SIXTY-EIGHTH ANNUAL MEETING

THE AMERICAN THYROID ASSOCIATION, INC.

THE FAIRMONT HOTEL

CHICAGO, ILLINOIS

****

Wednesday, September 28, 1994

Thursday, September 29, 1994

Friday, September 30, 1994

Saturday, October 1, 1994

****

PLEASE BRING THIS PROGRAM TO THE MEETING

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The Van Meter Prize, supported by Forest Pharmaceuticals, Inc., is awarded to a person who has made outstanding contributions to research on the thyroid gland or related subjects. The awardee must not have reached his or her 41st birthday before September 1st of this year.

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1973 Martin I. Surks
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1992 Margaret Shupnik
1993 J. Larry Jameson

PAUL STARR AWARD

The Paul Starr Award, supported by Daniels Pharmaceuticals, Inc. and Boris Catz, M.D., is specifically intended to acknowledge an outstanding contributor to clinical thyroidology.

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1982 Albert G. Burger
1983 Aldo Pinchera
1984 John Nicoloff
1986 Hugo Studer
1987 Reginald Hall
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1989 G. Hennemann
1991 Samuel Refetoff
1992 John Stanbury
1993 Orlo H. Clark
1994 Ian D. Hay

A.T.A. DISTINGUISHED PRIZE LECTURESHIP AWARD

This Distinguished Prize Lectureship Award is conferred upon an established investigator who has made major contributions in thyroid or related research. As of 1994, this award has been combined with the Sidney H. Ingbar Memorial Lectureship Award.

1978 Sidney H. Ingbar
1979 Jacob Robbins
1980 Alvin Taurog
1981 J. Maxwell McKenzie
1982 Delbert A. Fisher
1983 Jack H. Oppenheimer
1984 John Pierce
1986 Herbert H. Samuels
1987 Hans Cahnmann
1988 Inder J. Chopra
1989 P. Reed Larsen
1991 Robert Volpé
1992 Leonard Wartofsky
1993 Leslie J. De Groot

SIDNEY H. INGBAR DISTINGUISHED LECTURESHIP AWARD

Endowed by contributions to honor the memory of Sidney H. Ingbar and supported in part by Boots Pharmaceuticals. This Award recognizes outstanding academic achievements in the field of thyroidology in keeping with the innovation and vision that epitomized Dr. Ingbar's brilliant investigative career. The Award is conferred upon an established investigator who has made major contributions in thyroid or related research over many years.

1990 Lewis A. Braverman
1991 Basil Rapoport
1992 William W. Chin
1993 J. Maxwell McKenzie
1994 Bruce Weintraub

This program book is dedicated in

Memory of

Sidney Charles Werner, M.D.

The officers and members of The American Thyroid Association mourn the death of Sidney, our former president, esteemed colleague, dedicated teacher, eminent scientist, outstanding physician. His wisdom and friendship will be missed. We extend our sympathy and condolences to the family and friends of Dr. Werner.

We have established an Endowment Fund in Sidney's memory. Contributions to this fund can be sent to the ATA office, attention Manfred Blum, M.D., Treasurer

SPECIAL TOURS AND EVENTS - PROGRAM AT-A-GLANCE

TUESDAY, SEPTEMBER 27TH, 1994

WELCOME RECEPTION & BUFFET 7:00 pm

THE MID AMERICA CLUB
AMOCO BUILDING - ADJOINING THE FAIRMONT

WEDNESDAY, SEPTEMBER 28TH

FINANCIAL DISTRICT OF CHICAGO	8:45 am
HISTORIC CHICAGO	9:00 am
THE NORTH SHORE	1:00 pm
THE ART INSTITUTE	6:30 pm

THURSDAY, SEPTEMBER 29TH

THYROID ASSOCIATES LECTURE GOYA, CAPRICE & INVENTION	7:45 am
FAVORITE SONS OF OAK PARK	9:30 am
MICHIGAN AVENUE WALK	12:30 pm
GALLERY WALK	12:30 pm
1ST LADY CRUISE	6:30 pm
ALL THAT JAZZ...AND A BLUE NOTE	7:00 pm
CHICAGO SYMPHONY	7:15 pm

EN EVENING AT THE THEATER

GOODMAN THEATER - "MERCHANT OF VENICE" 7:30 pm - CURTAIN
SECOND CITY REVIEW - "SATIRE & COMEDY" 8:30 pm - SHOW TIME
BRIAR STREET THEATER - "LAUGHTER ON THE 23RD FLOOR" 8:00 pm - CURTAIN

FRIDAY, SEPTEMBER 30TH

THYROID ASSOCIATES LECTURE CURRENT STATE OF THE PRESIDENT'S HEALTH PLAN	7:30 am
UNIVERSITY OF CHICAGO AND ROBIE HOUSE	9:30 am
ETHNIC HERITAGE OF CHICAGO	9:30 am
ARCHITECTURAL INTERIORS WALKING TOUR	2:00 am
ANNUAL BANQUET RECEPTION & DINNER	6:30 pm

SATURDAY, OCTOBER 1ST

CHICAGO CUBS VS PITTSBURGH PIRATES 1:20 pm

THE AMERICAN THYROID ASSOCIATION, INC.

CORDIALLY INVITES YOU TO ATTEND
OUR "WELCOME RECEPTION & BUFFET"

AT THE PRESTIGIOUS

MID AMERICA CLUB

80TH FLOOR - AMOCO BUILDING ADJOINING THE FAIRMONT

TUESDAY - SEPTEMBER 27TH, 1994
From 7:00 PM to 9:30 PM

THIS EVENING IS SPONSORED BY:

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TICKETS ARE NOT REQUIRED FOR THIS EVENT

"PRE-MEETING"
SYMPOSIA
RECURRENT THYROID CANCER: MANAGEMENT DILEMMAS
Wednesday, September 28, 1994
Moulin Rouge Room - (lobby level) - The Fairmont Hotel

Continental Breakfast - 6:00 A.M.- 6:45 A.M.
Program - 6:45 A.M. to 7:45 A.M.

Join the experts in a discussion of practical clinical issues relating to the management of persistent/recurrent papillary Thyroid Cancer.
Thyroidologists will respond to a challenging case and provide recommendations for state-of-the-art diagnosis and treatment

Faculty

Leonard Wartofsky, M.D., MACP - Moderator
Professor of Medicine and Physiology
Uniformed Services University of the Health Sciences
Bethesda, Maryland
Chairman, The Department of Medicine
Washington Hospital Center
Washington, DC

Ernest L. Mazzaferri, M.D., FACP
Professor and Chairman of Internal Medicine
Professor of Physiology
The Ohio State University Hospitals
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Harry R. Maxon, III, M.D., FACP
Professor of Medicine
University of Cincinnati School of Medicine
Director
The Jean Mildred Paul Thyroid Cancer Registry
University of Cincinnati Medical Center
Cincinnati, Ohio

Space is limited
Please pre-register - 1 800 537-8087 extension 23

This program is made possible by an educational grant from the Department of Professional Education Boots Pharmaceuticals, Inc.

THE AMERICAN THYROID ASSOCIATION, INC.

CORDIALLY INVITES YOU TO ATTEND
A GALA EVENING FEATURING GALLERY VISITS, HORS D'OEUVRES AND MUSIC

AT

THE ART INSTITUTE OF CHICAGO

SOUTH MICHIGAN AVENUE AT ADAMS STREET

WEDNESDAY - SEPTEMBER 28TH, 1994
From 6:30 P.M. to 9:30 P.M.

THIS EVENING IS SPONSORED BY:

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ADMISSION BY TICKET ONLY

MEMBERSHIP APPLICATION FOR THE AMERICAN THYROID ASSOCIATION

Selection for membership in The American Thyroid Association, Inc. is based primarily on:

1. Demonstrated interest in pathophysiology of the thyroid.
2. Clinical and/or laboratory experience in thyroidology.
3. Publications related to the thyroid.
4. Attendance at meetings of The American Thyroid Association and national thyroid meetings.

IMPORTANT Completed applications must be submitted by July 1st to be considered for current year.

Membership Applied For: ACTIVE _____ CORRESPONDING _____ HONORARY _____ ASSOCIATE_____

DATE _____ Date of Birth _____ Social Security Number _____

Name _____ Phone / FAX _____

_____

Office Address

_____

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☐ 0 → plan to attend ☐ 1 → year _____ ☐ 2 → years _____ ☐ > 2 →years _____

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Teaching Appointments with Dates_____

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SENT TO: THE AMERICAN THYROID ASSOCIATION, INC.
MARTIN I. SURKS, M.D., SECRETARY
MONTEFIORE MEDICAL CENTER
111 EAST 210TH STREET
BRONX, NEW YORK 10467

THE AMERICAN THYROID ASSOCIATION, INC.

CORDIALLY INVITES YOU TO ATTEND OUR

"ANNUAL BANQUET"

RECEPTION AND DINNER

at the

FAIRMONT HOTEL
INTERNATIONAL BALLROOM

FRIDAY - SEPTEMBER 30TH, 1994

RECEPTION BEGINS AT 6:30

FOLLOWED BY:
AWARDS, DINNER AND DANCING

TICKETS: - $35.00 PER PERSON
$17.00 STUDENTS & FELLOWS

THIS SPECIAL PRICE IS MADE POSSIBLE BY THE GENEROUS CONTRIBUTIONS FROM:

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Tickets may be purchase at our registration booth

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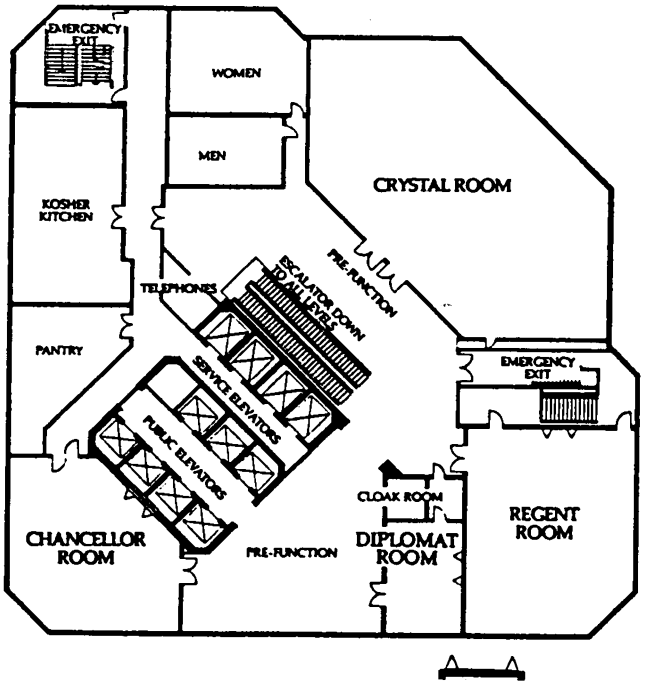
MEETING LOCATION MAPS

MEETING ROOM LEVEL
(2 levels above lobby)

HOSPITALITY
DIPLOMAT ROOM

MEET THE PROFESSOR LUNCHEONS
PRESIDENTS' RECEPTION
CHANCELLOR, REGENT, CRYSTAL ROOMS

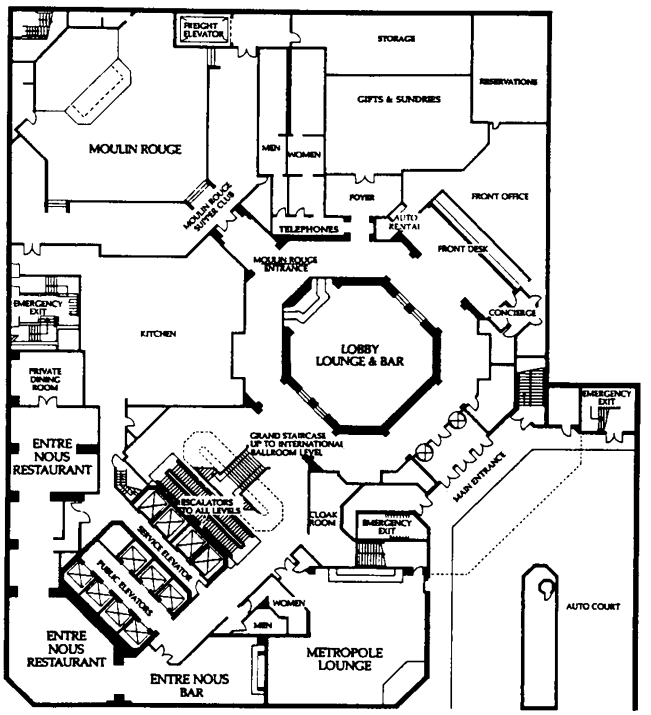
COUNCIL COMMITTEE MEETING
REGENT ROOM



LOBBY LEVEL

SATURDAY BREAKOUT SESSIONS
MOULIN ROUGE ROOM

MEET THE PROFESSOR LUNCHEON
METROPOLE LOUNGE



MEETING LOCATION MAPS

IMPERIAL BALLROOM LEVEL
(2 levels below lobby)

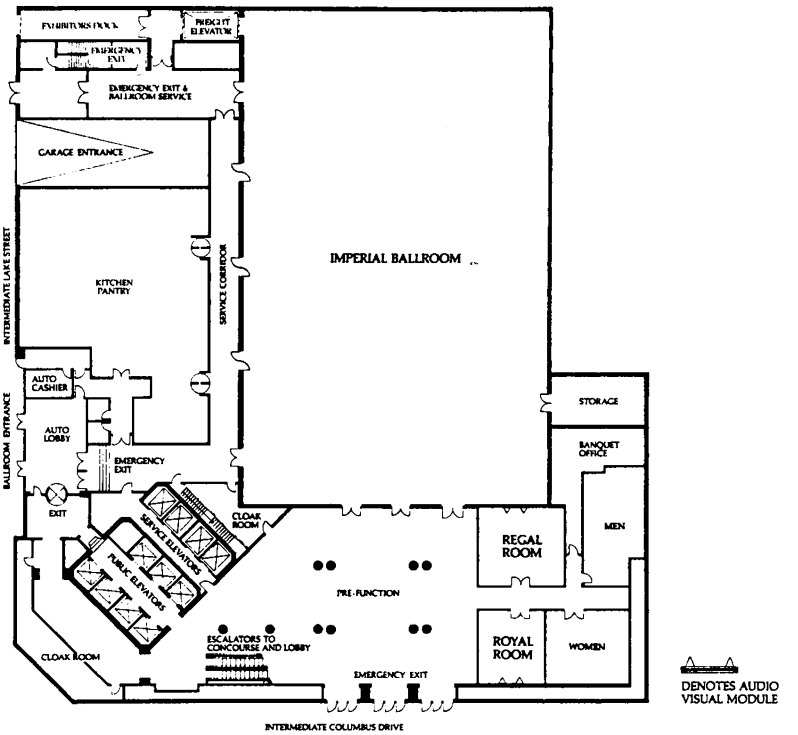
EXHIBITORS -
IMPERIAL BALLROOM

POSTERS -
IMPERIAL BALLROOM

COFFEE BREAKS -
IMPERIAL BALLROOM

SPEAKER READY ROOM -
ROYAL ROOM

INTERNATIONAL COMMITTEE MEETINGS &
EXECUTIVE COUNCIL MEETINGS -
REGAL ROOM



INTERNATIONAL BALLROOM LEVEL
(1 level above lobby)

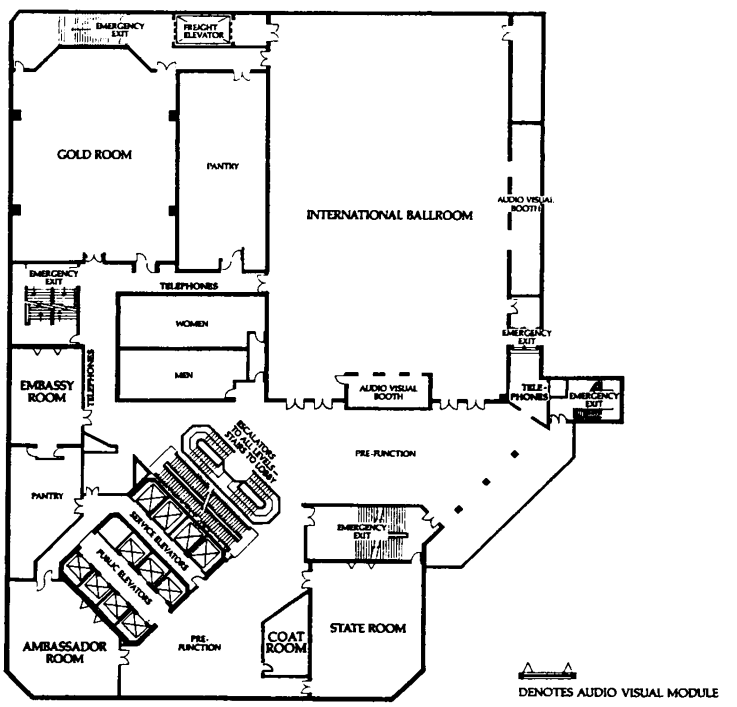
MEETING REGISTRATION -
INTERNATIONAL FOYER

GENERAL SESSIONS -
INTERNATIONAL BALLROOM

BREAKOUT SESSIONS -
GOLD ROOM

MEET THE PROFESSOR LUNCHEONS &
COUNCIL COMMITTEE MEETING
STATE, EMBASSY, AMBASSADOR ROOMS

THYROID ASSOCIATES LECTURES -
EMBASSY ROOM



A.T.A. TUESDAY SEPT. 27 - SATURDAY OCT. 1, 1994
MEETING AT A GLANCE

	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
7:00 am					
8:00 am			EARLY RISER LECTURE		EARLY RISER LECTURE (GOLD RM.)
9:00 am		WELCOME	ORALS	ORALS	SYMPOSIUM THYROID & MOOD
10:00 am		GRAND ROUNDS (8:10 - 10:15)	VAN METER LECTURE	INGBAR LECTURE	STATE OF THE ART CLONING
11:00 am		BREAK & POSTERS RECOMBINANT TSH SYMPOSIUM (10:30-12:00)	BREAK & POSTERS ORALS	BREAK & POSTERS ORALS	BREAK ORALS (GOLD RM) / SYMPOSIUM PEDIAT. (MOULIN)
noon					
1:00 pm		MEET THE PROFESSOR LUNCHEONS AND VISIT THE POSTERS	MEET THE PROFESSOR LUNCHEONS AND VISIT THE POSTERS	MEET THE PROFESSOR LUNCHEONS AND VISIT THE POSTERS	
2:00 pm					
3:00 pm		ORALS (INTERN'L) / PRIV. PRACT (GOLD RM)	ORALS CANCER (INTERN'L) / ORALS TH ACT'N (GOLD RM)	ORALS AUTOIM. (INTERN'L) / ORALS TSH-TRH (GOLD RM)	
4:00 pm	REGISTRATION AND EXHIBITS OPEN	POSTERS AND EXHIBITS	POSTERS & EXHIBITS ORALS (INTERN'L) / SYMPOSIUM TR'FICK (GOLD RM)	POSTERS & EXHIBITS ORALS (INTERN'L) / SYMPOSIUM TSH-R (GOLD RM)	
5:00 pm		CONTROVERSIES IN GRAVES' DISEASE			
6:00 pm		HISTORICAL	PAUL STARR LECTURE	PRESIDENTIAL ADDRESS & BUSINESS MEETING (GOLD RM)	
7:00 pm		ART INSTITUTE RECEPTION (BUSES FROM 6:00)		RECEPTION	
8:00 pm	WELCOME RECEPTION & BUFFET MID AMERICA CLUB			ANNUAL BANQUET AND DANCE (INTERN'L RM)	
9:00 pm					

THE AMERICAN THYROID ASSOCIATION, INC.

TUESDAY, SEPTEMBER 27, 1994

THE FAIRMONT HOTEL

CHICAGO, ILLINOIS

8:00 A.M. **EXHIBITORS' SET-UP**
Imperial Ballroom

10:00 A.M. **MEETING—INTERNATIONAL THYROID CONGRESS COMMITTEE**
Regal Room—Members Only

3:00 P.M. **REGISTRATION—International Foyer**

3:00 P.M. **EXHIBITS OPEN—Imperial Ballroom**
All Exhibits Will Remain Open Until 7:00 P.M.

7:00 P.M. **WELCOME RECEPTION AND BUFFET—MID AMERICA CLUB**
(Walk through The Fairmont Hotel. Follow signs to the 80th floor of the Amoco Building.)

8:00 P.M. **MEETING—THE EXECUTIVE COUNCIL**
Regal Room—Members Only

THE AMERICAN THYROID ASSOCIATION, INC.

