition and Processing System for Physiological Study** KW Lin, *National Cheng-Kung University, Taiwan*; MS Young, SK Liao 1987
6. **Computer-Aided Assessment of Vascular Reactivity in Subjects with Essential Hypertension** MB Chilukuri, *Vanderbilt University*; T Susawa, AB Yerby, LQ Zhang, JA Zimmer, RM Robertson, JC Collins 1989

Session 13.12: Expert Systems

Session Chair: C. Hernandez, University of Santiago, Spain

1. **Combination of a Neural Network Model and a Rule-Based Expert System to Determine Efficacy of Medical Testing Procedures** ME Cohen, *University of California, San Francisco*; DL Hudson, MF Anderson 1991
2. **Physical and Functional Integration System for Intelligent Processing and Prioritization of Variables in an ICU** B Arcay, *University of Santiago, Spain*; V Moret, R Balsa, C Hernandez 1993
3. **More Than Classical Patient Monitoring: Steps Towards Intelligent Approaches** S Barro, *University of Santiago, Spain*; R Ruiz, J Mira 1995
4. **Clinical Datafiles and Expert Systems: Knowledge Evaluation from Data Analysis** A Duhamel, *CERIM, Lille, France*; P Roussel, C Robert, L Moussu 1997

3. **More Than Classical Patient Monitoring: Steps Towards Intelligent Approaches** S Barro, *University of Santiago, Spain*; R Ruiz, J Mira **1995**
4. **Clinical Datafiles and Expert Systems: Knowledge Evaluation from Data Analysis** A Duhamel, *CERIM, Lille, France*; P Roussel, C Robert, L Moussu **1997**
5. **Expert System on Neonatal Birth Injuries** C Veena Kumari, *Andhra University, India*; G Sundara Rao, G Madhusudhana Rao, KVV S Reddy **1999**
6. **Fundamental Study of Auxiliary Diagnostic System** Y Yang, *Zhejiang University, China*; J Ge **2001**

Session 13.13: Systems, Standards and Information Management Systems

Session Chair: Dinkar Mukhedkar, *Ecole Polytechnique de Montreal*

1. **Physiological Implementation of the Basilar Membrane/Vocal Tract on Transputers** K Adamson, *University of Ulster, U.K.*; G Donnan, ND Black **2003**
2. **OB Information Management System: A Microcomputer Solution** S Subramanian, *University Hospital, Stony Brook* **2005**
3. **Multimodality Image Registration Techniques in Medicine** M Moshfeghi, *Phillips Laboratories, Briarcliff Manor, NY* **2007**
4. **An Automatic Medical Emergency Telephone Communications Device** WJ Jameson, *Montana State University*; M Kejarawal, T Herreid, C Mitten, JM Ray **2009**
5. **On Using Formal Software Engineering Techniques in an Academic Environment** CP Anson, *University of Washington*; RL Harrison, TK Lewellen, SB Gillispie, KP Pollard, AN Bice, RS Miyaoka, DH Haynor, J Zhu **2011**
6. **Requirements for an Interchange Format for Digitized EEG** EC Jacobs, *Cleveland Clinic Foundation*; TF Collura, RC Burgess **2013**

Track 14: Neural Networks

Chair: Evangelia Micheli-Tzanakou

Session 14.01: Neural Networks in Cardiology

Session Chair: M. Waldron, *Ohio State University*

1. **Detection of Abnormal Electrocardiograms Using a Neural Network Approach** JY Cheung, *University of Oklahoma*; SS Hull, Jr. **2015**
2. **Classification of QRS Pattern by an Associative Memory Model** KP Lin, *Chung-Yuan Christian University, Taiwan*; WH Chang **2017**
3. **Data Compression Using Neural Network for Digital Holter Monitor** Y Nagasaka, *Nagoya Institute of Technology, Japan*; A Iwata, N Suzumura **2019**
4. **Neural Nets for ECG Classification** E Pietka, *Technical University of Silesia, Poland* **2021**
5. **A Neural Network Weight Pattern Study with ECG Pattern Recognition** Q Xue, *University of Wisconsin - Madison*; Y Hu, WJ Tompkins **2023**

- | | | |
|----|---|------|
| 6. | Using a Translation-Invariant Neural Network to Diagnose Heart Arrhythmia SC
Lee, <i>Johns Hopkins University</i> | 2025 |
|----|---|------|