WEDNESDAY, SEPTEMBER 28, 1994

THE CLINICAL DAY PROGRAM

THE INTERNATIONAL BALLROOM
THE FAIRMONT HOTEL
CHICAGO, ILLINOIS

8:00 A.M. **WELCOME**
P. Reed Larsen, President A.T.A.
Terry Davies, Program Director

8:10 A.M. **THYROID GRAND ROUNDS**
Douglas Ross, Massachusetts General Hospital

10:00 A.M. ***INTERMISSION—COFFEE, POSTERS, EXHIBITS IN THE IMPERIAL BALLROOM***

10:30 A.M. **SYMPOSIUM—"Recombinant TSH"**
Chairperson: Arthur B. Schneider, University of Illinois

- **Synthesis of Recombinant TSH**
  Bruce Weintraub, National Institutes of Health
- **Clinical Trials Status**
  Lewis Braverman, University of Massachusetts Medical School
- **Case Presentations**
  Leslie De Groot, University of Chicago Medical Center

WEDNESDAY

MEET THE PROFESSOR LUNCHEONS

ADMISSION BY TICKET ONLY—$28.00
AT REGISTRATION
12:00 P.M. TO 1:30 P.M.

1. **Elaine Kaptein**
   Los Angeles General Hospital
   The Thyroid in Renal Failure
   Crystal Room
2. **George Schussler**
   State University of New York, Brooklyn
   Free Thyroid Hormone
   Regent Room
3. **Rebecca Bahn**
   Mayo Clinic and Foundation
   Graves' Eye Disease
   State Room
4. **Mitchell Lazar**
   University of Philadelphia
   Thyroid Hormone Receptors
   Ambassador Room
5. **James Baker**
   University of Michigan Medical Center
   TPO
   Chancellor Room

Wednesday, September 28, 1994

POSTER SESSION*

12:00 P.M. TO 2:00 P.M.

2. **Serum Osteocalcin Correlates with Triiodothyronine (T3) Levels in Hypothyroid Patients Having Abnormal TSH Responses to TRH Under Treatment with L-Thyroxine (L-T4).**
H. Niepomniszcze, S. Damilano, G. Faraj, and R. Lutfi
Service of Endocrinology, Complejo Médico (PFA) "Churruca-Visca," Buenos Aires, Argentina

5. **Manifestation of Hyperthyroidism in Different Postpartum Periods of the Same Patients with Graves' Disease.**
N. Momotani, Y. Gomi, J.H. Noh, N. Ishikawa, and K. Ito
Ito Hospital, Tokyo, Japan

17. **NA+K+ATPase Activity in Red Cells Predicts the Recurrence of Hyperthyroidism in Patients with Graves' Disease.**
C. De Riva, F. Virgili, and F. Frigato
Department of Endocrinology, Umberto I° General Hospital, Mestre-Venezia, Italy

23. **Relationships Between Pituitary and Thyroid Function in Patients with Central Hypothyroidism; Thyroid Hormone Concentrations, Bioactivity of TSH, and Response of TSH to TRH.**
M. Horimoto, M. Nishikawa, M. Yoshimura, T. Ishihara[2)], and M. Inada
Second Department of Internal Medicine, Kansai Medical University, 570 Osaka, Japan, and [2)]Internal Medicine, Kobe Central Municipal Hospital, Kobe 658, Japan

33. **Possible Involvement of Sympathetic Overactivity in Lid Retraction in Graves' Disease Even in the Euthyroid State.**
N. Hamada, J.Y. Noh*, Y. Nakamura**, and K. Ito*
Thyroid Study Unit, Sumire Hospital, Osaka 536, *Ito Hospital, Tokyo 150, and **NTT Osaka Central Health Administration Center, Osaka 530, Japan

38. **Inclusion of Serum Protein Bound T_4 in Measurements of Serum Free T_4 by Nondialysis Methods.**
Jerald C. Nelson and R. Bruce Wilcox
Loma Linda University School of Medicine, Loma Linda, CA 92354

52. **Methoxy-Iso-Butyl-Isonitrile (MIBI)-^{99m}Tc-Scanning: An Approach to the Localisation of Recurrent Medullary Thyroid Carcinoma (MTC)**
M. Colombo-Benkmann*, H. Elser†, P. Hartkorn*, P. Georgi†, and H.J. Buhr*
Departments of Surgery* and Nuclear Medicine†, University of Heidelberg, Germany

53. **Human T-Lymphotropic Virus Type I (HTLV-I) Associated Uveitis in Patients with Graves' Disease Treated with Methylmercaptoimidazole (MMI).**
T. Mizokami, K. Okamura, T. Kohno*, K. Sato, H. Ikenoue, T. Kuroda, K. Inokuchi, and M. Fujishima
Second Department of Internal Medicine and *Department of Ophthalmology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

54. **Detection of ras Oncogene Mutation in Rat Transplantable Thyroid Tumors.**
Y. Hiasa, Y. Kitahori, K. Yane, N. Konishi, M. Ohshima, H. Naitoh, K. Okaichi, T. Ohonishi, Y. Matsuda.
Department of Pathology, Biology and Otohinolaryngopharyngology, Nara Medical University, Kashihara, Nara-634, Japan

60. **Effects of Radioiodine on Thyrotropin Binding Inhibiting Immunoglobulins in Graves Disease: Long-Term Follow Up Study.**
Y. Aizawa, K. Yoshida, N. Kaise, K. Kaise, H. Fukazawa, Y. Kiso, K. Mori, N. Sayama, K. Kikuchi, and K. Abe
The Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan

71. **Pregnancy and Serum Non-Protein Bound Iodine.**
C. Liberman, S.C. Pino, and C.H. Emerson
University of Chile, Santiago, Chile and University of Massachusetts School of Medicine, Worcester, Massachusetts

80. **Clinical Utility of Ultrasound Guided Fine Needle Aspiration Biopsies (FNAB) of Thyroid Nodules.**
P.A. Burford, A.J. Van Herle, and E. Kingston
Department of Medicine, Division of Endocrinology, University of California, LA 90024

***All poster sessions will be held in the Imperial Ballroom. Posters should be in place by 9:00 A.M. and be removed by 8:00 P.M.**

Wednesday, September 28, 1994

POSTER SESSION

82. **Utility of Near Total Thyroidectomy in Reducing Potential Recurrences of Thyroid Cancer.**
J. Kolenda, I.B. Rosen, and P.G. Walfish
Departments of Surgery and Medicine, Mount Sinai Hospital and University of Toronto Medical School, Toronto, Ontario, Canada M5G 1X5

90. **Thyrotropin (TSH)-Measurement by Chemiluminescence: The New Order in Thyroid Function Testing.**
L. Duntas[1], B.M. Grab[2], T.P. Kemmer[1], and D.K. Nelson[1,3]
[1]Department of Internal Medicine I, [2]Department of Nuclear Medicine, University of Ulm, Ulm, Germany and [3]Mayo Clinic, Rochester, Minnesota

94. **The Study of the Transforming Mechanism of PTC-1 Oncogene.**
Q. Tong, Y.-S. Li, E.L. Mazzaferri, and S.M. Jhiang
The Ohio State University, Columbus, Ohio

124. **Calcitonin Gene-Related Peptide Response to Pentagastrin Stimulation in Normal Subjects and in Patients with Medullary Thyroid Carcinoma.**
H.M. Heshmati[1], R. Cohen[2], J. Taboulet[2], N. Bouyge[1], A. Jullienne[2], C. Calmettes[1], and E. Modigliani[1]
[1]Groupe d'Etude des Tumeurs à Calcitonine (GETC), Avicenne Hospital, University of Paris XIII, Bobigny, and [2]INSERM U 349, Lariboisière Hospital, Paris, France

137. **TSH and EGF Stimulate Thyroglobulin Secretion and Invasion of a Metastatic Hurthle Cell Carcinoma Cell Line.**
A. Zielke, S. Tezelman, G. Jossart, A.J. Van Herle, A.E. Siperstein, O.H. Clark, and Q.Y. Duh
VA Medical Center and Mt Zion Medical Center, UC San Francisco, and UC Los Angeles

153. **^{18}F-FDG-PET Scanning—A Diagnostic Tool for Detection of Recurrent and Metastatic Differentiated Thyroid Cancers.**
F.H. Baqai, P.S. Conti, P.A. Singer, C.A. Spencer, C.C. Wang, and J.T. Nicoloff
USC School of Medicine, Los Angeles, CA

159. **Analysis of a Female Phenotyped Complete Thyroxine-Binding Globulin Deficiency (TBG-CD): Unbalanced X Chromosome Inactivation as a Mechanism.**
H. Okamoto, Y. Mori, Y. Miura, Y. Tani, Y. Oiso, T. Sano[1], and K. Oyama[1]
First Department of Internal Medicine, Nagoya University, School of Medicine, Nagoya, [1]Department of Pediatrics, Yamanashi Medical University, Yamanashi, Japan

160. **Gene Analysis of Thyroxine-Binding Globulin (TBG) Deficiencies in Japanese: Only Two Mutations Account for TBG Deficiencies in Japanese.**
Y. Miura, H. Okamoto, Y. Tani, A. Inagaki, and Y. Oiso
First Department of Internal Medicine, Nagoya University, School of Medicine, Nagoya 466 Japan

167. **Altered Crosstalk Between TSH Receptor and Tyrosine Kinase Receptors Dependent Pathways in Thyroid Carcinoma Cells: The Role of Protein-Kinase C.**
M. Bröcker, G. Mayr*, and M. Derwahl
Medizinische Universitätklinik Bergmannsheil, Bochum and *Institut für Physiologische Chemie, Universität Hamburg, Germany

169. **Sulfated Thyroid Hormone Metabolites and Hepatic Deiodinase Expression in Fetal Sheep: Effect of Exogenous Dexamethasone.**
D.H. Polk, S.Y. Wu, A. Reviczky, M. Berry, and D.A. Fisher
Department of Pediatrics, Harbor–UCLA Medical Center, Torrance, California, Department of Nuclear Medicine, VA Medical Center, Long Beach, California, and Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts

172. **Expression of Transforming Growth Factor β (TGFβ) 1 and 3 in Benign and Malignant Human Thyroid Glands.**
E.T. Kimura[1], J. Zbaeren, and H. Studer
Experimental Laboratory of Endocrinology, Inselspital, Bern, Switzerland, [1]Department of Histology and Embriology, Institute of Biomedical Sciences, University of São Paulo, Brazil

Wednesday, September 28, 1994

POSTER SESSION

176. Tetracycline Versus Saline in Treatment of Thyroid Cysts: Comparison with Ethanol.
A. Antonelli, A. Campatelli*, M. Ferdeghini**, F. Bianchi***, B. Alberti, C. Gambuzza, C. Francese, and L. Baschieri
Institutes of Clinical Medicine II, *General and Experimental Surgery and **Nuclear Medicine, University of Pisa; ***Institute of Clinical Physiology, CNR, Pisa, Italy

181. Color-Flow Doppler Sonography in Thyroid Autoimmune Disease.
P. Vitti, E. Martino, S. Mazzeo, S. Brogioni, M. Lampis, T. Rago, A. De Liperi, and P. Bartolozzi
Istituto di Endocrinologia e Radiologia, Università di Pisa, Italia

183. A Follow Up Study of Untreated Graves' Patients with Undetectable Thyrotropin Receptor Antibodies and the Effects of Antithyroid Drugs.
H. Tamai, K. Kawai, S. Matsubayashi, T. Morita, Y. Matsumoto, S. Kubota, S. Fukata[1)], and K. Kuma[1)]
Department of Psychosomatic Medicine, Kyushu University, Faculty of Medicine, Fukuoka 812, [1)]Kuma Hospital, Kobe 650, Japan

184. Polymerase Chain Reaction Analysis of Growth Hormone Receptor Expression in Human Normal and Pathological Thyroid Tissues.
S.M. Villares, M.I. Gazzelli, E.T. Frazzato, A. Palomino, B.L. Wajchenberg, and W. Nicolau
Radioimmunoassay Laboratory, Endocrinology Department, University of Sao Paulo Medical School, PO 8091, Sao Paulo-SP, Brazil

185. P53 Oncogene Mutations in Thyroid Tumors: Evaluation of the Ability of SSCP to Detect Abnormalities.
K.D. Burman, Y.Y. Djuh, M. Galvin, P. Rhooms, D. Jaques, J. Anderson, H.B. Burch, and G. Jossart
Walter Reed Army Medical Center, Washington, D.C., PE Applied Biosystems Division, Foster City, CA, and University of California, San Francisco, CA

186. Methimazole, 3-Methyl-2-Thiohydantoin and Iodine in Thyroid Tissue.
D. Aktuna, A. Berger, O. Lorenz, and O. Eber
Hospital Barmherzige Brüder, Bergstrasse 27, A-8020 Graz, Austria

188. A Longitudinal Study of Changes in Body Mass Index After I-131 Treatment for Graves' Disease.
R.E. de la Rosa MD, J.V. Hennessey MD, and J.R. Tucci MD
Rhode Island Hospital/Roger Williams Medical Center/Brown University, Providence, Rhode Island

194. Low-Dose Iodine in Endemic Goitre—A Placebo-Controlled, Double Blind Trial.
G. Kahaly, F. Reiche, C. Molitor, J. Beyer, and C. Hansen
Dept of Medicine III and Endocrinology, University Hospital, Mainz, Germany

196. Treatment of Differentiated Thyroid Carcinoma with a Uniform Treatment Protocol; Outcome in 658 Patients Over 26 Years.
E.G. Wilmshurst, P. Clifton-Bligh, L.W. Delbridge, G.R. Fulcher, I.B. Hales, A. McElduff, A.G. Poole, T.S. Reeve, B.G. Robinson, J.N. Stiel, and J.C. Wiseman
Royal North Shore Hospital, St Leonards, NSW, Australia

198. Is the Long-Term Administration of Amiodarone Really Dangerous to Patients with Abnormal Thyroid Function Tests?
L.S. Ward, M.A.B. Teixeira, L.C. Oliveira, G.A. Fernandes, and R.M.B. Maciel
Departments of Medicine, University of Campinas School of Medical Sciences and Escola Paulista de Medicina, Campinas and São Paulo, Brazil

199. Elevated cAMP Levels Generate Growth Inhibitory Signals in a Thyroid Anaplastic Carcinoma Cell Line (ARO).
S. Misiti[1]*, F. Moretti#*, A. Farsetti#*, A. Sacchi*, A. Pontecorvi@*, and C. Gaetano*
[1]II Chair of Endocrinology, University of Rome "LA SAPIENZA," *Molecular Oncogenesis Laboratory, Ist. Regina Elena, #Dept. of Experimental Medicine, National Research Council, @Inst. of Medical Pathology, Catholic University, Rome, Italy

202. Relationship of Subclinical Hypothyroidism to Cardiovascular Risk Factors and Disease in an Elderly Population.
P.W. Ladenson, M.C. Wilson, J. Gardin, R. Krommal, L. Kuller, R. Tracy, G. Burke, and L.P. Fried
Divisions of Endocrinology and Internal Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287-4904

Wednesday, September 28, 1994

POSTER SESSION/AFTERNOON SESSION

212. **Methimazole, 3-Methyl-2-Thiohydantoin and Iodine in Thyroid Tissue.**
D. Aktuna, A. Berger, O. Lorenz, and O. Eber
Hospital Barmherzige Brüder, Bergstraβe 27, A-8020 Graz, Austria

215. **Changes in Body Weight, Body Composition and Collagen Related Peptides After Treatment for Thyreotoxicosis.**
E. Nyström, K. Stenlöf, L. Lönn, L.E. Tisell, P.A. Lundberg, G. Lindstedt, G. Berg, A. Michanek, and L. Sjöström
Departments of Endocrinology, Radiology, Surgery, Clinical Chemistry and Oncology, Sahlgrenska University Hospital, Göteborg, Sweden

218. **TSH Suppression: Daytime Values May Rise to Unacceptable Values at Night.**
V.J. Bernet, B.L. Solomon, T.S. Cranston, and K.D. Burman
Walter Reed Army Medical Center, Washington, D.C.

219. **Influence of Compensated Radioiodine Therapy on Thyroid Volume and Incidence of Hypothyroidism in Graves' Disease.**
L. Hegedüs, B. Nygaard, M. Gervil, H. Hjalgrim, B.M. Hansen, B. Søe-Jensen, and J.M. Hansen
Departments of Internal Medicine and Endocrinology and Ultrasound, Herlev University Hospital, DK-2730 Herlev, and Department of Internal Medicine and Endocrinology, Odense University Hospital, DK-5000 Odense C, Denmark

222. **Prevalence of Incidental Thyroid Disease in a Low Iodine Intake Area by Ultrasonography.**
F. Pedrinola, E. Tomimori, H. Cavaliere, N. Lima, and M. Knobel
Thyroid Laboratory, Hospital das Clinicas, Univ São Paulo Med School, São Paulo, Brazil

223. **Secondary Thyroid Failure May Be Underdiagnosed and Undertreated in Growth Hormone (GH) Deficient Adults.**
P. Laurberg, H.C. Hoeck, P.E. Jacobsen, and P. Vestergaard
Department of Endocrinology, Aalborg Hospital, DK-9000 Aalborg, Denmark

228. **Immunohistochemical Study in Differential Diagnosis of Thyroid Tumors.**
J. Hua, G. Yu, and YY. Jiang
Chinese Great Wall Hospital, Beijing, China

SIMULTANEOUS SESSIONS

2:00 P.M. **CELL BIOLOGY ORAL PRESENTATIONS—International Ballroom**

CHAIRPERSONS: Richard Haber and Stephen Spaulding

2:00 P.M. 27. **Mutual Antagonistic Interactions Between the cAMP and Protein Kinase C/Tyrosine Kinase Pathways in Human Thyroid Cell Proliferation, Differentiation, *c-jun* and *c-fos* Proto-Oncogene Expression.**
Z. Kraiem, O. Sadeh, M. Yosef, A. Aharon, and R. Heinrich
Endocrine Research Unit, Carmel Medical Center, Haifa 34362, Israel

2:15 P.M. 46. **Inhibiting Iodide Uptake in Rat Thyroid Cells Using Chloride Channel Blockers; Probes for the Study of Iodide Transport.**
A. Fanelli, W.K. Berlin, and E.F. Grollman
National Institutes of Health, Bethesda, Maryland

2:30 P.M. 91. **Novel Effectors of RAS in Thyroid Cells.**
E. Kupperman*, S. Ching*, N. Al-Alawi*, T. Tominaga*, M. White$^{\triangle}$, M. Wigler$^{\triangle}$, J.R. Feramisco$^{*\Theta\Phi}$, and J.L. Meinkoth$^{*\Theta}$
Departments of *Medicine, $^{\Theta}$Cancer Center, and $^{\Phi}$Pharmacology, University of California at San Diego, La Jolla, California 92093. $^{\triangle}$Cold Spring Harbor Laboratories, Cold Spring Harbor, New York 11724

2:45 P.M. 144. **Cholera Toxin A1 Subunit Gene Eliminates Ligand-Stimulated Inositol Phospate Production in Stably Transfected Rat FRTL-5 Thyroid Cells.**
G. Laglia, M. Saji, M.A. Zeiger, P. Caturegli, M.A. Levine, and L.D. Kohn
National Institutes of Health, Bethesda and Johns Hopkins University, Baltimore, Maryland

Wednesday, September 28, 1994

AFTERNOON SESSION

2:00 P.M. **SYMPOSIUM—"Private Practice"–The Gold Room**

Chairperson: Michael Kaplan, Southfield, Michigan

- **Resources, Equipment, and Services**
  Jack Baskin, Orlando, Florida
- **Changing Spectrum of Thyroid Diseases**
  Michael Kaplan, Southfield, Michigan
- **Patient Education and Support Groups**
  Shelly Rubenfeld, Houston, Texas

3:00 P.M. ***INTERMISSION—COFFEE, POSTERS, EXHIBITS IN THE IMPERIAL BALLROOM***

3:30 P.M. **SYMPOSIUM—"Controversies in Graves' Disease"–The Gold Room**

Chairperson: Paul Ladenson, Johns Hopkins Hospital, Baltimore

- **Immunomodulation**
  Anthony Weetman, Northern General Hospital, Sheffffield, U.K.
- **Ophthalmopathy and RAI Treatment**
  Colum Gorman, Mayo Clinic, Rochester
- **Remissions in Graves' Disease**
  David Cooper, Sinai Hospital of Baltimore

5:00–5:30 P.M. **HISTORICAL SESSION—"Iodine and Goiter"**
The Early Work of David Marine
Clark T. Sawin, V.A. Medical Center, Boston

6:30 P.M. **THE ART INSTITUTE OF CHICAGO—RECEPTION**
Buses will depart at 6:20 P.M. Admission by ticket only.

THE AMERICAN THYROID ASSOCIATION, INC.

THURSDAY, SEPTEMBER 29, 1994

THE MORNING PROGRAM

THE INTERNATIONAL BALLROOM
THE FAIRMONT HOTEL
CHICAGO, ILLINOIS

7:00 A.M. **EARLY RISER LECTURE:** "Combinatorial Libraries"
Basil Rapoport, Veterans Administration Medical Center, San Francisco, California

7:00 A.M. **COMMITTEES—BREAKFAST MEETING**
Regent and State Rooms

8:00 A.M. **AUTO IMMUNITY ORAL PRESENTATIONS**

CHAIRPERSONS: Rebecca Bahn and Jim Baker

8:00 A.M.
57. **Analysis of the T-Cell Antigen Receptor V-Gene Repertoire in Lymphocytes Infiltrating the Pretibial Lesions of Patients with Graves' Ophthalmopathy and Pretibial Dermopathy.**
A.E. Heufelder and R.S. Bahn*
Molecular Thyroid Research Unit, Medizinische Klinik, Klinikum Innenstadt, Ludwig-Maximilians-Universität, München, Germany, and *Division of Endocrinology, Department of Internal Medicine, Mayo Clinic and Foundation, Rochester, MN

8:15 A.M.
109. **CTLA-4 Gene Polymorphism Associated with Graves' Disease.**
T. Yanagawa and L.J. DeGroot
Thyroid Study Unit, The University of Chicago, Chicago, IL

8:30 A.M.
139. **Epitopic "Fingerprints" of Thyroid Peroxidase Autoantibodies in Hashimoto's Thyroiditis: Evidence for Conservation Over Time and in Families.**
J.C. Jaume, S.M. McLachlan, C.L. Burek, W.H. Hoffman, N. Rose, and B. Rapoport
Thyroid Molecular Biology Unit, VA Medical Center, University of California, San Francisco and Johns Hopkins University, Baltimore, MD

8:45 A.M.
141. **Human Thyroid Peroxidase Autoantibodies of Lambda Light Chain Type Cloned by Phage Display: Immunoglobulin V Gene Usage.**
S. Portolano and S.M. McLachlan
Thyroid Molecular Biology Unit, VA Medical Center and University of California, San Francisco

9:00 A.M. **VAN METER LECTURE**
The Gold Room

10:00 A.M. ***INTERMISSION—COFFEE, POSTERS, EXHIBITS IN THE IMPERIAL BALLROOM***

10:30 A.M. **ORAL PRESENTATIONS**

TSH Receptor

CHAIRPERSONS: Bernard Rees-Smith and Gregorio Chazenbalk

10:30 A.M.
14. **Molecular Cloning and Functional Analysis of the Thyroropin Receptor from Non-Thyroid Tissue.**
T. Endo, K. Ohta, K. Haraguchi, and T. Onaya
Third Department of Internal Medicine, University of Yamanashi Medical School, Tamaho, Yamanashi 409-38, Japan

10:45 A.M.
55. **A Genomic TSH Receptor Point Mutation Is Highly Associated with Autoimmune Thyroid Disease in Females.**
R.M. Cuddihy, C.M. Dutton, and R.S. Bahn
Division of Endocrinology, Mayo Clinic and Foundation, Rochester, MN

Thursday, September 29, 1994

MORNING PROGRAM

11:00 A.M. **Novel Mutations of TSH-Receptor Gene in Thyroid Hyperfunctioning Adenomas.**
88. S. Pannain, A. Porcellini, I. Ciullo, G. Amabile, V.E. Avvedimento, and G.F. Fenzi
CEOS-CNR, Dipartimento di Biologia e Patologia Molecolare e Cellulare; Cattedra di Endocrinologia, II Facoltà di Medicina, via S. Pansini 5, 80131 Naples, Italy; Dipartimento di Medicina Sperimentale, Facoltà di Medicina di Catanzaro, 3 via T. Campanella, Catanzaro, Italy

11:15 A.M. **Functional Characterization of Two New Somatic Mutations in the Thyrotropin Receptor in Hyperfunctioning Thyroid Adenomas.**
89. R. Paschke, M. Tonacchera, J. Dumont, and G. Vassart
Service de Génétique Médicale and IRIBHN Université Libre de Bruxelles, Bruxelles, Belgique

11:30 A.M. **Human TSH Receptor Variant 1.3 mRNA in Human Extraocular Muscles.**
122. M. Nakashima, D.L. Kendler*, J. Rootman*, and P. Graves
Departments of Medicine, Mt. Sinai School of Medicine, NY, NY, and *University of British Columbia, Vancouver, BC

11:45 A.M. **Congenital Non-Autoimmune Hyperthyroidism Caused by a Mutation in the Thyrotropin (TSH) Receptor Gene.**
132. P. Kopp, J. van Sande, J. Parma, L. Duprez, K. Zuppinger, J.L. Jameson, and G. Vassart
Department of Internal Medicine and Laboratory of Endocrinology (P.K.), Clinic of Pediatrics (K.Z.), Inselspital, University of Berne, Switzerland; Center for Endocrinology, Northwestern University, Chicago, USA (P.K., J.L.J.); Institut de Recherche Interdisciplinaire, Faculty of Medicine, University of Brussels, Belgium (J.S., J.P., L.D., G.V.)

THURSDAY

MEET THE PROFESSOR LUNCHEONS

ADMISSION BY TICKET ONLY—$28.00
AT REGISTRATION
12:00 P.M. TO 1:30 P.M.

1. **Nadir Farid**
   Durham, NC
   Immunogenetics and You
   State Room
2. **Leonard Wartofsky**
   Washington Hospital Center
   Thyroid Hormone Therapy: Pearls and Baroques for the Thyroidologist
   Regent Room
3. **Ralph Cavalieri**
   VA Medical Center, San Francisco
   Radioiodine in 1994
   Ambassador Room
4. **J. Larry Jameson**
   Northwestern University
   Automated DNA Sequencing and Mutational Analysis
   Chancellor Room
5. **Marla Berry**
   Brigham & Women's Hospital
   Deiodinase
   Embassy Room

Thursday, September 29, 1994

POSTER SESSION

12:00 P.M. TO 2:00 P.M.

3. **Regulation of Motility and Adhesion of Follicular and Papillary Thyroid Cancer Cells: A Possible Mechanism for Inhibition of Invasion by Transforming Growth Factor-β1.**
Th. Hölting, Q.Y. Duh, A.E. Siperstein, Ch. Herfarth, and O.H. Clark
Surgical Departments of the Universities of Heidelberg, Germany and California at San Francisco

4. **Double Point Mutations in the Promoter Region of the Thyroglobulin Gene in a Patient with Congenital Goiter.**
A. Hishinuma, K. Kasai, K. Kobayashi, A. Yoshida, T. Ieiri, and S.I. Shimoda
Department of Clinical Pathology, Dokkyo University School of Medicine, Mibu, Toguchi, Japan

8. **Increased Hepatic Inner Ring Deiodinating Type III Activity After Partial Feed Restriction in the Rat and the Chicken.**
V.M. Darras, E. Dewil, M. Cokelaere*, S. Arnout, E.R. Kühn, and E. Decuypere
Leuven Poultry Research Group, KUL, 3000 Leuven, Belgium and *KULAK, 8500 Kortrijk, Belgium

10. **Regional Anesthesia for Thyroidectomy and Parathyroidectomy.**
R. Kulkarni, L.E. Braverman, and N. Patwardhan
Departments of Anesthesiology, Medicine, and Surgery, University of Massachusetts Medical Center, Worcester, MA

18. **Preparation and Characterization of Monoclonal Anti-Thyrotropin Receptor Antibodies (TSH-R Ab) Obtained from Peripheral Lymphocytes of Hypothyroid Patients with Primary Myxedema.**
J. Okuda*, T. Akamizu*, H. Sugawa*, F. Matsuda†, L. Hua*, and T. Mori*
*Department of Laboratory Medicine, Faculty of Medicine, and †Center for Molecular Biology and Genetics, Kyoto University, Kyoto, Japan

21. **The Selenium Analog of Propylthiouracil; Measurement of Its Inhibitory Effect on Type I 5′-Deiodinase and of Its Antithyroid Activity.**
A. Taurog, M.L. Dorris, L.J. Guziec, and F.S. Guziec, Jr.
University of Texas Southwestern Medical Center, Dallas, TX 75235 and New Mexico State University, Las Cruces, NM 88003

32. **Interleukin-6 Regulates Type 1 5′Deiodinase in Rat Liver Cells.**
P.H. Davies, M.C. Sheppard, and J.A. Franklyn
Department of Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham, B15 2TH, United Kingdom

40. **Integrin Expression in Human Thyroid Cell Lines and Effect of N-*ras* Overexpression.**
M. Vitale, M. Illario, A. Casamassima, V. Bassi, S. De Riu, C. Sandomenico, G. Rossi, and G.F. Fenzi
Dipartimento Biologia e Patologia Cellulare e Molecolare, Departimento Endocrinologia e Oncologia Molecolare e Clinica, Università Federico II, Naples, Italy

41. **Retinoic Acid and Proinflammatory Cytokines Induce Up-Regulation of Cellular ICAM-1 Molecule and Parallel Shedding of Soluble ICAM-1 Form in Human Thyroid Cell Lines.**
V. Bassi*, M. Maio†, M. Altomonte†, M. Vitale°, S. De Riu*, and G. Rossi°
*Dipartimento di Endocrinologia ed Oncologia Clinica e Molecolare, °Dipartimento di Biologia Cellulare e Molecolare, Università degli Studi di Napoli "Federico II," †Advanced Immunotherapeutics Unit, CRO, Aviano (PN), Italy

43. **Phenotypic Characterization of Thyroglobulin-Specific T Cell Lines Derived from Thyroiditis-Prone BB/WOR Rats.**
E.M. Allen and J.N. Thupari
Baltimore VA Medical Center and University of Maryland Medical Center

45. **Inhibition of TSH-Stimulated Iodide Organification In Vitro Following Diacylglycerol Kinase Inhibition.**
J. Ginsberg, W. Matowe, and K. Chen
Department of Medicine, University of Alberta, Edmonton, Alberta, Canada

Thursday, September 29, 1994

POSTER SESSION

56. **Early Cellular Events in the Thyroid After Exposure to Iodine.**
N. Bagchi, T.R. Brown, P. Anand, and R.S. Sundick
Departments of Internal Medicine and Immunology and Microbiology, Wayne State University, Detroit, MI 48201

61. **The Kinetics of Rat Type III Iodothyronine Deiodinase Present in the Placenta Is Quite Different from That in the Cerebral Cortex.**
K. Mori, K. Yoshida, H. Fukazawa, Y. Kiso, N. Sayama, K. Kikuchi, Y. Aizawa, and K. Abe
Tohoku University School of Medicine, Sendai, Japan

65. **Expression of the Thyrotropin-Receptor in Orbital Tissues from Patients with Graves' Disease.**
L. Tallstedt[1], A. Janson[2], C. Marcus[2], O. Törring[3], and M. Brönnegård[2]
Department of [1]Ophthalmology and [2]Pediatrics, Huddinge University Hospital and [3]Department of Endocrinology, Karolinska Hospital, Stockholm, Sweden

66. **Studies Toward an Immunoassay for Thyrotropin Receptor Antibodies (TRAb): Production of Antibodies to the TRAb Epitope.**
P. Ward, B.M. Luttrell, and D. Williams
Department of Endocrinology, Royal North Shore Hospital and Department of Biochemistry, University of Technology, Sydney, St. Leonards NSW 2065 Australia

68. **TSH Stimulates ^{3}H-Choline Incorporation into Sphingomyelin of Aged but Not Young FRTL-5 Rat Thyroid Cells.**
A.E. Pekary, L. Berg, and J.M. Hershman
Endocrinology Research Laboratory, West Los Angeles VA Medical Center and UCLA Department of Medicine, Los Angeles, CA 90073

70. **A Point Mutation of the Gs-α Gene in Thyroid Tissue from Patients with Graves' Disease (GD): Correlation with the Level of Expression of Gsα-Proteins.**
V. Gorelov, N. Barteneva[#], K. Dumon, D. Palm, H.-D. Röher, P. Goretzki, and B.E. Wenzel[#]
Dept. of Surgery A, University of Düsseldorf; [#]Cell and Immune Biological Laboratory, Department of Internal Medicine, Medical University Lübeck, Germany

74. **Localization of HLA-DR Antigen and Heat Shock Protein 70 and CD44 Antigen Expression in Upper Eye Lids from Patients with Thyroid-Associated Ophthalmopathy.**
Y. Hiromatsu[1], J. Kamachi[1], K. Tanaka[1], T. Kuroki[1], Y. Inoue[2], and K. Nonaka[1]
[1]Department of Medicine, Kurume University School of Medicine, Kurume, Japan and [2]Olimpia Clinic, Tokyo, Japan

76. **Prostaglandin E_2-Mediated Shape Changes in Orbital Fibroblasts from Patients with Graves' Ophthalmopathy.**
H-S Wang, MG Hogg, and TJ Smith
Molecular and Cellular Medicine, Departments of Medicine and Biochemistry, Albany Medical College and the Department of Veterans Affairs Medical Center, Albany, NY 12208

78. **Coculture of Peripheral Blood Mononuclear Cells with Allogeneic Human Extraocular and Skeletal Muscle Cells: Possible Relevance to Thyroid Orbitopathy.**
D.L. Kendler, P. Dolman, C. Cordeiro, and J. Rootman
University of British Columbia, Vancouver BC, Canada

79. **Enzyme Activity and Blood Lymphocytes in Graves' Disease**
M.C. Werner, J.H. Romaldini, L.F.C. Rosa, and R. Curi
Department of Endocrinology, HSPE-IAMSPE and Institute of Biomedical Sciences, University of Sao Paulo, S.P., Brazil

87. **Characteristics of the Immune Response to Thyroid Peroxidase in Patients with Post-Partum Thyroiditis.**
J. Janssen, P. Arscott, R. Smallridge, and J.R. Baker, Jr.
University of Michigan Medical School, Ann Arbor, MI, and Walter Reed Army Institute of Research, Washington, DC

Thursday, September 29, 1994

POSTER SESSION

93. Role of GRB2 in TSH Signal Transduction.
D. Wofford*, J.R. Feramisco, and J.L. Meinkoth
Departments of *Biology, Pharmacology and Medicine, Cancer Center, University of California, San Diego, La Jolla CA 92093

96. Relationship Between Eye Muscle Autoantibodies and Severity of Thyroid-Associated Ophthalmopathy.
A. Boucher, F. Ertug, C. Corriveau, P. Gauvin, H. Beauregard, and R. Comtois
Notre-Dame Hospital, Montreal, Canada

103. Comparative Studies of Human Thyroid Xenografts from Graves' Disease in Severe Combined Immunodeficient (SCID) Mice and NIH-Beige-Nude-XID (NIH-3) Mice.
T. Mukuta, G. Arreaza, M. Nishikawa, N. Yoshikawa, E. Resetkova, and R. Volpé
Endocrinology Research Laboratory, Department of Medicine, The Wellesley Hospital, University of Toronto, Toronto, Ontario, M4Y1J3 Canada

106. Does Thyroidectomy, RAI Therapy or Antithyroid Drug Treatment Alter Reactivity of Patients T Cells to Epitopes of Thyrotropin Receptor in Autoimmune Thyroid Diseases?
M. Soliman, E. Kaplan, and A. Abdel-Latif
Thyroid Study Unit, The University of Chicago and Mansoura University Hospital, Egypt

116. T Lymphocyte Reactivity to Eye Muscle Proteins and Their Predicted Peptides in Patients with Thyroid-Associated Ophthalmopathy.
J. Kiljanski, C. Stolarski, D. Scalise, V. Nebes, M. Hayes, and J.R. Wall
Thyroid Center, Allegheny-Singer Research Institute, Pittsburgh, PA

119. Region Specific T Cell Intolerance to TSH Receptor Extracellular Domain.
A. Martin, J.C. Morris[1], S. Yeung, and T.F. Davies
Department of Medicine, Mount Sinai School of Medicine, New York, NY, and [1]Division of Endocrinology, Mayo Clinic, Rochester, MN

121. Intrathyroidal T Cell Receptor Gene Expression in Murine Autoimmune Thyroiditis Induced by Transfer of Mouse Thyroglobulin-Activated Lymphocytes.
M. Nakashima, N. Matsuoka, and Y.M. Kong*
Department of Medicine, Mount Sinai School of Medicine, New York, NY, and *Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI

134. ETa and ETb Endothelin Receptor Messenger RNAs Are Expressed in the Rat Thyroid Gland and Regulated in Goiter Formation and Iodide-Induced Involution.
D. Toussaint-Demylle, J. Weiss, D.M. Maiter, J-F. Denef, and I.M. Colin
Center for Endocrinology and Molecular Medicine (JW, IMC), Northwestern University, Chicago, IL, and Histology (DT-D, J-FD), Diabetology and Nutrition Research Units (DMM), University of Louvain, Brussels, Belgium

136. Evidence for Nitric Oxide Activity in the Rat Thyroid Gland.
I.M. Colin, E. Nava, D. Toussaint-Demylle, J-F. Denef, D.M. Maiter, T.F. Lüscher, and J.L. Jameson
Center for Endocrinology and Molecular Medicine (IMC, JLJ), Northwestern University, Chicago, IL, Histology (DT-D, J-FD), Diabetology and Nutrition Research Units (DMM), University of Louvain, Brussels, Belgium, and Cardiology, (EN, TFL), Inselspital, University of Berne, Switzerland

143. Structure and Function of the TSH Receptor Extracellular Region Expressed Under the Influence of Different Baculovirus Vector Promoters.
G.D. Chazenbalk and B. Rapoport
Thyroid Molecular Biology Unit, VA Medical Center, UC San Francisco, California

147. Preferential Development of Blocking Antibodies to TSH Receptor After Ablative ^{131}I Therapy in Graves' Disease.
M.K. Gupta, R. Beham, M. Gliga, M. Secic, G. Kosmorsky, J. Perl, A. Licata, L. Kohse, B. Hoogwerf, and C. Faiman
The Cleveland Clinic Foundation, Cleveland, OH 44195

Thursday, September 29, 1994

POSTER SESSION

148. **Thyroid Hormone Plasma Membrane Transport: Structural Homology and Binding Site Interactions with Amino Acids and Benzodiazepines.**
M. Lakshmanan, M. McCourt, Y. Liu, and L. Kragie
Department of Medicine, MetroHealth Medical Center, Cleveland, OH, Electron Diffraction Department, Medical Foundation of Buffalo, Buffalo, NY, and ADARC, McLean Hospital, Belmont, MA

150. **Divergent cAMP Responses to HCG in Different Cell Lines Expressing the Recombinant Human TSH Receptor (hTSHr).**
S. Poertl, M. Broecker*, J. Hammer*, K. Mann†, and R. Hoermann†
Medical Department II, Klinikum Grosshadern, University of Munich, 81377 Munich, *Clinic of Internal Medicine, University of Bochum, 44789 Bochum and †Dep. of Endocrinology, University of Essen, 45122 Essen, Germany

151. **Primary Hormonogenic Sites as Conserved Autoepitopes in Murine Autoimmune Thyroiditis: Role of Iodination.**
Y.M. Kong, D.J. McCormick°, Q. Wan, R.W. Motte*, B.E. Fuller, A.A. Giraldo*, and C.S. David°
Wayne State University School of Medicine and *St. John Hospital, Detroit, MI, and °Mayo Clinic, Rochester, MN

152. **Dynamics of T4 and T3 Deiodination in Man—Dominant Role of Extrahepatic Metabolism.**
S.J. Eng, J.S. LoPresti, H. Liang, and J.T. Nicoloff
USC School of Medicine, Los Angeles, CA

156. **Imaging of Calcium Fluxes in Individual Thyroid Cells.**
R.W. Lash, C.A. Zimmerman, C.W. Balke, and P.S. Shacklock
Divisions of Endocrinology and Cardiology, Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland

157. **Thyrotropin (TSH) Regulates Thyroid Growth Factors in Hypophysectomized (Hypox) Rats.**
G.P. Becks*, M. Forsyth**, A. Logan**, and D.J. Hill*
*Department of Medicine, St. Joseph's Health Centre and the Lawson Research Institute, University of Western Ontario, London, Canada and **Department of Clinical Chemistry, The University of Birmingham, Birmingham, UK

164. **Thyroid Peroxidase (TPO) Activities and TPO mRNA Levels in the Presence of Propylthiouracil and Methimazole in Cultured Porcine Thyroid Follicles.**
M. Sugawara, H. Murai, and P.E. Cizdziel
VA Medical Center, West Los Angeles, University of California Los Angeles, School of Medicine, Los Angeles, CA, and Life Technology Inc., Germantown, MD

166. **Melittin, a Membrane Active Peptide, Increases Iodide Efflux Independently of Phospholipases C and A_2 in FRTL-5 Cells.**
R.C. Smallridge, X-D. Wang, and I.D. Gist
Walter Reed Army Institute of Research, Washington, DC

171. **Both 3′ and 5′ Regulatory Elements Are Involved in the Tissue-Specific Hormonal Regulation of EGF mRNA.**
L.G. Sheflin, E.M. Brooks, and S.W. Spaulding
Buffalo VA Medical Center and SUNY at Buffalo, Buffalo NY, 14215

175. **Delineation of Amino Acid Residues Within hTSHr 256-275 That Participate in Hormone Binding.**
W.P. Bryant, E.R. Bergert, and J.C. Morris
Endocrine Research Unit, Mayo Clinic and Foundation, Rochester, MN 55905

180. **Involvement of Thyroid-Specific Transcription Factors in Hormone- and Serum-Dependent Regulation of the Thyroglobulin Gene Expression in FRTL-5 Cells.**
F. Kambe and H. Seo
Department of Endocrinology and Metabolism, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