Session 14.02: General Applications of Neural Networks

Session Chair: O. Ozdamar, University of Miami

- | | | |
|----|--|------|
| 1. | Evaluation of a Neural Network for Fault-Tolerant, Real-time, Adaptive Control DJ
Wasser, <i>University of North Carolina, Chapel Hill</i> ; DW Hislop, RN Johnson | 2027 |
| 2. | A Micropopulation Model Adaptation for Neural Network Studies E Ackerman,
<i>University of Minnesota</i> ; D Kilis, GA Hatfield | 2029 |
| 3. | Wormhole-type Routing in Neural Networks A Shaout, <i>Univeristy of Michigan - Dearborn</i> ; K Akingbehin | 2032 |
| 4. | Use of Neural Networks for Detection of Artifacts in Arterial Pressure Waveforms
AV Sebald, <i>University of California, San Diego</i> | 2034 |
| 5. | An Electronic Neurocomputer Using General Purpose Floating Point Digital Signal Processors A Iwata, <i>Nagoya Institute of Technology, Japan</i> ; Y Sato, N Suzumura, S Matsuda, Y Yoshida | 2036 |
| 6. | Decomposing Neural Networks into Systems of Cooperating Subnets S Mukherjee,
<i>North Carolina State University</i> ; M White | 2038 |

Session 14.03: Vision and Neural Networks

Session Chair: S. Usui, Toyohashi University of Technology, Japan

- | | | |
|----|---|------|
| 1. | Efficient Presentations of Learning Samples to Accelerate the Convergence of Learning in Multilayer Perceptron A Okamoto, <i>Nagoya University, Japan</i> ; N Ohnishi, N Sugie | 2040 |
| 2. | Need for a Knowledge-based Subsystem in Evoked Potential Neural-Net Recognition System I Bruha, <i>McMaster University, Canada</i> ; GP Madhavan | 2042 |
| 3. | Neural Network Model of Color Vision S Usui, <i>Toyohashi University of Technology, Japan</i> ; S Nakauchi, S Miyake | 2044 |
| 4. | EEG Waveform Analysis Using CaseNet RC Eberhart, <i>Johns Hopkins University</i> ; RW Dobbins, WRS Webber | 2046 |
| 5. | Convergence of Images in the Alopex Process with Moment Invariants and Probabilities E Micheli-Tzanakou, <i>Rutgers University</i> ; TS Chon | 2048 |

Session 14.04: Modeling Using Neural Networks

Session Chair: R. Eberhart, Johns Hopkins University

- | | | |
|----|---|------|
| 1. | Experimental Study on Origin of Cerebral Bioelectric Rhythm D Pinzhong,
<i>Tianjin University, China</i> ; G Yian | 2050 |
| 2. | Confusion Test on Content Addressable Memory Model W Hu, <i>University of North Carolina, Chapel Hill</i> ; HS Hsiao | 2052 |
| 3. | Phase Dependent Output in a Time Varying Neural Net D Pollock, <i>Ohio State University</i> ; MB Waldron | 2054 |

4. **Construction of Multiplier By Neural Network** N Toda, *Toyohashi University of Technology, Japan*; S Usui **2056**
5. **Connectionist Modeling vs. Bayesian Procedures for Sparse Data Pharmacokinetic Parameter Estimation** R Shadmehr, *University of Southern California*; DZ D'Argenio **2058**
6. **A Neural Network Model of Transformations in the Somatosensory System** IN Bankman, *Johns Hopkins University*; KO Johnson, SS Hsiao **2060**

Authors' Index

A1

Keywords Index

K1

THE DYNAMIC BREAKDOWN OF HEART CELL MEMBRANES EXPOSED TO RAMP INCREASES IN TRANSMEMBRANE POTENTIAL

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ABSTRACT

Membrane patches of single frog ventricular cells drawn into glass micropipettes were exposed to relatively large transmembrane potentials using a custom-built whole cell patch clamp unit. The current through the pipette was measured during the application of voltage ramps which enabled the observation of rapid changes in membrane impedance due to electrical breakdown. Our results show that the dynamic electrical breakdown of heart cell membrane involves threshold levels of transmembrane potential above which there exist, 1) a period of reversible membrane instability, and 2) a rapid, irreversible increase in membrane permeability.