189. **Changes of Serum Sulfated Iodothyronines and the Severity of Liver Cirrhosis.**
W.S Huang, T.H. Yung, S.W. Kuo, H.S. Tang, W.L. Chen, and S.Y. Wu
Departments of Nuclear Medicine and Internal Medicine, Tri-Service General Hospital, Taipei, Taiwan and Nuclear Medicine Service, VA Medical Center, Long Beach, CA

Thursday, September 29, 1994

POSTER SESSION/AFTERNOON SESSION

190. **The Mechanism of Peroxidase-Catalyzed Coupling and Its Stimulation by Low Concentrations of Free Diiodotyrosine.**
D.R. Doerge, M.L. Dorris, and A. Taurog
National Center for Toxicological Research, Jefferson, AR 72079 and University of Texas Southwestern Medical Center, Dallas, TX 75235

197. **Comparison of the Uptake of Triiodothyroacetic Acid (TRIAC) and T3 in Cultured Anterior Pituitary Cells.**
M.E. Everts, T.J. Visser, R. Docter, M. de Jong, E.P. Krenning, and G. Hennemann
Departments of Internal and Nuclear Medicine, Erasmus University Medical School, Rotterdam, The Netherlands

201. **Inhibitory Role of Insulin-Like Growth Factor Binding Proteins in the Regulation of Thyroid Function.**
M.C. Eggo
Department of Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham, United Kingdom

203. **Increased Metastatic Potential of Poorly Differentiated Human Hepatocellular Carcinoma Cells Is Associated with an Overexpression of Thyroid Horomone β1 Nuclear Receptor.**
K-H. Lin[1] and S-Y. Cheng[2]
[1]Chang-Gung College of Medicine and Technology, Taoyuan, Taiwan; [2]Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

207. **Neurothyronines: Morphologic Evidence Demonstrating a Non-Genomic Role for T3 in Locus Ceruleus (LC) and All Other Brain Stem Noradrenergic (NA) Nuclei.**
M.B. Dratman
Philadelphia VA Medical Center, Philadelphia, PA

208. **Heterogeneity in mRNA Encoding Thyroglobulin's C Terminus.**
ME Mason, SJ Hoback, and JT Dunn
University of Virginia, Charlottesville, VA

214. **Clinical Relevance of Autoantibodies with Dual Specificity for Thyroglobulin and Thyroperoxidase.**
U. Feldt-Rasmussen, L. Hegedüs, M. Ferrand, J. Ruf, and P. Carayon
Department of Medical P, Division of Endocrinology, Rigshospitalet, and Medical Department of Endocrinology, Herlev Hospital, Copenhagen University, Copenhagen, Denmark; U38 INSERM, Faculté de Médecine, Marseille, France

220. **Expression and TSH Binding of Mutant TSH-Receptor (PRO52 → THR) Occurring in Autoimmune Hyperthyroidism.**
U.R.M. Bohr
Abteilung Innere Medizin I, Universität Ulm, Ulm, Germany

221. **Heterozygous Segregation of a Cytosine to Thymine Transition in the Thyroglobulin Gene in a Family with Congenital Goiter.**
H.M. Targovnik, A.E.C. Billerbeck, G.D. Frechtel, G.E. Cerrone, J. Vono, F. Mendive, F. Pedrinola, and G.A. Medeiros-Neto
Cátedra Génetica y Biologia Molecular, Hospital de Clinicas José de San Martin, Fac. Farm. Bioq. Univ. Buenos Aires, Argentina and Lab. Tireoide, Hospital Clínicas, Fac. Med. Univ. São Paulo, Brazil

SIMULTANEOUS SESSIONS

2:00 P.M. **THYROID CANCER ORAL PRESENTATIONS—International Ballroom**

CHAIRPERSONS: James Fagin and Nadir R. Farid

2:00 P.M. **Radiation-Induced Thyroid Cancer: A Pooled Analysis of Seven Studies.**
48. E. Ron, J.H. Lubin, R.E. Shore, K. Mabuchi, B. Modan, L.M. Pottern, A.B. Schneider, M.A. Tucker, and J.D. Boice, Jr.
Epidemiology and Biostatistics Program, NCI, NIH, Bethesda, MD, Department of Environmental Medicine, New York University Medical Center, NY, NY, Departments of Epidemiology and Epidemiologic Pathology, Radiation Effects Research Foundation, Hiroshima, Japan, Department of Clinical Epidemiology, Chaim Sheba Medical Center, Tel Hashomer, Israel, and Department of Endocrinology and Metabolism, Michael Reese Hospital, University of Illinois, Chicago, Illinois

Thursday, September 29, 1994

AFTERNOON SESSION

2:15 P.M. 158. **RET Mutations and Pentagastrin Testing in Familial Medullary Thyroid Carcinoma and Multiple Endocrine Neoplasia 2A.**
B.G. Robinson, D. Marsh, V. Hyland, S. Andrew, D. McDowell, and P. Clifton-Bligh
Royal North Shore Hospital, Sydney, Australia

2:30 P.M. 182. **Screening for RET Gene Mutations in Multiple Endocrine Neoplasia (MEN) Type 2 and in Sporadic Medullary Thyroid Carcinoma (MTC): Clinical Applications.**
F. Pacini, I. Ceccherini*, E. Martino, C. Romei, R. Elisei, E. Molinaro, F. Mancusi, G. Romeo*, and A. Pinchera
Istituto di Endocrinologia, University of Pisa, and *Istituto G. Gaslini, Genova, Italy

2:45 P.M. 200. **Overexpression of Stimulatory Guanine Nucleotide-Binding Protein ($G_{s\alpha}$ Protein) in Papillary Thyroid Carcinomas Is Not Restricted to Tumors with $G_{s\alpha}$ Oncogenes.**
M. Derwahl*, C. Hamacher*, G. Papageorgiou†, N. Speidel*, K.M. Müller#, and H. Schatz*
*Medizinische Universitätsklinik and #Institut für Pathologie, 44789 Bochum, Germany and †Evangelismos Hospital, Athens, Greece

2:00 P.M. THYROID HORMONE ACTION—The Gold Room

CHAIRPERSONS: Samuel Refetoff and Marla J. Berry

2:00 P.M. 50. **Human Transthyretin (TTR) Ligand Complexes: Crystal Structure of TTR-3′,5′-Dichloro-2-Carboxy-Diphenylamine Complex.**
V. Cody, J.R. Luft, W. Pangborn, S. Munro*, D. Chalmer*, and D. Criak
Medical Foundation of Buffalo, Buffalo, NY 14203 and *Monash University, Parkville, Australia

2:15 P.M. 75. **The Same Missense Mutation in the Albumin Gene Is Associated with Familial Dysalbuminemic Hyperthyroxinemia in 8 Unrelated Families.**
T. Sunthornthepvarakul, P. Angkeow, R.E. Weiss, and S. Refetoff
Departments of Medicine and Pediatrics, The J.P. Kennedy Jr. Mental Retardation Research Center, The University of Chicago, Chicago, IL

2:30 P.M. 149. **Role of Hetero- Versus Homodimers in the Dominant Negative Action of Human Mutant Thyroid Hormone β1 Receptors in Patients with Resistance to Thyroid Hormone (RTH).**
R. Wong, X. Zhu*, M.A. Pineda, S-Y Cheng*, and B.D. Weintraub
NIDDK and *NCI, National Institutes of Health, Bethesda, MD 20892

2:45 P.M. 161. **Gene Amplification As a Cause for Inherited Thyroxine-Binding Globulin (TBG) Excess.**
Y. Mori, A. Inagaki, H. Takeuchi[1], Y. Igarashi[1], and J. Sugiura[2]
First Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, [1]Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu and [2]Toyota Memorial Hospital, Toyota, Japan

3:00 P.M. ***INTERMISSION—COFFEE, POSTERS, EXHIBITS IN THE IMPERIAL BALLROOM***

SIMULTANEOUS SESSIONS

3:30 P.M. CLINICAL ORAL PRESENTATIONS—International Ballroom

CHAIRPERSONS: Leonard Wartofsky and Jerome M. Hershman

3:30 P.M. 13. **Effects of Acute Hypothyroidism on Human Brain Phosphorus Metabolism.**
K.B. Ain and C.D. Smith
Departments of Internal Medicine and Neurology, University of Kentucky Medical Center, Lexington, Kentucky and Department of Medicine, Veterans Administration Medical Center, Lexington, Kentucky

3:45 P.M. 51. **The Diagnostic Value of ^{99M}TC-Sestamibi in the Localization of Recurrent Differentiated Thyroid Cancer.**
H. Elser, I. Mattern-Alvarez, and P. Georgi
Department of Nuclear Medicine, University of Heidelberg, Germany

4:00 P.M. 86. **Treatments with 131-I Impart Less Absorbed Radiation to Thyroid Cancer than Predicted by Dosimetry.**
J.C. Sisson, R. Ackermann, S. Zempel, and S. Spaulding
University of Michigan, Ann Arbor, MI

Thursday, September 29, 1994

AFTERNOON SESSION

4:15 P.M. **High Prevalence of Thyroid Nodules in Occupationally Radiation Exposed Subjects.**
173. L. Baschieri
Instituto di Clinica Medica II, Universitá di Pisa, Pisa, Italy

4:30 P.M. **Bone Mass Is Normal in Men Chronically Treated with Suppressive Doses of Levothyroxine.**
209. C. Marcocci, F. Golia, G. Bruno-Bossio, E. Vignali, and A. Pinchera
Istituto di Endocrinologia, Università di Pisa, Pisa, Italy

4:45 P.M. **Follow Up of Graves' Disease Patients Treated with Antithyroid Drugs Alone Combined with TSH**
217. **Suppression Therapy by Triiodothyroacetic (TRIAC) or Triiodothyronine (T3).**
C. Jaffiol, N. Khalifah, J.C. Manderscheid, L. Baldet, J. Bringer, and R. Martinel
Service d'Endocrinologie, Hôpital Lapeyronie, Montpellier, France

3:30 P.M. **SYMPOSIUM: "Protein Trafficking"—The Gold Room**

Chairperson: Margaret Eggo, University of Birmingham, UK

- **Overview**
  Angela Wandinger-Ness, Northwestern University, Illinois
- **Thyroglobulin**
  Peter Arvin, Brigham & Women's Hospital, Boston, MA
- **TRH Receptor**
  Marvin Gershengorn, Cornell Medical Center, New York

5:00 P.M. **PAUL STARR LECTURE**
Ian D. Hay, M.D., Ph.D., The Mayo Clinic, Rochester, Minnesota

THE AMERICAN THYROID ASSOCIATION, INC.

FRIDAY, SEPTEMBER 30, 1994

THE MORNING SESSION

THE INTERNATIONAL BALLROOM
THE FAIRMONT HOTEL
CHICAGO, ILLINOIS

7:00 A.M. **MEETING—The EXECUTIVE COUNCIL**
Regal Room—Council Members Only

8:00 A.M. **CLINICAL ORAL PRESENTATIONS**

CHAIRPERSONS: Corbin P. Roudebush and Ian Hay

8:00 A.M.
24. **Prediction of Postpartum Onset of Graves' Thyrotoxicosis by Measurement of Thyroid Stimulating Antibody in Early Pregnancy**
N. Amino, Y. Hidaka, H. Tada, H. Tamaki, T. Kashiwai, Y. Iwatani, and N. Mitsuda
Departments of Laboratory Medicine and Obstetrics and Gynecology, Osaka University Medical School, Osaka, Japan

8:15 A.M.
49. ***Extremely* Low Doses of Heparin Can Cause Artifactual Elevations in the Serum Free Thyroxine Concentration as Measured by Equilibrium Dialysis.**
C.M. Mendel, J.C. Jaume, P.H. Frost, F. Greenspan, and C. Laughton
Cardiovascular Research Institute and Thyroid Molecular Biology Unit, VA Medical Center, Department of Medicine, University of California, San Francisco CA

8:30 A.M.
77. **Follicular Lesions of the Thyroid: The Futility of Frozen Section Evaluation.**
H. Chen, M.D.*, T. Nicol, M.D.†, A. Busseniers, M.D.†, and R. Udelsman, M.D.*
*The Division of Endocrine and Oncologic Surgery, Department of Surgery and †The Department of Pathology, The Johns Hopkins Hospital, Baltimore, Maryland 21287

8:45 A.M.
195. **Cardiac Valve Involvement in Autoimmune Thyroid Disease.**
S. Mohr-Kahaly* and G. Kahaly
Departments of Cardiology* and Endocrinology/Metabolism, Johannes Gutenberg-University Hospital, Mainz, Germany

9:00 A.M. **SIDNEY H. INGBAR DISTINGUISHED LECTURESHIP**
Bruce D. Weintraub, M.D., Chief, Molecular and Cellular Endocrinology Branch, National Institutes of Health, Bethesda, Maryland

10:00 A.M. ***INTERMISSION—COFFEE, POSTERS, EXHIBITS IN THE IMPERIAL BALLROOM***

10:30 A.M. **HORMONE ACTION ORAL PRESENTATIONS**

CHAIRPERSONS: Mitchell A. Lazar and William Chin

10:30 A.M.
16. **Synergistic Interaction Between Muscle Enhancer Factor 2 (MEF2) and T_3 Receptors (TR) in Stimulating Rat Cardiac Sarcoplasmic Endoplasmic Reticulum Ca^{2+} ATPase (SERCA2) Gene Transcription.**
A. Moriscot, M.R. Sayen, R. Hartong, and W.H. Dillmann
University of California, San Diego, CA

10:45 A.M.
26. **Identification of a *cis*-Acting Destabilizing Region Within Rat TSHβ mRNA That Mediates T_3-Induced Degradation of TSHβ mRNA.**
P.J. Leedman, R.A. Spanjaard, A.R. Stein, and W.W. Chin
Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Howard Hughes Medical Institute and Harvard Medical School, Boston, Massachusetts, 02115

11:00 A.M.
84. **Thyroid Hormone Response Elements and T_3 Receptor Cross Talk Modulate 9-cis Retinoic Acid Induced Activation of Retinoid X Receptors: A New Paradigm of Dual Hormonal Regulation?**
P.G. Walfish*, Y-F. Yang*, X. Zhu*, L. Xia*, and T.R. Butt†
*Samuel Lunenfeld Research Institute of Mount Sinai Hospital and University of Toronto Medical School, Toronto, Ontario, Canada M5G 1X5 and †SmithKline Beecham, King of Prussia, PA 19403

11:15 A.M.
85. **The Importance of Sequence Versus Spacing for Direct Repeat Thyroid Hormone Response Elements.**
R.W. Katz and R.J. Koenig
Endocrinology Division, University of Michigan Medical Center, Ann Arbor, Michigan

Friday, September 30, 1994

MORNING SESSION

11:30 A.M. 107. **Amino-Terminal Phosphorylation of Thyroid Hormone Receptor Beta Modulates Its Binding to and Function on Thyroid Hormone Response Elements.**
O. Cohen, A.N. Hollenberg, T.R. Flynn, M.K. Hegarty, J.S. Flier, and F.E. Wondisford
Thyroid Unit and Endocrine Division, Beth Israel Hospital and Harvard Medical School, Boston MA

11:45 A.M. 216. **Functional Analysis of a Transactivation Domain in the Thyroid Hormone β Receptor.**
V.K.K. Chatterjee, Y. Tone, T.N. Collingwood, and M. Adams
Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Hills Rd, Cambridge CB2 2QQ, U.K.

FRIDAY

MEET THE PROFESSORS LUNCHEONS

ADMISSION BY TICKET ONLY—$28.00
AT REGISTRATION
12:00 P.M. TO 1:30 P.M.

1. **Gregory Brent**
   Brigham & Women's Hospital
   Pregnancy and Thyroid Disease
   Crystal Room
2. **Ernest Mazzaferri**
   Ohio State University
   Treatment of Thyroid Cancer
   Metropole
3. **John Wilber**
   University of Maryland Metropole
   TRH
   Embassy Room
4. **James Fagin**
   Cedar-Sinai Medical Center
   Oncogenes
   Regent Room
5. **Sandra Mc Lachlan**
   VA Medical Center, San Francisco
   Thyroid Auto Antibodies
   State Room

Friday, September 30, 1994

POSTER SESSION

12:00 P.M. TO 2:00 P.M.

7. **Hypofunction of the Hypothalamus–Pituitary–Thyroid Axis in Cadmium-Treated Rats.**
M. Pavia, Jr., B. Paier, M.I. Noli, M.Gonzalez Pondal, K. Hagmüller, and A.A. Zaninovich
University of Buenos Aires, Hospital de Clínicas, Buenos Aires, Argentina and University of Graz, Department of Zoology, Graz, Austria

9. **Galactosyltransferase and Mannosidase II mRNA Levels Increase with Different Kinetics in Thyrotrophs of Hypothyroid Mice.**
T.E. Helton and J.A. Magner
Section of Endocrinology, East Carolina University (ECU) School of Medicine, Greenville, NC 27858

22. **Interaction of Thyroid Hormone on α- and β-Adrenergic Stimulation on Sarcoplasmic Reticulum (SR) Ca^{2+} ATPase Gene Expression in Cardiac Myocytes.**
P.S. Wu and W.H. Dillmann
University of California, San Diego, CA

31. **Thyroid Hormone Increases the Partitioning of Glucose Transporters to the Plasma Membrane in ARL 15 Cells: Surface GLUT1 Photolabeling with [^{3}H]ATB-BMPA.**
R.S. Haber*, S.P. Weinstein*, A. Pritsker*, C. Wilson†, and S.W. Cushman†
*Mount Sinai School of Medicine, New York, New York, and †National Institutes of Health, Bethesda, Maryland

37. **Effect of Thyroid Hormone on Synaptosomes: Coupling of Synaptosomal T3 Receptor to G-Proteins in Chick Embryos.**
A. Giguère, C. Beaudry, S. Fortier, N. Gallo-Payet, and D. Bellabarba
Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec, Canada

39. **Sequence of Nuclear and Mitochondrial Actions in Thyroid Hormone Activation of Metabolic Effects.**
K. Sterling and M.A. Brenner
Bronx VA Medical Center, Bronx, NY and the Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY

44. **Annexin IV Is Induced by Thyroid Hormone in α1-Receptor-Dependent Human Fibroblasts: Application of Differential Display Analysis.**
J. Menke, B. Torres, R. Klann, J. Wiley, B. Bercu, and S. Usala
East Carolina University, Greenville, NC, University of South Florida, Tampa, FL

59. **Characterization of a New Family with Inherited Pituitary Resistance to Thyroid Hormone.**
O.E. Janssen and A.E. Heufelder
Medizinische Klinik, Klinikum Innenstadt, Ludwig-Maximilians-Universität, Munich, Germany

62. **Circadian Variation in Circulating TSH Oligosaccharides: Observations from Frequent Blood Sampling in Four Human Subjects.**
J.A Magner, J. Kane, and N. Scherberg
East Carolina University School of Medicine, Greenville, NC, Michael Reese Hospital, Chicago, IL, and University of Chicago, Chicago, IL

64. **Pulsatile Thyrotropin (TSH) and Prolactin (PRL) Secretion During 10-Day Continuous Thyrotropin-Releasing Hormone (TRH) Infusions.**
M.H. Samuels and P. Kramer
Oregon Health Sciences University, Portland, Oregon and University of Texas Health Sciences Center, San Antonio, Texas

67. **Epidermal Growth Factor (EGF) Transcriptionally Down-Regulates Thyrotropin-Releasing Hormone Receptor Messenger Ribonucleic Acid in Rat Pituitary Cells.**
S. Konaka, M. Yamada, T. Monden, T. Satoh, T. Iwasaki, M. Murakami, T. Iriuchijima, and M. Mori
First Department of Internal Medicine, Gunma University School of Medicine, Maebashi, Gunma, Japan

Friday, September 30, 1994

POSTER SESSION

72. **Comparison Among Three Truncated T3 Receptors Identified in Patients with Generalized Resistance to Thyroid Hormone (GRTH).**
H. Nakamura, Y. Miyoshi*, S. Sasaki*, T. Tagami*, K. Nakao*, M. Taniyama†, T. Yoshimi
Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu; *Kyoto University School of Medicine, Kyoto; †Showa University School of Medicine, Tokyo, Japan

73. **Is the Clinical Presentation of Resistance to Thyroid Hormone (RTH) Predictable Through the Functional Analysis of Mutant Thyroid Hormone Receptor (TRβs)?**
Y. Hayashi, R.E. Weiss, E.C. Wilcox[§], T. Sunthornthepvarakul, C. Marcocci[#], and S. Refetoff
Departments of Medicine and Pediatrics, University of Chicago, [§]The Division of Genetics, Department of Medicine, Howard Hughes Medical Institute and Harvard Medical School, Boston and [#]Istituto di Endocrinologia, University of Pisa, Italy

81. **Evidence That 125I-Thyroxine (T4*) Uptake in Rat Brain Reflects Binding to Type II 5′-Deiodinase.**
J.T. Gordon, L.V. Outterbridge, E.E. Tomlinson, and M.B. Dratman
Medical College of Pennsylvania, University of Pennsylvania, and V.A. Medical Center, Philadelphia, PA

92. **Characterization of Expression and Function of T3 Receptor α and β Variants in Normal and Chronically Diseased Human Liver.**
A. Chamba, J. Hopkins, A. Strain*, J. Neuberger*, R. Bland, M.C. Sheppard, and J.A. Franklyn
Department of Medicine and *Liver Unit, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, UK

97. **Steroid Hormone Receptors Block Thyroid Hormone-Mediated Transcriptional Activation.**
P.M. Yen, E.C. Wilcox, and W.W. Chin
Division of Genetics, Brigham and Women's Hospital, Howard Hughes Medical Institute and Harvard Medical School, Boston, MA

98. **9-*cis* Retinoic Acid Regulation of Rat Growth Hormone Gene Expression: Potential Role of Multiple Nuclear Hormone Receptors.**
A. Sugawara
Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Howard Hughes Medical Institute and Harvard Medical School, Boston, MA

100. **Effect of 3,5-Diiodo-L-Thyronine on Pituitary Gene Expression In Vitro.**
S. Ball, C. Horst*, H. Rokos*, and W.W. Chin
Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Howard Hughes Medical Institute and Harvard Medical School, Boston, MA, and *Marion Merrell Dow Research Institute, Henning Berlin R&D, Berlin

104. **DNA Binding Affinity of hTRβ1 Mutants as Heterodimers with Traps from Different Tissues.**
T. Takeda and R-T. Liu
Thyroid Study Unit, The University of Chicago, Chicago, IL 60637

108. **The C-Terminus of the Beta-Subunit Is Important for Thyrotropin Secretion.**
D.T. Herodotou and F.E. Wondisford
Thyroid Unit, Beth Israel Hospital, Harvard Medical School, Boston, MA

111. **An Artificial Thyroid Hormone Receptor Mutant Without DNA Binding Can Have Dominant Negative Effect.**
R-T. Liu, S. Suzuki, and T. Takeda
Thyroid Study Unit, The University of Chicago, Chicago, IL 60637

113. **Importance of the GC Box for Constitutive Activity of the Promoter of Human Thyroid Hormone Receptor β1.**
S. Suzuki
Thyroid Study Unit, Department of Medicine, The University of Chicago, IL 60637

115. **Thyroid Hormone Potentiates rHuIFN-γ-Induced HLA-DR Expression in HeLa Cells by a Protein Kinase-Dependent Mechanism.**
H.-Y. Lin, F.B. Davis, L.J. Martino, M.G. Hogg, H.R. Thacore, and P.J. Davis
Albany Medical College, Albany, and SUNY at Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY

Friday, September 30, 1994

POSTER SESSION

118. **The Potentiation by Thyroid Hormone of rHuIFN-γ-Induced Antiviral Activity is Protein Kinase and Phospholipase C-Dependent.**
H.-Y. Lin, F.B. Davis, H.R. Thacore and P.J. Davis
Department of Medicine, Albany Medical College, Albany and Department of Microbiology, SUNY at Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY

120. **proTRH Gene Expression in the Hypothalamus Is Stimulated by Glucocorticoids *In Vitro* Within One Hour.**
IMD Jackson and L-G Luo
Division of Endocrinology, Brown University, Rhode Island Hospital, Providence RI 02903

123. **Response of Triiodothyronine-Dependent Enzyme Activities to Insulin Like Growth Factor-1 (IGF-I) and Growth Hormone (GH) in Primary Culture of Rat Liver Cells.**
C.G. Pellizas, A. Coleoni, A.M. Cabanillas, A. Masini-Repiso, and M.E. Costamagna
Clinical Biochemistry Department, School of Chemical Sciences, National University of Cordoba, Argentina

127. **Protein Kinase Inhibitor (H7) Blocks the Effect of Triiodothyronine (T3) on Acetylcholinesterase Activity (AChE) and Its mRNAs, in Neuroblastoma Cells That Overexpress the Thyroid Receptor β1.**
J. Puymirat and J.H. Dussault
CHU Laval Research Center, Quebec, Canada

128. **A Thyrotrope-Derived Cell Line Which Has Lost Thyroid Hormone Regulation Lacks TRβ2 and RXRγ mRNA.**
B.R. Haugen, W.M. Wood, D.F. Gordon, V.D. Sarapura, and E.C. Ridgway
University of Colorado Health Sciences Center, Denver, Colorado

129. **Regulation of the Human TRH Gene (hTRH) by Human Thyroid Hormone β_1 Receptor (hTRβ_1) Mutants.**
P. Feng, Q.L. Li, T. Sato, R. Wong*, and J.F. Wilber
Division of Endocrinology, University of Maryland School of Medicine, Baltimore, MD 21201, and *NIDDK, NIH, Bethesda, MD 20892

142. **Asparagine-Linked Oligosaccharide Structures Determine Clearance and Organ Distribution of Pituitary and Recombinant Thyrotropins (TSH).**
M.W. Szkudlinski, J.E. Tropea, M. Grossmann, and N.R. Thotakura
MCEB, NIDDK, National Institutes of Health, Bethesda, MD 20892

145. **Mutant Allele PCR & Sequencing (MAPS): A Novel Method for Identification of Mutations of the Human Thyroid Receptor-β (hTR-β) Gene in Patients with Resistance to Thyroid Hormone (RTH).**
M.B. Grace and G.S. Buzard*
MCEB/NIDDK and *BCDP, PRI/DynCorp NCI-FCRDC, National Institutes of Health, Bethesda, MD 20892

154. **Selective Affinity Labeling of Thyroid Hormone Receptor (TR) Monomers with a Carboxy-Esterified Derivative of Bromoacetyl T_3 (BrAcT_3-OMe).**
M. Safran, P.M. Yen, H. Rokos, and J.L. Leonard
Molecular Endocrinology Laboratory, University of Massachusetts Medical Center, Worcester, MA, Division of Genetics, BWH and Harvard Medical School, Boston, MA and Marion Merrell Dow Research Institute, Henning Berlin GMBH, GFR

155. **T_4-Dependent Regulation of the Secretion and Extracellular Organization of Laminin and Fibronectin in Astrocytes.**
A.P. Farwell
Molecular Endocrinology Laboratory, University of Massachusetts Medical School, Worcester, MA

162. **A Novel Mutation at Outside of the Hot Spot Regions of the Thyroid Hormone β Receptor in a Family with Thyroid Hormone Resistance.**
K. Onigata, A. Sakurai*, H. Yagi, T. Miyamoto*, K. Hashizume*, K. Nagashima, A. Morikawa
Department of Pediatrics, Gumma University School of Medicine, Maebashi, and *Department of Geriatrics, Endocrinology and Metabolism, Shinshu University School of Medicine, Matsumoto, Japan

163. **Homo- and Hetero-Dimerization Ability of Two Defective Dominant Negative Mutant Thyroid Hormone Receptor β: ARG311HIS and 422.**
T. Miyamoto, R. Sekine, A. Sakurai, and K. Hashizume
Department of Geriatrics, Endocrinology and Metabolism, Shinshu University School of Medicine, Matsumoto, Japan

Friday, September 30, 1994

POSTER SESSION/AFTERNOON SESSION

165. **Identification of a Retinoic Acid Response Element in the Rat Thyrotropin Beta Subunit Gene.**
J.J. Breen and J.A. Gurr
Department of Biochemistry and Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140

170. **Effects of Thyroid Hormones on the Colonic and Brown Adipose Tissue Thermal Response to Norepinephrine.**
N. Negrão, F.L.A.S. Lebrun, and A.C. Bianco
Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

174. **Indirect Evidence for a Role of the 5'-Monodeiodinase on the Regulation of GH mRNA Levels.**
C.B. Volpato and M.T. Nunes
Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, Brazil

178. **T3-Dependent Ontogeny of a T3-Activated Stretch Channel in Rat Atriocytes.**
W.L. Green, C.A. Shuman, and V. Chen
Veterans Affairs Medical Center and SUNY/Brooklyn, Brooklyn, NY

179. **Differentially Expressed RXRs Modulate Thyroid Hormone Action in Primary Hepatocyte Cultures.**
T. Nagaya, Y. Murata, M. Menjo, A. Sugawara, and H. Seo
Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan, and Brigham and Women's Hospital and Harvard Medical School, Boston, MA

187. **Chronic Treatment with Vitamin A Does Not Modify Clinical and Biochemical Features of Patients with Thyroid Hormone Resistance.**
P.Beck-Peccoz, D. Cortelazzi, L. Persani, D. Preziati and V.K.K. Chatterjee*
Institute of Endocrine Sciences, University of Milan, Milan, Italy, and *Department of Medicine, University of Cambridge Clinical School, Cambridge, UK

191. **Sex Differences in the Action of TRH on Rat Pituitary TSH and PRL Secretion In Vivo and In Vitro.**
X. Wang, M.A. Greer, and S.E. Greer
Section of Endocrinology, Department of Medicine, Oregon Health Sciences University, Portland OR 97201

192. **Osmotic Cell Swelling Stimulates Hypothalamic Median Eminence TRH Secretion Which Is Independent of Ca^{2+} Influx.**
M.A. Greer, S.E. Greer, and X. Wang
Oregon Health Sciences University, Portland, OR

193. **Structure-Function Relationship of Amiodarone Analogues in the Inhibition of Thyroid Hormone Binding to α_1 and β_1 T_3 Receptors.**
W.M. Wiersinga, H.C. van Beeren, and O. Bakker
Department of Endocrinology, University of Amsterdam, The Netherlands

224. **The Effect of Retinoic Acid (RA) on T_3-Induced Responses in HepG2 Cells.**
JW Barlow, TC Crowe, NM Loidl, NL Cowen, DJ Topliss, and JR Stockigt
Ewen Downie Metabolic Unit, Alfred Hospital, Commercial Rd., Melbourne, Australia, 3181

230. **Thyroid Hormone Regulation of Mitochondrial Adenine Nucleotide Translocase and Glycerol-3-P Dehydrogenase.**
K. Dümmler#, S. Müller#, A. Harneit#, F. Buck¶, and H.-J. Seitz#
#Inst. Physiol. Chemie, ¶Inst. Zellbioch., Univ.-Krankenhaus Eppendorf, 20246 Hamburg, Germany

SIMULTANEOUS SESSIONS

2:00 P.M. **AUTOIMMUNITY ORAL PRESENTATIONS—International Ballroom**

CHAIRPERSONS: Jack Wall and Anthony Weetman

2:00 P.M. 19. **Establishment of an Anti-Human Thyrotropin Receptor (TSHR) Specific $CD4^+$ T Cell Line from a Patient with Graves' Disease: Evidence for Multiple T Cell Epitopes on TSHR.**
T. Akamizu*, Y. Ueda†, L. Hua*, and T. Mori*
*Department of Laboratory Medicine, Kyoto University School of Medicine, Kyoto, Japan, †Central Research Laboratory, Ishihara Industrial Co., Kusatsu, Japan

Friday, September 30, 1994

AFTERNOON SESSION

2:15 P.M. 110. **Thyroid Atrophy with Extensive Fat Replacement and Hypothyroidism in Mice Immunized with the Extracellular Domain of the Human TSH Receptor.**
V.C. Guimaraes, Y. Hidaka, J. Quintans, F.H. Strauss, Y. Okamoto, G. Medeiros-Neto, and L.J. DeGroot
Thyroid Study Unit, The University of Chicago, Section of Endocrinology of Osaka City General Hospital, and Hospital das Clinicas, University of San Paulo Medical School, Brazil

2:30 P.M. 114. **Native Gel Electrophoresis of the 64 kDa Protein Shows That It Is Eye Muscle Specific and an Important Target for Autoantibodies in Patients with Thyroid-Associated Ophthalmopathy.**
J.R. Wall, D. Scalise, M. Hayes, C. Stolarski, M. Sato, M. Salvi, and V. Nebes
Thyroid Center, Allegheny-Singer Research Institute, Pittsburgh, PA

2:45 P.M. 117. **Human Autoantibodies to the TSH Receptor: Polyclonal Recognition of Linear, Folded and Glycosylated Recombinant Extracellular Domain.**
H. Vlase, P.N. Graves, R. Magnusson, and T.F. Davies
Departments of Medicine and Pharmacology, Mount Sinai School of Medicine, New York, New York

2:00 P.M. **TSH–TRH ORAL PRESENTATIONS—The Gold Room**

CHAIRPERSONS: James A. Magner and Alan C. Moses

2:00 P.M. 42. **TRH Stimulates TSHβ Promoter Activity by Two Distinct Mechanisms Including Ca^{2+} Uptake Through L-Type Ca^{2+} Channels and Activation of Protein Kinase C.**
M.A. Shupnik and P.M. Hinkle
Department of Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, VA and Department of Pharmacology, University of Rochester Medical School, Rochester, New York

2:15 P.M. 125. **MSX-1 and OCT-1 Interact with 5′ Flanking Sequences Important for Thyrotropic α-Subunit Gene Expression.**
V. Sarapura
University of Colorado Health Sciences Center, Denver, CO

2:30 P.M. 126. **Construction of a 3-Dimensional Model of the Thyrotropin-Releasing Hormone Receptor Binding Site at an Atomic Level.**
J.H. Perlman, L. Laakkonen, R. Osman, and M.C. Gershengorn
Cornell University Medical College and Mt Sinai Medical Center, NY, NY

2:45 P.M. 140. **Role of the Carboxyterminus of the Alpha Subunit in the Expression and Bioactivity of Human TSH: Identification of Deletion Mutants That Act as Competitive Antagonists.**
M. Grossmann, M.W. Szkudlinski, H. Zeng*, R.N. Thotakura, J.E. Tropea, T.H. Ji*, and B.D. Weintraub
MCEB, NIDDK, National Institutes of Health, Bethesda, MD 20892, and *Department of Molecular Biology, University of Wyoming, Laramie, WY 82071

3:00 P.M. ***INTERMISSION—COFFEE, POSTERS, EXHIBITS IN THE IMPERIAL ROOM***

SIMULTANEOUS SESSIONS

3:30 P.M. **THYROID HORMONE METABOLISM ORAL PRESENTATIONS—International Ballroom**

CHAIRPERSONS: Inder Chopra and Elaine Kaptein

3:30 P.M. 58. **Molecular Modeling and Site-Directed Mutagenesis of the Ligand Binding Domain of Thyroxine-Binding Globulin.**
C. Büttner, B. Treske, and O.E. Janssen
Medizinische Klinik Innenstadt, Ludwig-Maximilians-Universität, Munich, Germany

3:45 P.M. 69. **Effect of 6-Anilino-2-Thiouracil, a Potent Inhibitor of Hepatic 5′ D-I Activity, and Selenium Deficiency on Thyroid Hormone Economy in the Neonatal Rat.**
I.E. Veronikis, S. Alex, C.H. Emerson, S.L. Fang, G. Wright, and L.E. Braverman
University of Massachusetts Medical School, Worcester, MA

4:00 P.M. 101. **The Type III 5-Deiodinase in *Rana Catesbeiana* (RC) Tadpoles Is Encoded by a Thyroid Hormone-Responsive Gene.**
V.A Galton, M.J. Schneider, K.B. Becker, and J.C. Davey
Dartmouth Medical School, Lebanon, NH 03756

Friday, September 30, 1994

AFTERNOON SESSION

4:15 P.M. **Identification of a cDNA for the Rat Type III Iodothyronine 5-Deiodinase (5DIII).**
102. W. Croteau, S.L. Whittemore, M.J. Schneider, and D.L. St. Germain
Departments of Medicine and Physiology, Dartmouth Medical School, Lebanon, New Hampshire

4:30 P.M. **Comparison of Amino Acid Requirements for 5′ and 5 Deiodination of Iodothyronines by Type 1**
133. **Deiodinase (D-1).**
N. Toyoda, P.R. Larsen, M.J. Berry, J.W. Harney, C. Horst#, and T.J. Visser*
Brigham and Women's Hospital, Boston, MA, #MMDRI, Henning, Berlin, Germany and *Erasmus University, School of Medical, Rotterdam, The Netherlands

4:45 P.M. **Structure and Function of Normal and Defective Mouse Type I Deiodinase (*dio1*) Genes.**
135. A.L. Maia, M.J. Berry, R. Sabbag, J.W. Harney, and P.R Larsen
Brigham and Women's Hospital, Boston, MA

3:30 P.M. **SYMPOSIUM: "The TSH Receptor"—The Gold Room**

Chairperson: Leonard Kohn, National Institutes of Health, Bethesda

- **Protein Folding and the TSHR**
  Peter Graves, Mt. Sinai Medical Center, New York
- **Immune Responsiveness of Insect Cell HTSHR**
  Paul Banga, Kings College School of Medicine, London
- **Mutational Analysis**
  Gilbert Vassart, Université Libre de Bruxelles

5:00 P.M. **PRESIDENT'S ADDRESS AND THE ANNUAL BUSINESS MEETING OF THE AMERICAN THYROID ASSOCIATION, INC.**
For Members Only
The Gold Room

6:30 P.M. **RECEPTION AND BANQUET—International Ballroom**

THE AMERICAN THYROID ASSOCIATION, INC.

SATURDAY, OCTOBER 1, 1994

THE MORNING SESSION

THE FAIRMONT HOTEL
CHICAGO, ILLINOIS

7:00 A.M. **EARLY RISER LECTURE: "Thyroid Hormone and the Brain"—The Gold Room**
Jack H. Oppenheimer, University of Minnesota, MN

7:00 A.M. **MEETING—INTERNATIONAL ORGANIZING COMMITTEE**
Regal Room—Members Only

8:00 A.M. **SYMPOSIUM: "Thyroid and Mood Debate"—The Gold Room**

CHAIRPERSON: E. Chester Ridgway, University of Colorado Health Science Center, Denver, CO

- **For**
  Russell Joffe, Clark Institute of Psychiatry, Toronto
- **Against**
  Ivor Jackson, Brown University, Providence, RI

9:00 A.M. **STATE OF THE ART LECTURE: "Molecular Cloning and Human Disease"—The Gold Room**
Graeme Bell, Ph.D.

10:00 A.M. ***INTERMISSION—COFFEE IN THE GOLD ROOM***

SIMULTANEOUS SESSIONS

10:30 A.M. **SYMPOSIUM: "Pediatric Thyroid Disease"—Moulin Rouge Room**

Chairperson: Rosalind Brown, University of Massachusetts, MA

- **Congenital Hypothyroidism—Current Controversies**
  Stephen La Franchi, Oregon Health Sciences University, OR
- **Graves' Disease in the Young**
  Donald Zimmerman, Mayo Clinic, Rochester, MN
- **Chernobyl—Implications for Children**
  Thomas P. Foley, University of Pittsburgh, PA

10:30 A.M. **THYROID HORMONE ACTION ORAL PRESENTATIONS—The Gold Room**

CHAIRPERSONS: Fredric E. Wondisford and Stephen Jon Usala

10:30 A.M. 1. **The Effect of Thyroid Hormone Receptors on the 9-*cis* Retinoic Acid Responsiveness of Retinoid X Receptors Is Cell Type and Isoform-Specific.**
A.L. O'Donnell and A. Burakowski
Department of Medicine, VA Medical Center and SUNY, Buffalo, NY

10:45 A.M. 47. **Interaction of the Thyroid Hormone Receptor with a Novel Oncoprotein Associated with a Pediatric Malignancy.**
W. Seol and D.D. Moore
Department of Molecular Biology, Massachusetts General Hospital, Boston, MA

11:00 A.M. 99. **Relationship of Isoform-Specific T3 Binding Capacities to mRNA Levels in Rat Pituitary and Extrapituitary Tissues.**
J.H. Oppenheimer, H.L. Schwartz
Department of Medicine, University of Minnesota, Minneapolis

11:15 A.M. 105. **Characterization of an Element in the 5′ Flanking Region of a Purkinje Cell Gene (PCP2) Which Abolishes Triiodothyronine (T3) Regulation in Transient Transfection Studies.**
G.W. Anderson, K.A. Strait, and J.H. Oppenheimer
University of Minnesota, Minneapolis, MN

Saturday, October 1, 1994

MORNING SESSION

11:30 A.M. **Negative Thyroid Hormone Response Elements Are Distinguished by Their Interactions with the**
112. **Retinoid X Receptor (RXR).**
A.N. Hollenberg, T.R. Flynn, and O. Cohen
Thyroid Unit, Beth Israel Hospital and Harvard Medical School, Boston, MA

11:45 A.M. **Brown Adipose Tissue β_3-Adrenergic Receptors Are Down-Regulated by Thyroid Hormone.**
138. A. Rubio, A-L.S. Maia, A. Raasmaja, and J.E. Silva
Division of Endocrinology, Jewish General Hospital, McGill University, Montreal, Canada

12:00 P.M. **END OF 68TH ANNUAL MEETING**

PLEASE REMEMBER TO PICK UP YOUR CME CREDIT CERTIFICATE

2. SERUM OSTEOCALCIN CORRELATES WITH TRIIODOTHYRONINE (T3) LEVELS IN HYPOTHYROID PATIENTS HAVING ABNORMAL TSH RESPONSES TO TRH UNDER TREATMENT WITH L-THYROXINE (L-T4). H. Niepomniszcze, S. Damilano, G. Faraj and R. Lutfi. Service of Endocrinology, Complejo Médico (PFA) "Churruca-Visca", Buenos Aires, Argentina.