The data suggest that the threshold voltages for the onset of these events are independent of the polarity of the applied voltage ramp. The data support the theory that membrane breakdown is a function of transmembrane potential, i.e. a process of electroporation. The advantage of using ramp waveforms for membrane breakdown studies is discussed.

INTRODUCTION

Electroporation of the cell membrane by high intensity, pulsed electric fields appears to be a universal phenomenon and is critically dependent on the magnitude of the induced transmembrane potential [1,2]. A transmembrane potential around 1 volt appears to be the threshold level for electroporation to occur [3]. Direct studies have not, however, been performed in cardiac muscle, despite the common clinical situation in which heart muscle is subjected to intense electric fields during defibrillation. Electrophysiological studies in intact heart [4] and heart cell aggregates [5] provide indirect evidence for an increase in the permeability of the cell membrane as a function of field intensity. Our laboratory has recently used cell contractility as a biological assay of cell permeability and shown it to be a hyperbolic function of field intensity and duration [6]. One difficulty with this approach is that the relation of transmembrane potential to the applied electric field is not known exactly. We have therefore adopted a variation of the whole cell voltage clamp technique, using a suction pipette with a "loose seal" around a membrane patch. In this way we can control the membrane potential across a portion of the cell membrane and observe the dynamic change in membrane resistance as the threshold for breakdown is exceeded. The aim of our study is to obtain information relevant to the understanding of the possible role of electroporation in high energy shock of cardiac muscle.

METHODS

Single ventricular heart cells (5 - 10 μm diameter, 200 - 400 μm long) isolated from adult frogs (*Rana pipiens*) were drawn into a glass micropipette using suction until a seal resistance of 10 - 50 $\text{M}\Omega$ was attained (see Figure 1 inset diagram) [7]. Seal resistances <10 $\text{M}\Omega$ allowed too much shunting of current to permit the

changes in the cell membrane impedance to be sensed. Cell size and pipette lumen diameter determined how much of the cell entered the pipette; the range was approximately 5 - 15 μm .

The experimental set-up included an inverted microscope, a custom-built whole cell patch clamp unit, and a ramp generator circuit. The experimental protocol was to 1) apply suction to the pipette while imposing 10 mV, 2.0 msec rectangular pulses to monitor the seal resistance; 2) apply ± 100 mV, 7.0 msec ramps to assess the linearity of the total pipette impedance; and 3) deliver a single ± 2.0 V, 7.0 msec ramp shock. A transmembrane potential of 1 volt corresponds to a peak field intensity on the order of 10^6 V/cm across the cell membrane. The total pipette impedance consists of three major parallel pathways (see Figure 1 below): 1) the distributed capacitance across the glass pipette, 2) the cell-to-glass seal resistance, and 3) the cell membrane impedance. Changes in 3) would then be reflected in changes in pipette impedance.

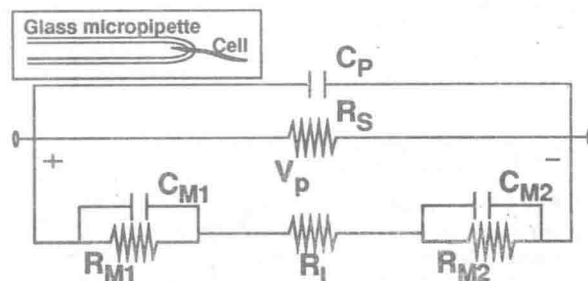


FIGURE 1 - Equivalent electrical circuit for the total pipette impedance: C_p is the distributed pipette capacitance, R_s is the cell-to-pipette seal resistance, $R_{M(i)}$, $C_{M(i)}$ are the membrane resistance and capacitance (subscript 1 denotes membrane patch within pipette and 2 denotes the membrane within the bath), R_i is the intracellular resistance and V_p is the pipette voltage. The inset diagram depicts the cell inserted into the pipette.

EXPERIMENTAL RESULTS

Following the formation of the seal the cell was exposed to the low level voltage ramps shown in Figure 2 A. For the case of a cell depolarized by using a bath solution which mimicked the intracellular environment (high potassium, low sodium, zero calcium), the pipette current responded linearly and symmetrically (Figure 2 B). This current most likely flows through the seal resistance pathway (Figure 1). Depolarization of the cell was used to inhibit ionic action currents which would interfere with the measurement of membrane impedance.