In previous studies we have shown that osteocalcin is not a useful clinical marker for increased bone turnover in patients slightly overtreated with L-T4. However, our preliminary results have opened the possibility that a positive correlation between osteocalcin and serum T3 can take place under such conditions. For this reason we investigated 18 hypothyroid women, aging 51±18 (SD) yrs., who were taking a single daily dose of L-T4 since, at least, one year prior to this study. T3, T4, basal TSH and 25´ post 200 µg., i.v., TRH, and osteocalcin were measured in serum samples of all these women, by RIA techniques. According to the results of TRH-TSH tests they were divided in 3 groups: a) hyperresponse of TSH to TRH (n=5) (subclinical hypothyroidism); b) normal TRH-TSH test (n=7) (euthyroidism); and c) blunted response of TSH to TRH (n=6) (subclinical hyperthyroidism). The daily dose of L-T4 for each group was; a) 135±34 (SD) µg, b) 125±43 µg, and c) 165±38 µg. Basal TSH levels showed: a) 8.6±(SEM) 2.7 µU/ml, b) 1.7±0.3 µU/ml, and c) 0.8±0.2 µU/ml. Peak values of TSH post TRH: a) 40.3±(SEM) 3.8 µU/ml, b) 12.2±2.1 µU/ml, and c) 1.7±0.4 µU/ml. There were no differences in osteocalcin levels among the groups, ranging values between 2.4-13 ng/ml (normals < 14 ng/ml). The mean ages of the women were similar for all groups, as well as the mean levels of circulating thyroid hormones. The average values, for all patients, were: T3=117±(SD) 23 ng/dl, and T4=10.5±2.2 µg/dl. There were no correlations between serum osteocalcin and serum levels of T4 or the L-T4 daily dose. However, a positive correlation was observed between osteocalcin and T3 in groups a (r=0.99; p<0.002) and c (r=0.97; p<0.001), but not in group b.

We concluded that osteocalcin correlates with serum T3 only in cases where L-T4 replacement therapy is not adjusted to allow a normal status of the pituitary-thyroid axis, rising the possibility that bone turnover is rather independent of thyroid activity when hormonal production is strictly normal.

5. MANIFESTATION OF HYPERTHYROIDISM IN DIFFERENT POSTPARTUM PERIODS OF THE SAME PATIENTS WITH GRAVES' DISEASE. N.Momotani, Y.Gomi, J.H.Noh, N.Ishikawa, K.Ito. Ito Hospital, Tokyo Japan.

The forms of hyperthyroidism seen in women with a history of Graves' disease during postpartum periods are various: Graves' hyperthyroidism alone, silent thyroiditis alone, and Graves' hyperthyroidism preceded by silent thyroiditis. To know whether susceptibility resulting from genetic factors is related to the variety, we investigated the forms of hyperthyroidism that developed within a year after delivery in two different postpartum periods in the same women with Graves' disease. Graves' hyperthyroidism and silent thyroiditis were differentiated on the basis of radioiodine uptake, urinary iodine excretion, and/or the duration of hyperthyroidism. TBII had been detected in all of the 12 women who were included in this study when they were diagnosed as having Graves' disease. TBII around the time of conception was positive in one woman and negative in 7 women in both pregnancies. In the other 4 women TBII was positive in one pregnancy and negative in the other. Thionamides were discontinued before or during pregnancy. All of the women were euthyroid at the time of delivery in both pregnancies. In 3 women TBII was positive at or near term in both pregnancies, negative in 6 in both pregnancies; and in the other 3 women TBII was positve in one pregnancy and negative in the other. Hyperthyroidism developed during all of the 24 postpartum periods. The same women manifested the same forms of hyperthyroidism during the two different postpartum periods: in 5 women, only Graves' hyperthyroidism, in the other 6 women, only silent thyroiditis occurred, and in the remaining 1 woman, Graves' hyperthyroidism developed following silent thyroiditis. The peak values of TBII and the times when TBII peaked after delivery were similar in both pregnancies in most of the cases. There was no relationship between the forms of hyperthyroidism and the results of TBII around the time of conception and at or near term. It is concluded from these findings that genetic factors and not the activities of Graves' disease before or during pregnancy may account for postpartum immunological changes that cause the distruction of the thyroid and the production of thyroid stimulating antibodies.

17. NA+K+ATPASE ACTIVITY IN RED CELLS PREDICTS THE RECURRENCE OF HYPERTHYROIDISM IN PATIENTS WITH GRAVES' DISEASE. C.De Riva, F.Virgili and F.Frigato, Department of Endocrinology Umberto I° General Hospital, Mestre-Venezia,Italy.

The clinical course of hyperthyroidism dependent by Graves' disease is still unpredictable in the patients treated with antithyroid drugs. Several parameters have been evaluated for the prediction of relapse of hyperthyroidism after discontinuation of therapy. They include TSH receptor antibodies, thyroglobulin, T3 suppression test, HLA determination, T3/T4 ratio, thyroidal technetium-99m uptake. Among these, the T3 suppression test and the measurement of TSH receptor antibodies (TR-AB) have been considered the most sensitive. Recently the thyroid hypoechogenic pattern at ultrasonography and the response of plasma FT3 for 3 h. after TRH administration have been proposed.We recently demonstrated that in hyperthyroid patient the activity of Na+K+ATPase in the erythrocytes is impaired and it may be normalised by thionamide therapy during a period of 150 days with a concomitant normalisation of FT3 levels. We observe, elsewhere, a linear regression between FT3 and Na+K+ ATPase activity in red cells. With the aim to clarify the relationship between Na+K+ATPase activity and the recurrence of disease after the outcome of antithyroid therapy we followed up 24 patients affected by Graves' disease during a period of 2 years studying the pattern of ATPase activity and the incidence of relapse of hyperthyroidism . At the start of the study the patients had a reduced Na+K+ ATPase activity in red cells (1.35±0.2 vs 2.09±0.3 mmol Pi h-1.L-1 RBC, $p<0.01$). Thionamide therapy restored in all the subjects a normal level of Plasma T4, FT4, FT3 in a median period of 180 days . Antithyroid treatment replaced a normal level of red cells Na+K+ ATPase activity in all subjects after a period of 150 days (2.04±0.4). The dosage of drugs was progressively reduced until to stop it when the patients became clinically and biochemically euthyroid (median length of therapy period was 304+-15 days). The following determinations (day 450) of erythrocytes Na+K+ATPase activity showed normal values only in 16 patients (Group Tx), where it was newly reduced in 8 subjects (Group Tx-R). On the contrary in 450th day all the patients were still euthyroid. During the time-interval between 450th and 600th day the patients in Group Tx-R developed a recurrence of hyperthyroidism as demonstrated by clinical grounds and thyroid hormone determinations in the presence of an impaired Na+K+ ATPase activity. The patients in Group Tx were still euthyroid and Na+K+ ATPase in red cells was normal. Conclusions:1) the determination of Na+K+ ATPase activity in the erythrocytes seem to be an early marker of relapse of hyperthyroidism. 2) the alteration is obvious before the clinical or biochemical relapse of hyperthyroidism suggesting an action to the red cells during the maturation (in the bone marrow?).

23. RELATIONSHIPS BETWEEN PITUITARY AND THYROID FUNCTION IN PATIENTS WITH CENTRAL HYPOTHYROIDISM;THYROID HORMONE CONCENTRATIONS, BIOACTIVITY OF TSH, AND RESPONSE OF TSH TO TRH. M.Horimoto, M.Nishikawa, M.Yoshimura, T.Ishihara[2)] and M Inada. Second Department of Internal Medicine, Kansai Medical University, 570 Osaka, Japan and Internal Medicine, Kobe Central Municipal Hospital[2)], Kobe 658, Japan.

To investigate cause(s) of central hypothyroidism with normal or elavated TSH concentrations, we evaluated that pituitary function and thyroid function as well as bioactivity of serum TSH.

Subjects were seven patients with documented deficiency(ies) of anterior pituitary hormones other than TSH and hypothyroidism. Their basal TSH concentrations were from 2.2 to 14.8mU/L. Six of these patients had low T4 and free-T4 concentrations, and the remaining one had low free-T4 and low normal T4 with elevated TSH concentrations of 14.4mU/L. Mean increments of TSH at 30, 60, and 90 min. after TRH administration(mean-ΔTSH) in these patients, being 13.5±9.1mU/L(mean±SD), were not significantly different from those in normal subjects, being 9.2±3.5 mU/L (mean±SD). On the other hand, ratios of T_3-increment at 120 min. (ΔT3) to mean-ΔTSH (ΔT3/mean-ΔTSH) in these patients of 8.2±4.4nmol/U(mean±SD), were significantly lower than those in eight normal subjects of 39.3±22.3nmol/U(mean±SD) ($p<0.01$), suggesting that thyroid response was reduced. Serum T4 concentrations were correlated with mean-ΔTSH in these patients ($r=0.78$, $p<0.05$), while ΔTSH is exaggerated in patients with primary hypothyroidism, suggesting that hypothyroidism in these patients depended on conserved pituitary function in spite of normal or elavated TSH levels. The bioactivity to immunoreactivity-ratios of TSH in these patients, being 0.97±0.27(mean±SD), were not significantly different from those in eight normal subjects, being 1.05±0.22(mean±SD), and all within the range of the mean± 2×SD of normal subjects(0.61 to 1.49), suggesting that bioactivities of TSH in these patients were not decreased.

The above findings suggest that thyroid function in the patients with central hypothyroidism was reduced because of pituitary function disorder, not reduced bioactivity of TSH, in spite of their normal or even slightly increased serum TSH levels as well as non-reduced response of TSH secretion to TRH.

33. **POSSIBLE INVOLVEMENT OF SYMPATHETIC OVERACTIVITY IN LID RETRACTION IN GRAVES' DISEASE EVEN IN THE EUTHYROID STATE.** N. Hamada, J. Y. Noh,* Y. Nakamura,** and K. Ito.* Thyroid Study Unit, Sumire Hospital, Osaka 536, *Ito Hospital, Tokyo 150, and ** NTT Osaka Central Health Administration Center, Osaka 530, Japan

We have reported that pupil size (PS) is large, pupillary unrest (PU) is slight, and accommodation (AC) amplitude is low in hyperthyroid Graves' disease, suggesting sympathetic overactivity in the eye. In this report, the effect on PS, PU, and AC of restoration of thyroid function to normal was investigated in patients with Graves' disease. In addition, PS, PU, and AC in the euthyroid state were compared in patients with and without lid retraction. Four hyperthyroid patients with Graves' disease were treated with antithyroid drugs. Before treatment and at least 3 months after euthyroidism was restored, PS, PU, and AC were measured with a computer-assisted infrared television camera (Iriscorder) and an infrared optometer (Nidek, Aichi, Japan). The area of pupils was measured at a 12.5- Hz sampling rate for 100 seconds during continuous illumination. The mean and coefficient of variation of the areas were calculated and used to express the PS and the degree of PU, respectively. AC was measured in substatic (AC-S) and dynamic (AC-D) conditions, and the results are expressed as the change in refractive power of the eye in response to movements of a target. PS, PU, and AC were measured in two euthyroid Graves' patients with lid retraction, also. As thyroid function returned to normal, PS tended to decrease, and PU increased from 4.46 ± 1.47: mean ± SD (%) to 10.5 ± 1.8 ($P < 0.01$), AC-S increased from 3.33 ± 0.99 diopters to 5.43 ± 0.58 ($P < 0.01$), and AC-D increased from 1.97 ± 1.09 diopters to 2.74 ± 1.18 ($P < 0.05$; $n = 6$ eyes, by paired t-test). PS was significantly greater, PU was significantly smaller, and AC-S and AC-D amplitudes were significantly less in the euthyroid Graves' patients with lid retraction (n=4 eyes, 47.12 ± 2.90 mm^2, 2.22 ± 1.74%, 3.11 ± 0.68 diopters, and 2.03 ± 0.83 diopters, respectively) than in the euthyroid Graves' patients without lid retraction (n=6 eyes, 34.3 ± 9.0, 10.5 ± 1.8, 5.42 ± 0.58, and 2.74 ± 1.18, respectively) by Student's t-test. Pupillary unrest and accommodation become normal during treatment of hyperthyroidism in some Graves' patients, but in those patients with lid retraction, the abnormalities continued when a euthyroid state was reached. Sympathetic overactivity may play a role in lid retraction of Graves' disease even in the euthyroid state.

38. INCLUSION OF SERUM PROTEIN BOUND T_4 IN MEASUREMENTS OF SERUM FREE T_4 BY NONDIALYSIS METHODS. Jerald C. Nelson and R. Bruce Wilcox, Loma Linda University School of Medicine, Loma Linda, CA. 92354

Measurements of serum free T_4 (FT_4) by nondialysis methods are often inaccurate (JCEM in press). These methods systematically underestimate [FT_4] in simple solutions that do not contain serum protein bound T_4 (PBT_4) (see below). In order to obtain FT_4 measurements similar to generally accepted values for serum [FT_4], these assays require the presence of PBT_4. Because the inaccurate FT_4 measurements they produce are proportional to [PBT_4], it is likely that the magnitude of the contribution by PBT_4 to these measurements introduces bias (JCEM in press). This study examined the quantity of serum PBT_4 included in measurements of serum FT_4 by nondialysis methods.

DESIGN: FT_4 measurements were obtained on two series of test solutions containing identical [TT_4] ranging between 0.031-24.0 μg/dL. One series contained T_4 in buffer without serum (BUFFER), in which [TT_4] = [FT_4]; the other contained T_4 in a pool of T_4-free normal human serum (SERUM), in which 0.022% of TT_4 was FT_4 (as determined by equilibrium dialysis). Measurements were made using six free T_4 immunoassays. Four were one-step and two were two-step in design. Three were manual and three were automated. Because serum proteins bind T_4, the addition of serum to T_4 lowers FT_4 measurements by excluding a portion of TT_4 from these measurements. The included or remaining portion was quantified as the ratio of measurements on SERUM to measurements on BUFFER at identical [TT_4], expressed as a percent. All measurements were repeated three times.

RESULTS: FT_4 measurements ranged between 0.16-6.56 ng/dL on BUFFER, and 0.08-6.14 ng/dL on SERUM, providing in each assay measurements on both at equal [TT_4]. FT_4 measurements on BUFFER ranged between 0.016%-0.960% of [TT_4], a striking apparent loss of FT_4. FT_4 measurements on SERUM were 3.3%, 4.7%, 33%, 55%, 58%, and 67% of [TT_4], a striking inclusion of TT_4. The quantity of serum TT_4 included in these measurements of serum FT_4 exceeded the quantity of FT_4 150 to 3045 fold, the difference being attributable to PBT_4.

CONCLUSION: The large quantities of PBT_4 (relative to FT_4) included in measurements of serum FT_4 by these methods were more than sufficient to explain their PBT_4 dependent bias.

52. Methoxy-Iso-Butyl-Isonitrile (MIBI)-^{99m}Tc-Scanning: An Approach to the Localisation of Recurrent Medullary Thyroid Carcinoma (MTC)

M.Colombo-Benkmann*, H.Elser+, P. Hartkorn*, P.Georgi +, H.J. Buhr*
Depts of Surgery* and Nuclear Medicine +, University of Heidelberg, Germany

Due to improved surgical therapy, MTC has become potentially curable. A challenge to current diagnostic methods available, is the exact localisation of MTC-recurrencies.They are usually located by ultrasonography (US), computed tomography (CT), magnetic resonance tomography (MRT) and selective venous catheterisation (SVC). MIBI is a recently developed radiotracer which has been demonstrated to have an increased uptake in MTC-recurrencies, as described in some case reports. However these reports failed to obtain regular histological confirmation of the detected lesions.
The objective of our prospective study was to determine the diagnostic value of MIBI-scanning in local or mediastinal recurrencies of MTC. 11 patients with previous total thyroidectomy and with known or suspected cervical or mediastinal MTC-recurrencies were prospectively studied. Each patient underwent MIBI-scanning, SVC, CT and US within one week preoperatively. One patient was correctly diagnosed as being free of recurrence. In the remaining ten patients 14 areas were correctly localised by MIBI, as confirmed histologically. In one of these patients 2 enhanced uptake areas due to asymmetrical uptake in salivary glands without any pathological histology were observed. In one patient one additional retrosternal micrometastasis could not be demonstrated in any of the applied diagnostic tests.
Conclusion: According to our results MIBI seems to be of considerable value in the diagnosis of MTC-recurrencies in the neck and the mediastinum. However more patients are to be studied in order to confirm our results and in order to determine the exact sensitivity and specificity of this method.

53. HUMAN T-LYMPHOTROPIC VIRUS TYPE I (HTLV-I) ASSOCIATED UVEITIS IN PATIENTS WITH GRAVES' DISEASE TREATED WITH METHYLMERCAPTOIMIDAZOLE (MMI). T. Mizokami, K. Okamura, T. Kohno*, K. Sato, H. Ikenoue, T. Kuroda, K. Inokuchi and M. Fujishima, Second Department of Internal Medicine and *Department of Ophthalmology, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

Human T-lymphotropic virus type I (HTLV-I) causes adult T-cell leukemia lymphoma (ATLL) and HTLV-I associated myelopathy/ tropical spastic paraparesis (HAM/TSP). Recently, it has been proposed that HTLV-I infection might be primarily responsible for certain forms of pulmonary alveolitis, chronic arthropathy, and uveitis of heretofore undefined etiologies. Moreover, it was reported that the patients with HTLV-I associated uveitis sometimes had a previous history of either Graves' disease or hyperthyroidism. In this work we clinically evaluated the relationship between Graves' disease, HTLV-I associated uveitis, and the administration of methylmercaptoimidazole (MMI). Eight hundred and nineteen consecutive patients with thyroid disorders who visited our thyroid clinic at the Second Department of Internal Medicine, Kyushu University Hospital (392 with Graves' disease, 257 with chronic thyroiditis, and 170 with nodular goiter) examined between January 1991 and December 1992. Most of the patients were residents of Fukuoka Prefecture, located in the northern district of Kyushu Island, which is in the south-western part of Japan, where HTLV-I infection is comparatively endemic. All patients who complained of eye symptoms were referred to ophthalmologists. In all patients, the thyroid functional tests were studied. The presence of serum anti-HTLV-I antibody was also screened by the particle agglutination (PA) method, and PA-positive sera were further examined by the western blot method. HTLV-I antibody was found in 25 patients with Graves' disease, 19 with chronic thyroiditis, and 3 with nodular goiter. Five patients either had or developed uveitis during the study period, which was considered to be HTLV-I associated uveitis by the presence of HTLV-I antibody in the serum after an exclusion of other established clinical etiologies of uveitis. The initial symptoms of uveitis were either acute or subacute foggy vision or blurred vision. All of these 5 patients had Graves' disease treated with MMI for 2 months to 16 years. The dosage of MMI was between 5mg every two days and 15mg every day. Within a few months before the onset of the initial symptoms of uveitis, 3 patients were found to be hyperthyroid, and 2 were hypothyroid. In 2 of 5 patients, an exacerbation of uveitis occurred soon after the relapse of Graves' hyperthyroidism. All uveal lesions were resolved in a few weeks by systemic and/or topical corticosteroid therapy. During the study period, no further relapse was observed while they remained euthyroid by taking MMI.

In conclusion, it was suggested that Graves' disease, thyroid dysfunction and/or MMI administration may thus play some precipitating role in the pathogenesis of HTLV-I associated uveitis.

54. DETECTION OF RAS ONCOGENE MUTATION IN RAT TRANSPLNTABLE THYROID TUMORS. Y. Hiasa, Y. Kitahori, K. Yane, N. Konishi, M. Ohshima, H. Naitoh, K. Okaichi, T. Ohonishi, Y. Matsuda. Dept. of Pathology, Biology and Otohinolaryngopharyngology, Nara Medical University, Kashihara, Nara-634, Japan.

Thyroid tumors were induced in Whistar rats treated with N-bis (2-hydroxypropyl) nitrosamine (DHPN). The tumors were implanted in the back subcutaneous tissue of same strain rats. Sixteen transplantable thyroid tumors have been established from these tumors and cultured cell lines have been established from them.

The purpose of the present studies are to detect point mutation or ras oncogene in these transplantable thyroid tumors and the cultured cell lines, using PCR amplication and direct sequencing.

The activated form of the ras oncogenes were present in 5 (26%) of a total of 15 cell lines. Four of these gene mutations occured in Ki-ras codons 12 and 63 and the other involved the first nucleotide of Ha-ras codon 12. Three of the Ki-ras mutations were located in codon 12, 2 in the first nucleotide and one in the second position and the other affected the first nucleotide of codon 63. In addition, mutation in codon 63 was detected in TRTC-G1-C that is one of the cultured cell line with mutation in codon 63. All these mutations were G → A transitions of the first or second nucleotide. Histologically, 3 of these 4 carcinomas with Ki-ras point mutations only were diagnosed as well differentiated and the other as poorly differentiated. Mutations of the ras gene are relatively uncommon in these histological types.

These data indicate that, of the ras family, DHPN induces Ki-ras gene activation preferentially and that G → A transitions are the predominant mutation type. It is, therefore, suggested that tumorigenesis in DHPN-induced thyroid carcinoma cells may involve Ki-ras activation as a part of multistep pathway, although Ki-ras involvement is not obligatory for tumor development.

60. EFFECTS OF RADIOIODINE ON THYROTROPIN BINDING INHIBITING IMMUNOGLOBULINS IN GRAVES DISEASE : LONG-TERM FOLLOW UP STUDY. Y.Aizawa,K.Yoshida,N.Kaise,K.Kaise,H.Fukazawa,Y.Kiso,K.Mori,N.Sayama,K.Kikuchi, K.Abe. The Second Department of Internal Medicine,Tohoku University School of Medicine, Sendai,Japan.

The long-term effects of I-131 therapy on thyrotropin binding inhibiting immunoglobulins (TBII) have been studied. Sera were obtained from 225 patients (age: 28-77,158 females and 67 males) with hyperthyroid Graves disease treated with I-131 1-13 years before. Patients were classified on the basis of times after I-131 treatment into 4 groups, 1 year (Group1: 27 patients), 2-5 years (Group 2 : 42 patients), 6-9years (Group 3 : 79 patients), and 10-13 years (Group 4 : 77 patients).The TBII index was measured by a radioreceptor assay kit based on a preparation of porcine thyroid membranes and I-125 labeled TSH. Prior to I-131 therapy , TBII was detected in 78% of pa--tients. In 12 patients whose blood samples were obtained at yearly intervals for 4 years, TBII index decreased gradually.The mean pre-treatment value was 42%, and the values at the end of first , second, third, and fourth years were 50%,19%,10% and 8% , respectively.The frequency of positive TBII was 85% in Group 1 and this figure decreased to 40, 19 and 17% in Group 2, 3 and 4, respec--tively. The incidences of hyperthyroidism and hypothyroidism after I-131 treatment were 37% and 15% in Group 1, 21% and 26% in Group 2, 19% and 23% in Group 3, and 9% and 42% in Group 4, respectively.The incidence of positive TBII correlated significantly with the incidence of hyper--thyroidism. Pre-treatment TBII value was significantly higher in those patients who had persistent hyperthyroidism (45.9%) compared to those who became eu- or hypo-thyroidism (34.2%) after I-131 treatment.The incidence of hyperthyroidism after I-131 treatment in patients with negative TBII before treatment (7%) was significantly lower than that (29%) in patients with positive TBII. On the other hand, the antithyroglobulin and antimicrosomal antibodies results before treatment were not predictive of the long-term outcome of I-131 treatment.These results indicate that 1) the TBII index is one of the important factors which influence the outcome of I-131 therapy for Graves disease, and 2) the high incidence of later-onset of hypothyroidism in I-131 treated patients might be due not only to destruction of thyroid follicular cells by I-131, but also to a decrease in TBII index.

71. PREGNANCY AND SERUM NON-PROTEIN BOUND IODINE. C. Liberman, S.C. Pino, and C.H. Emerson, University of Chile, Santiago, Chile and University of Massachusetts School of Medicine, Worcester, Massachusetts.

Pregnancy is characterized by thyroid enlargement (TE), even in regions of iodide (I^-) sufficiency. It is widely held that a decline in serum inorganic I^- (SII) is important in the genesis of TE. There are remarkably few measurements of SII in pregnant women, however. The most frequently cited study (Aboul-Khair et al, 1964) employed an indirect technique (plasma RAI/urine RAI times urine I^-) to estimate the SII. We measured the serum non-protein bound iodine (N-PBI) during gestation and after delivery. N-PBI (Total serum iodine minus PBI) is a standard method for estimating the SII, comparing favorably with other methods. Data (Mean ± SEM) are presented from 15 residents of the Santiago region of Chile who made donations, at all periods shown, of 24 hr urine and fasting blood.

	N-PBI µg/dl	Urine I^-, µg/g Cr	T_4, µg/dl	FTI	TSH U/l
Trimester 1	1.7 ± 0.2	756 ± 137	10.2 ± 0.5	8.5 ± 0.3*	1.97 ± 0.36†
Trimester 2	1.8 ± 0.2	473 ± 70	11.5 ± 0.4	7.2 ± 0.2	2.40 ± 0.39
Trimester 3	1.9 ± 0.1	724 ± 143	10.5 ± 0.5	6.6 ± 0.3	2.44 ± 0.38
Post Partum	1.4 ± 0.2	529 ± 53	7.2 ± 0.2*	6.8 ± 0.2	3.30 ± 0.80

*$p < 0.001$ vs 2nd and 3rd trimesters; † $p < 0.05$ vs 3rd trimester for log of data

The FTI was increased and the TSH decreased in the 1st compared to the 3rd trimesters, consistent with thyroid stimulation in the first trimester. Mean SII (N-PBI) was actually slightly lower, but not significantly so, after delivery than during pregnancy. There was wide and apparently random intra-subject variation in urine I^- excretion and SII (mean cv = 62 and 41 % respectively). For the group as a whole, taking the mean of all data for each subject, there was less variability (cv = 30 and 25 % respectively). These studies fail to document a decline in SII during pregnancy and raise the question of whether new approaches to the measurement of SII should be considered. Even if more accurate methods for measuring SII are developed it is likely that, in iodine sufficient regions, the large variation in iodine intake is of greater consequences for thyroid economy than are pregnancy-induced changes in iodide kinetics.

80. CLINICAL UTILITY OF ULTRASOUND GUIDED FINE NEEDLE ASPIRATION BIOPSIES (FNAB) OF THYROID NODULES. P.A.Burford, A.J.Van Herle, E.Kingston, Department of Medicine, Division of Endocrinology, University of California, LA 90024

During a two year period (1991-1993) 79 patients were referred for the evaluation of thyroid nodules, which had either an inadequate FNAB (n=25) or had a nodule which was not palpable but was previously detected by ultrasound (n=54). 36 of the patients had more than one nodule. Nodules greater than 2mm in diameter underwent FNAB. A total of 120 nodules were biopsied. The FNAB was performed using local anesthesia and aspiration was performed with a 20cc syringe in a CAMECO syringe holder.18-27 gauge needles were used and the optimal biopsy site was determined by an ultrasonographer with a 10 Hz transducer.3-4 aspirations were obtained from each nodule. The majority of the biopsied nodules were not palpable (68%,n=82).In the nonpalpable nodules 56%(n=46) were $\leq$1 cm2 in area. Area is defined as the multiplication of both perpendicular diameters. Inadequate biopsies were observed in 20/46 of these lesions and 2/46 were carcinomas. In the nonpalpable group larger than 1 cm2, 7/36 biopsies were inadequate and 29/36 delivered a definite cytological diagnosis. 31 of the patients had palpable nodules, 42% were $\leq$1 cm2 in area, 58% were > than 1 cm2. 10.5% of the palpable lesions $\leq$1 cm2 yielded samples which were inadequate for diagnosis, 15.7% had a palpable lesion > 1 cm2 which also yielded samples which were inadequate for diagnosis. In summary;(1) A total of 3 cancers were found by FNAB, an additional 4 were diagnosed with atypical cells and proceeded to surgery, 2 were confirmed to be carcinomas. A total of 5 cancers were found in 120 nodules.(2) Patients with palpable nodules with one inadequate FNAB have a 52% chance of a correct cytological diagnosis if the FNAB is done under US guidance, decreasing the # of patients requiring surgery for diagnosis;(3) Nodules > than 1 cm2 which are not palpable can be diagnosed cytologically using US guided biopsy.(4)In the nonpalpable lesions, a successful biopsy is more likely to occur in subjects who have nodules > 1 cm2 (29/36) than in those $\leq$1 cm2(20/46). However an attempt should be made to biopsy those ultrasound lesions $\leq$1 cm2 since 1 cancer was found in a nonpalpable nodule $\leq$1 cm2.(5)The study indicated that multinodular goiters should not be considered an entirely benign entity since 1 subject had thyroid cancer on FNAB, and 2 had atypical cells which were confirmed carcinomas on surgical pathology.

82. **UTILITY OF NEAR TOTAL THYROIDECTOMY IN REDUCING POTENTIAL RECURRENCES OF THYROID CANCER.** J. Kolenda, I.B. Rosen, P.G. Walfish. Departments of Surgery and Medicine, Mount Sinai Hospital & University of Toronto Medical School, Toronto, Ontario, Canada M5G 1X5.

To determine the efficacy of near total thyroidectomy compared to lobectomy in reducing the potential recurrence of unsuspected residual well-differentiated (papillary and follicular) thyroid cancer (WDTC), available clinical and pathology records of 95 patients who had undergone bilateral thyroidectomy during (1988-1993) were reviewed and subdivided as follows: Group 1 (n=64) had near total thyroidectomy for an apparent solitary nodule; Group 2 (n=17) had completion thyroidectomy following a previous lobectomy for detected WDTC; and Group 3 (n=14) had near total thyroidectomy for palpable bilateral disease. Also, a retrospective analysis was performed in the pre-operative localization of unsuspected lesions by palpation, neck ultrasound and a thyroid radioisotope scan.

In Group 1 patients, 47 of 64 (73.5%) had WDTC confined to the index lobe only, while 12 of 64 (18.8%) had WDTC in both the index and contralateral lobes, and only 5 of 64 (7.8%) had benign disease in the index lobe with unsuspected WDTC in the contralateral lobe. Among the Group 1 patients with clinically unsuspected contralateral lobe disease, 11 were papillary and 1 papillary-follicular WDTC of which $\approx$ 50% were between 0.5 and 1.5cm in size and the remainder at $<$0.5cm, whereas 4 of 12 (33%) were multicentric. Most contralateral lobe pathology was not detected by clinical palpation or radioisotope scanning, and when performed, ultrasound had a detection rate only 3 of 11 (27%). In Group 2 patients, (completion thyroidectomy following a previous partial thyroidectomy for known WDTC), residual cancer was detected in 5 of 17 (29%) of which $\approx$ 60% were both $<$1.5cm in size and multicentric. In Group 3 patients (near total thyroidectomy for palpable bilateral disease), WDTC was documented to be bilateral in 8 of 14 (57%) of which $>$90% had co-existing multicentric papillary carcinoma.

From these observations it is concluded that: **1)** Since WDTC had a significant prevalence of bilateral disease, near total thyroidectomy can be effective in preventing recurrences. **2)** Although the unsuspected lesions were most often of small size and could not be easily detected by palpation, radioisotope scanning or neck ultrasound, such lesions could nevertheless be a potential source of recurrent WDTC. **3)** Near total thyroidectomy ensures a more complete eradication of residual WDTC and enhances the effectiveness of radioiodine ablation therapy as well as the subsequent utility of serum thyroglobulin as a reliable marker of recurrent WDTC.

90. THYROTROPIN (TSH)-MEASUREMENT BY CHEMILUMINESCENCE: THE NEW ORDER IN THYROID FUNCTION TESTING. L.Duntas[1], B.M.Grab[2], T.P.Kemmer[1], D.K.Nelson[1,3], [1]Dept.Internal Medicine I, [2]Dept.Nuclear Medicine, University of Ulm, Ulm, Germany and [3]Mayo Clinic, Rochester, Minnesota, USA

The refinement of immunoassay technology has led to the use of TSH measurement in front-line testing for thyroid disorders. However, problems like heterophilic antibodies, the specificity of current TSH assays and other ill- defined technical problems may still be a concern. **AIM:** To evaluate the ability of 3rd generation immunochemiluminometric (ICMA) TSH assay to differentiate clinical from subclinical hyper/hypothyroidism and to evaluate automated TSH testing at levels below 0.1 μU/ml. **METHODS:** 205 euthyroid subjects (ES; 18-68 yrs.), 51 pts. with various types of overt hyperthyroidism (HYPER; 29-58 yrs.), 34 pts. suspected of subclinical hyperthyroidism (S-HYPER; 32-71 yrs.), 48 pts. with overt hypothyroidism (HYPO; 26-74 yrs.) and 26 pts. suspected of subclinical hypothyroidism (S-HYPO; 30-52 yrs.) were studied. All subjects and pts. were reevaluated after a standard TRH test (200 μg iv) was performed. Serum TSH levels were measured by a 3rd generation ICMA assay (LUMItest-TSH, Henning-Berlin GmbH, Berlin, Germany) with a functional sensitivity of 0.037 μU/ml and analytical sensitivity of 0.03 μU/ml. All samples with TSH levels below 0.1 μU/ml as well as 43 ES samples and all HYPO samples were measured manually and by automation (Lumat LB 9501 and Autolumat LB 953, Fa.Berthold, Wildbad, Germany). **RESULTS:** In ES the TSH value was 1.4 $\pm$ 0.4 μU/ml mean $\pm$ SEM; range 0.23-3.5. All ES subjects presented a normal δ-TSH ($>$ 2 μU/ml). HYPER pts. had a TSH value of 0.06 $\pm$ 0.02 μU/ml and a δ-TSH of 0.08 $\pm$ 0.02 μU/ml. One of these pts. had normal δ-TSH (7.4μU/ml); no correlation to the clinical findings was found and the relative light units (LU) were very low compared to the standard zero of the assay, suggesting interferring substances in the serum (heterophilic antibodies?). The S-HYPER pts. had a TSH of 0.18 $\pm$ 0.07 μU/ml and a δ-TSH of 1.38 $\pm$ 0.3 μU/ml but 3 (10.7%) of these pts. showed a normal response to TRH and no thyroid disease in the following examination. The HYPO pts. had a TSH of 9.6 $\pm$ 1 μU/ml and a δ-TSH of 24.3 $\pm$ 3.6 μU/ml. Finally, the S-HYPO had a TSH of 3.9 $\pm$ 1.1 and a δ-TSH of 15.4 $\pm$ 2.7 μU/ml. None of the pts. with a TSH above 3.5 μU/ml had a normal response to TRH. In the comparison of manual and automated measurement a good correlation ($r = 0.9042$; $p < 0.001$) was found by linear regression analysis within ES and HYPO groups but not for the HYPER values. **CONCLUSIONS:** These results support a lower normal range than that established previously. In clinical practice, detection of hyperthyroidism remains unreliable on the basis of TSH values falling in the "grey zone" (0.1-0.2 μU/ml); clinical examinations including TRH-testing and thyroid hormone concentrations are still required. Heterophilic antibodies in the current ICMA do not present a serious problem. Automated testing provides a reliable paradigm for practical application of 3rd generation ICMA in the clinical setting.

94. THE STUDY OF THE TRANSFORMING MECHANISM OF PTC-1 ONCOGENE. Q. Tong, Y.-S. Li, E.L. Mazzaferri and S. M. Jhiang, The Ohio State University, Columbus, Ohio.

Three forms of PTC oncogene, resulting from the rearrangement of the *ret* proto-oncogene with different genes, have been detected in a minority of human papillary thyroid carcinomas (PC). PTC-1, the major form of PTC oncogene in human PC, encodes a fusion protein containing the N-terminus of H4 fused with the *ret* tyrosine kinase domain. Therefore, the PTC-1 expression is driven by the H4 gene promoter. The expression of proto-*ret* is reported to be restricted to embryonic neural crest cells and tumors of neural crest origin. However, our study showed that H4 is expressed in various human tissues investigated by RT-PCR. Furthermore, we have localized the transcription start site of H4 to a region 110 to 120 bp upstream of the translation initiation site (ATG) by primer extension assay and RT-PCR. We have also localized the H4 promoter region within 258 bp upstream of the ATG by luciferase assay. Interestingly, protein sequence analysis indicated a potential coiled-coil motif in the N-terminal region of H4. Indeed, oligomerization was demonstrated by an *in vitro* assay with recombinant protein containing this region. As dimerization is considered to be an important step for receptor tyrosine kinase activation, we hypothesize that both unscheduled expression of truncated *ret* and oligomerization of PTC-1 products are responsible for PTC-1 transforming activity in thyroid.

124. **CALCITONIN GENE-RELATED PEPTIDE RESPONSE TO PENTAGASTRIN STIMULATION IN NORMAL SUBJECTS AND IN PATIENTS WITH MEDULLARY THYROID CARCINOMA.** H.M. Heshmati[1], R. Cohen[2], J. Taboulet[2], N. Bouyge[1], A. Jullienne[2], C. Calmettes[1] and E. Modigliani[1], Groupe d'Etude des Tumeurs à Calcitonine (GETC), Avicenne Hospital[1], University of Paris XIII, Bobigny, and INSERM U 349, Lariboisière Hospital[2], Paris, France.

Calcitonin gene-related peptide (CGRP) is a peptide encoded by the calcitonin (CT) gene in the thyroid and in the nervous system. Data on the simultaneous evolution of CGRP and CT after C cell stimulation are scarce and controversial. The aim of this study was to evaluate the response of CGRP to pentagastrin stimulation in normal subjects and in patients with medullary thyroid carcinoma (MTC). Thirteen patients (7 males, 6 females) with MTC were studied. The diagnosis was confirmed by histological examination. Sixteen normal subjects (6 males, 10 females) were used as controls. Plasma was collected before and 3 minutes after a slow intravenous injection of pentagastrin (0.5 μg/kg in 3 minutes) to measure CGRP (RIA, synthetic $hCGRP^{1-37}$, sheep polyclonal antibodies, ^{125}I [tyr^0]-hCGRP, Amersham - normal value < 100 ng/L) and CT (IRMA Bio Cis - normal value < 10 ng/L). Statistical analysis was performed with the nonparametric Wilcoxon test. Results were expressed as the mean ± SD. Mean basal plasma CT level was 2 ± 3 ng/L (range, undetectable - 8 ng/L) in control subjects, and 1404 ± 2980 ng/L (range, 7 - 10856 ng/L) in MTC patients. Mean stimulated plasma CT level was 8 ± 11 ng/L (range, undetectable - 41 ng/L) in control subjects ($P < 0.01$), and 14951 ± 38525 ng/L (range, 53 - 141825 ng/L) in MTC patients ($P < 0.002$). In MTC patients, the mean increment was 1631 % over basal values. Mean basal plasma CGRP level was 45 ± 30 ng/L (range, undetectable - 136 ng/L) in control subjects, and 2427 ± 6815 ng/L (range, undetectable - 24675 ng/L) in MTC patients. Mean stimulated plasma CGRP level was 92 ± 41 ng/L (range, undetectable - 178 ng/L) in control subjects ($P < 0.003$), and 3541 ± 8075 ng/L (range, undetectable - 23660 ng/L) in MTC patients (*NS*). Only 3 MTC patients had an increment higher than 100 % over basal values (+ 196 %, + 235 %, + 297 %). In conclusion, unlike the usual and dramatic plasma CT increase after pentagastrin stimulation in MTC, the CGRP increase is less common and of lower amplitude. This favors a dissociation between CGRP and CT secretion in most patients with MTC. (Supported by INSERM/APHP)

137. TSH AND EGF STIMULATE THYROGLOBULIN SECRETION AND INVASION OF A METASTATIC HURTHLE CELL CARCINOMA CELL LINE. A. Zielke, S. Tezelman, G. Jossart, A. J. Van Herle, A. E. Siperstein, O. H. Clark, Q. Y. Duh, VAMC and Mt Zion MC, UC San Francisco, and UC Los Angeles

Thyroid stimulating hormone(TSH) causes differentiation and epidermal growth factor (EGF) causes dedifferentiation of the thyroid cells. We used a continuous cell line (XTC-UC1) derived from a breast metastasis of a patient with a Hürthle cell carcinoma, to test the hypotheses that 1) TSH would stimulate the thyroglobulin secretion (a differentiated function) more than EGF and 2) EGF would stimulate in vitro invasion (a dedifferentiated state) more than TSH. Invasion was measured by the MTT assay, and defined as the % of cells penetrating the 8 μm pore Matrigel coated polycarbonate membrane. All experiments were performed in triplicates.

XTC-UC1	Basal	TSH (mU/ml)				EGF (ng/ml)		
24 hour incubation		0.1	1	10	100	1	10	100
Thyroglobulin (ng/ml)	90	108	149	173	165	148	167	145
Invasion (%)	1.1	1.3	3.4	8.8	5.0	3.0	3.4	2.9

TSH induced differentiated morphologic changes in XTC cells, whereas EGF did not. Protease activity as measured by zymography showed bands at 57, 72 and 92 kDa, but the protease activity was not altered by TSH or EGF.

Conclusions: 1) XTC-UC1 is the first Hürthle cell thyroid cancer line that secretes thyroglobulin. 2) Both TSH and EGF stimulated thyroglobulin secretion and invasion, although only TSH stimulated differentiated morphology. 3) These data suggest that induction of a differentiated morphology and differentiated functions by a growth factor does not always correlate with attenuation of the malignant phenotype.

153. ^{18}F-FDG-PET SCANNING - A DIAGNOSTIC TOOL FOR DETECTION OF RECURRENT AND METASTATIC DIFFERENTIATED THYROID CANCERS. F.H. Baqai, P.S. Conti, P.A. Singer, C.A. Spencer, C.C. Wang, J.T. Nicoloff, USC School of Medicine, Los Angeles, CA

Morphologically based modalities (CT and MRI), imaging studies employing radioiodine (^{131}I) and 201-thallium (201TL), together with serial serum thyroglobulin (Tg) measurements, serve as major testing parameters in monitoring differentiated thyroid cancer (DTC). However, discordance between these parameters, especially negative ^{131}I diagnostic scans with persistently detectable Tg, often present a management problem. Since ^{131}I/serum Tg discordance potentially reflects DTC dedifferentiation associated with the loss of ^{131}I trapping, we explored the possibility that PET imaging employing ^{18}F-FDG, a substrate preferentially utilized by more primitive and less differentiated tumors, might be useful in such cases. Study: Eleven patients with DTC (M/F=5/6; age 48±11(+SD) yrs), who had negative 2-5 mCi ^{131}I diagnostic scans and persistently detectable Tg (>4.0 ng/ml) while on T_4 suppression (TSH < 0.1mU/L) following initial therapy (thyroidectomy + ^{131}I ablation) were imaged using F^{18}-FDG employing a Siemens 953 Whole Body PET scanner. Result: All patients showed abnormal ^{18}F-FDG uptake in one or more sites. 4/10, who had 201-TL uptake in the neck area, displayed extensive ^{18}F-FDG-PET uptake in other sites. In 4 with positive 30-100mCi ^{131}I post-treatment scans, ^{18}F-FDG uptake was also noted in sites not revealed by ^{131}I. In 2 patients with suspected residual tumor, both of whom had negative ^{131}I scans and low serum Tg's (<4ng/ml) ^{18}F-FDG uptake in neck and chest as were noted. Conclusions: Results suggest that ^{18}F-FDG-PET scanning provides a sensitive tool for diagnosing persistent or recurrent thyroid cancers in patients with detectable serum Tg and negative diagnostic ^{131}I scans or in cases where residual tumor is suspected on other grounds. The technique avoids the need for withdrawal from T_4 suppression therapy and provides an excellent scan images. However, as it is very expensive, it should be employed in those instances where tumor staging and localization is critical for clinical management.

159. ANALYSIS OF A FEMALE PHENOTYPED COMPLETE THYROXINE-BINDING GLOBULIN DEFICIENCY (TBG-CD): UNBALANCED X CHROMOSOME INACTIVATION AS A MECHANISM. H. Okamoto , Y. Mori , Y. Miura, Y. Tani, Y. Oiso , T. Sano[1] , K, Oyama[1], First Dept. of Internal Medicine, Nagoya University, School of Medicine, Nagoya, [1] Dept.of Pediatrics, Yamanashi Medical University, Yamanashi, Japan.

We have reported a nucleotide deletion at codon 352 of the TBG gene (CDJ) was a common cause in Japanese CD (J Clin Endocrinol Metab 73:262,1991). Heterozygous females are usually phenotyped as partial deficiency (PD), as the TBG gene locates on X chromosome (q21-22).

In this study, unbalanced X chromosome inactivation was shown to be a cause of CD in one female carrying normal and CDJ alleles. Confirmation of a normal allele was performed by sequencing the coding regions and measuring the promoter activity. For the analysis of X chromosome inactivation, the phosphoglycerate kinase (PGK) gene was used, which locates on Xq13 and had a polymorphic *Bst*X I site and methylation sensitive sites, *Hpa* II, in its intron 1. Two heterozygous females of CDJ in this family were also heterozygotes as to the *Bst*X I site which segregated with the CDJ allele. Genomic DNA was digested with *Hpa* II, then applied to PCR in which 530 bp region of the PGK gene containing *Hpa* II and *Bst*X I sites and finally digested with *Bst*X I. The PCR product from the phenotypical CD female was resistant to *Bst*X I digestion, indicating selective inactivation of the normal TBG allele. On the other hand, the product from the PD female was partially digested, indicating inactivation of both alleles.

In addition, heterozygous females belonging to 5 CDJ and a PDJ families, all heterozygotes as to the *Bst*X I site, were analyzed. PDJ is a mutant discovered in Japanese PD families (Endocr J 40:127,1993). Only in the PD family, unbalanced X-chromosome inactivation was recognized. This female heterozygous of PDJ was phenotypically hemizygous as a result of selective inactivation of the normal TBG allele.

160. GENE ANALYSIS OF THYROXINE-BINDING GLOBULIN (TBG) DEFICIENCIES IN JAPANESE: ONLY TWO MUTATIONS ACCOUNT FOR TBG DEFICIENCIES IN JAPANESE. Y. Miura, H. Okamoto, Y. Tani, A. Inagaki and Y. Oiso, First Dept of Internal Medicine, Nagoya University, School of Medicine, Nagoya 466 JAPAN.

Thyroxine-binding globulin (TBG) is the major transport protein of thyroid hormones in human serum. The TBG gene is located on the long arm of the X-chromosome, and most of the TBG abnormalities have been shown to inherited as X-linked traits. Complete TBG deficiency (TBG-CD) is defined as undetectable TBG in serum with a sensitive assay, while partial TBG deficiency (TBG-PD) is characterized as a low TBG level. We have reported the sequences of TBG gene occurring in Japanese families with TBG-CD (TBG-CDJ) and TBG-PD (TBG-PDJ). TBG-CDJ consists of a nucleotide deletion at codon 352 of the TBG gene resulting in C-terminal truncation due to a frameshift and premature termination. TBG-PDJ consists of a nucleotide substitution at codon 363 of the TBG gene resulting in substitution of normal amino acid leucine(CTT) by proline(CCT). We have screened 42 affected subjects, 28 male and 14 female, from 42 unrelated Japanese families manifesting TBG-CD or TBG-PD from various areas of Japan regarding with these known mutations of the TBG gene. Genomic DNAs were extracted from peripheral white blood cells and specific mutatios at codons 352 and 363 were identified by the polymerase chain reaction with a pair of allele specific oligonucleotides primers toward to TBG-CDJ and TBG-PDJ. Twenty four male subjects manifesting TBG-CD were shown to have the mutation at codon 352 as a hemizygote of TBG-CDJ. Twelve female subjects with reduced TBG concentration in serum were shown to have the mutation at codon 352 as a heterozygote of TBG-CDJ. Four male subjects manifesting TBG-PD were shown to have the mutation at codon 363 as hemizygote of TBG-PDJ. No other mutations were found in these subjects. Thus, only two mutations account for all TBG deficiency in Japanese. In conclusion: In Japanese, TBG-CDJ and TBG-PDJ may be a common cause of TBG-CD and TBG-PD, respectively. And these have originated from common ancestors of the Japanese race after the divergence of human races.

167. ALTERED CROSSTALK BETWEEN TSH RECEPTOR AND TYROSINE KINASE RECEPTORS DEPENDENT PATHWAYS IN THYROID CARCINOMA CELLS: THE ROLE OF PROTEIN-KINASE C. M. Bröcker, G. Mayr* and M. Derwahl, Medizinische Universitätklinik Bergmannsheil, Bochum and *Institut für Physiologische Chemie, Universität Hamburg, Germany.

We have recently established the human HTC-TSHr thyroid carcinoma cell line that lacks an endogenous, but expresses the recombinant human TSH receptor. In contrast to normal thyroid cells, TSH activates the adenylate cyclase but not the phospholipase C (PLC) cascade in these cells. In addition, these cells express the platelet-derived growth factor (PDGF) α and ß receptors, whose expression is physiologically restricted to mesenchymal tissue, and the epidermal growth factor receptor. The former are activated by PDGF A and B, the latter by transforming growth factor α (TGFα). All factors are synthesized in these cells and stimulate cells by autocrine mechanisms. Since these tyrosine kinase receptors activate proteinkinase C (PKC) and PKC isoenzymes are able to inhibit PLC-Gq protein coupling, we investigated the effect of staurosporine and calphostin C, potent inhibitors of PKC, on the TSH-dependent generation of inositol phosphates. For measurement of inositol phosphates (InsP) cells were incubated with myo-[2-^{3}H]-inositol for 48 h, preincubated with staurosporine or calphostin C, and then stimulated with 10-300 mU/ml bTSH for 1, 3, and 5 min. After extractions InsP1, InsP2, InsP3 and InsP4 were separated by HPLC and labelled fractions measured in a ß-counter. Inhibition of PKC by both staurosporine and calphostin C led to a significant increase of InsP1 (130%), InsP2 (145%), InsP3 (240%) and InsP4 (265%) in response to TSH stimulation. These data demonstrate that in HTC-TSHr thyroid carcinoma cells the TSH receptor is coupled to the PLC pathway although the sensitivity of activation is diminished. We conclude that coupling of the TSH receptor to Gq-PLC cascade is dependent on the activity of PKC that in turn is regulated, at least in part, by autocrine activation of tyrosine kinase receptors in HTC-TSHr cells.

169. SULFATED THYROID HORMONE METABOLITES AND HEPATIC DEIODINASE EXPRESSION IN FETAL SHEEP: EFFECT OF EXOGENOUS DEXAMETHASONE. D.H. Polk, S.Y. Wu, A. Reviczky, M. Berry, D.A. Fisher. Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, California, Department of Nuclear Medicine, VA Medical Center, Long Beach, California, and Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts.

We have previously reported that sulfated thyroid hormone derivatives represent a significant fraction of circulating thyroid hormone levels in developing sheep (Wu, et.al., Am. J. Physiol. 265, 1993). Using steady-state infusion techniques and sensitive, specific radioimmunoassays, we have demonstrated that high fetal thyrosulfoconjugate levels are due to both increased production and decreased clearance of these metabolites in comparison to rates observed after birth. We have hypothesized that the alterations in clearance observed after birth are due to changes in hepatic thyroid hormone monodeiodinase activities. Previous studies have suggested that fetal exposure to exogenous glucocorticoids may augment expression of hepatic type I monodeiodinase. In the present studies, fetal sheep (126 d GA) were treated with either betamethasone (0.5 mg/kg fetal weight) or saline followed in 48 hrs by sacrifice. Blood samples were obtained and processed for thyroid hormone assays; hepatic tissue was processed for RNA which was subsequently analyzed using Northern blot techniques for specific expression of type I monodeiodinase. Values reported are mean ± SEM.

	(n)	T_3(ng/dl)	T_4(μg/dl)	T_4S (ng/dl)
Control	8	12±1	13.6±1.7	251±42
Betamethasone	12	36±3*	12.5±1.1	112±11*

*$p<0.01$ vs control

Densitometric analyses of Northern blots hybridized to a riboprobe specific for the type I monodeiodinase revealed a nearly 3 fold increase in expression in fetuses treated with dexamethasone. In a separate set of experiments, induction of type I monodeiodinase activity was found to be dose dependent between 0.1 μg and 2 μg/kg of betamethasone.

Conclusions 1) Betamethasone therapy of fetal lambs is associated with augmented circulating T_3 levels and decreased circulating levels of T_4-S. 2) This effect is due in part to increased type I monodeiodinase mRNA expression.

172. EXPRESSION OF TRANSFORMING GROWTH FACTOR ß (TGFß) 1 AND 3 IN BENIGN AND MALIGNANT HUMAN THYROID GLANDS. E.T. Kimura[1], J. Zbaeren and H. Studer, Experimental Laboratory of Endocrinology. Inselspital, Bern, Switzerland, [1]Department of Histology and Embriology, Institute of Biomedical Sciences, University of São Paulo, Brazil.

The TGFß family is composed of 25 kDa homodimeric peptides that are multifunctional growth factors with proliferative and anti-proliferative effects in a wide variety of cell types. Although the presence of TGFß1 has been described in thyroid carcinoma tissue, the TGFß-family expression in human thyroid tissue is yet to be fully characterized; its assumed that it plays a predominat inhibitory effect. In the present investigation, benign (n=10) and malignant (n=10) human thyroid glands were studied by immunohistochemistry using polyclonal TGFβ1 (British Bio-technology Ltd.) and monoclonal TGFß3 (Oncogene Science) antibodies. Staining was performed by the indirect 3-stage immunoenzymatic procedure with streptoavidin-biotin/alkaline phosphatase. Normal pig thyroid glands were used as controls and revealed a faint positivity for both TGFßs, restricted to the interfollicular tissue. In the human glands, light microscopy indicated that both benign and malignant tumor cells were immunoreactive for TGFß1 and 3, in a pattern similar to the p21*ras* protein (JCEM 75:1151,1992). In the malignant tissue, staining was homogeneously distributed throughout the tissue for both TGFßs. On the other hand, benign tumor tissue showed a wide variation of staining patterns: it was heavily immunostained in morphological proliferative areas, e.g. focal and solid buds, micronodular areas, papilliferous structure, high ephitelium, while surrounding normal follicles were almost always negative. **In conclusion**, TGFß1 and 3 are widely expressed in human thyroid tumor cells and may contribute, along with other growth factors, in the modulation of abnormal growth of neoplastic thyroid cells (supported by Swiss National Foundation and CAPES).

176. TETRACYCLINE VERSUS SALINE IN TREATMENT OF THYROID CYSTS: COMPARISON WITH ETHANOL. A. Antonelli, *A. Campatelli, **M. Ferdeghini, ***F. Bianchi, B. Alberti, C. Gambuzza, C. Francese, L. Baschieri. Institutes of Clinica Medicine II, *General and Experimental Surgery and **Nuclear Medicine, University of Pisa; ***Institute of Clinical Physiology, CNR; Pisa, Italy

Bachground. The medical treatment of thyroid cysts with tetracycline hydrochloride is up to now debated. Recently ethanol sclerotherapy has been studied in treatment of recurrent thyroid cysts. *Methods;* In a prospective study, 75 consecutive patients were randomized to ultrasonically guided cyst aspiration and subsequent injection with isotonic saline (n=38) or tetracycline hydrochloride (n=37). Subsequently 20 consecutive patients were treated with cyst aspiration followed by ethanol sclerotherapy. The patients were followed up clinically and ultrasonically until 12 months after treatment. Cure was defined as the absence of any residual cystic lesion and an ultrasonic cyst volume less than 50% with respect to basal after 12 months from the start of treatment. Cytologic study showed all of the lesions to be benign. *Results.* 14/38 (36.8%) patients in the saline group and 24/37 patients (64.8%) in the tetracycline group were cured (Chi-square: $p<0.05$). Among patients treated with ethanol 17/20 (85%) were cured (Chi-square with respect to saline: $p<0.05$). Both tetracycline and ethanol resulted more effective in patients with haemorrhagic fluid, with multinodular goiter and from iodine deficient areas. Slight neck pain was observed in 2 patients treated with saline (5%), in 7 with tetracycline (19%) and 5 (25%) with ethanol; 3 patients treated with ethanol presented severe neck pain. *Conclusion.* We conclude that tetracycline hydrochloride seems effective and safe in treatment of thyroid cysts especially in multinodular endemic goiter. Ethanol seems more effective than tetracycline and may be used in larger or relapsed cysts.

181. COLOR-FLOW DOPPLER SONOGRAPHY IN THYROID AUTOIMMUNE DISEASE

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Thyroid ultrasound was already shown to be an useful adjunct for the diagnosis of Graves' disease (GD) and goitrous (HT) Hashimoto's thyroiditis. Both GD and HT are characterized by thyroid enlargement with a diffusely hypoechogenic pattern that in most cases is indistinguishible in these two diseases.

Aim of the present study was to evaluate the usefulness of color-flow doppler sonography (CFDS) in differentiating the thyroid hypoechogenicity at ultrasound due to GD or HT. 37 patients with GD (36/37) with hypoecogenic pattern at ultrasound) and 55 (53/55 hypoecogenic pattern at ultrasound) with HT were submitted to CFDS using 7,5 MHz probe. Results were expressed as thyroid parenchimal blood flow speed (cm/sec) and referred to as normal, reduced or increased with respect to the blood flow observed in 14 normal subjects. Blood flow was cleary increased 21/23 (91,3%) patients with untreated GD, sligthly increased in 11/14 (78,5%) euthyroid under methimazole treatment. In HT patients blood flow was cleary reduced in 21/23 (91,3%) untreated euthyroid patients, in 25/29 (86,2%) patients under L-T4 therapy and in 2/3 (66,6%) patients with untreated hypothyroidism. When the whole groups of patients were compared irrespective of treatment 31/37 (83,7%) patients with GD (and none of those with HT) had the combination hypoechogenicity/increased blood flow, while 47/55 (85,4%) patients with HT (and none of those with GD) had the combination hypoechogenicity/decreased blood flow. The patient with GD and normal thyroid echogenic pattern had an increased blood flow at CDFS, while both HT patients with normal echogenic pattern had a decreased blood flow at CFDS.

In conclusion, the color flow doppler sonography in addition to the thyroid echo pattern, gives also informations on the thyroid blood flow leading to a clear distinction between GD and HT.

183. A FOLLOW UP STUDY OF UNTREATED GRAVES' PATIENTS WITH UNDETECTABLE THYROTROPIN RECEPTOR ANTIBODIES AND THE EFFECTS OF ANTITHYROID DRUGS.

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We studied the clinically meaningful differences between undetectable and detectable TRAb hyperthyroid Graves' disease in the relationship to the effect of antithyroid drug medication. We identified 94 TRAb negative patients. Eighty-three TRAb positive patients were randomly selected as a comparison group. The trial was conducted as a retrospective study with a maximum treatment period of 36 months and a follow up period of another 12 months. 56 patients in the TRAb negative group were treated with methimazole only (Group A: 13 men and 43 women, age$\pm$SD 35.6$\pm$14.7 years). Fifty-two patients of TRAb positive group were treated with only methimazole and served as a control group(Group B: 8 men and 44 women, age$\pm$SD 38.1$\pm$14.1 years). Furthermore, we classified group A into two subgroups according to the clinical course taken. One was the maintained TRAb negative group during methimazole treatment(Group A-1: 43 patients). The other group experienced a change to TRAb positive (Group A-2: 13 patients). Serum FT4 and FT3 levels in Group A, A-1 and A-2, before treatment, were significantly lower than those of in Group B ($P<0.001$). The thyroid volumes in Group A, A-1 and A-2 were significantly smaller than that of Group B($P<0.01$). The values of TRAb in Group A, A-1 and A-2 were significantly lower than that of Group B(8.4$\pm$4.5, 8.3$\pm$4.7 and 8.8$\pm$4.1 vs 57.0$\pm$19.9%, respectively, $P<0.001$). The values of TRAb in Group A, A-1 and A-2 were significantly lower than that of Group B (550$\pm$496,478$\pm$466 and 761$\pm$505 vs 2143$\pm$280%, respectively, $P<0.01$), and there was a significant difference in TSAb activities between Group A-1 and A-2. The remission ratios in group A, A-1, A-2 and B were 66, 77.4, 34.5, and 36.5%, respectively. The remission ratios in Group A and A-1 were significantly higher than those in Group B and there were significant differences between Group A-1 and A-2. The maintained TRAb negative group has a good prognosis but the experienced a change to TRAb positive group has the same prognosis as a control group. The patients of untreated TRAb negative Graves' disease may consist of some subtypes.

184. POLYMERASE CHAIN REACTION ANALYSIS OF GROWTH HORMONE RECEPTOR EXPRESSION IN HUMAN NORMAL AND PATHOLOGICAL THYROID TISSUES.

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The factors that regulate thyroid cell proliferation have been extensively studied. TSH is the main regulator of thyrocyte function and growth; furthermore it has been demonstrated that growth factors such as IGFs, EGF and cytokines are involved in the mitogenic activity of the thyroid cell. Clinical evidence suggest that the thyroid gland may be a target for growth hormone (GH). Moreover, nontoxic goiter and thyroid nodules are commonly seen in patients with untreated acromegaly. GH may have a direct effect on thyroid cells or this effect may be directly mediated by IGF-I through local secretion (paracrine/autocrine) or indirectly by circulating IGFs. It has been demonstrated that in hypophysectomized rats, GH administration induces an immediate increase of c-myc expression, followed by a late increase of IGF-I expression in liver and kidney tissues. This suggests that GH is responsible for initiating the mitotic response and later, for the expression of other genes. The cloning of cDNA growth hormone receptor (GH-R) in human liver provided data about the structure of GH-R (Lung and cols., 1987), enabling the study of GH action in specific tissue through the analysis of receptor gene expression. PCR has been extensively used to amplify and detect gene transcripts . We have used PCR to evaluate GH-R gene expression in normal and pathological thyroid tissue. Total RNA was extracted according to Chomczynski and Sachi method (1987) from normal thyroid tissue, adenomatous goiter, Grave's disease, thyroid cancer and multinodular goiter. Five μg of total RNA were converted into cDNA by reverse transcription with Moloney murine leukemia virus reverse transcriptase primed with 5'TTCACCTCCTCTAAT3' [specific for an exon 9 region in the GH-R cDNA]. The PCR reaction resulted in amplified cDNA fragments of GH-R. We used forward primer that spanned exon 7 (nt 624-647) and reverse primer that spanned exon 9 (nt 931-954). After amplification 330 bp fragments were generated. PCR products were evaluated through the densitometric analysis of amplified fragments applied to agarose gels, previously stained with ethidium bromide or analysed by Southern blot. GH-R gene expression was present in all human thyroid tissues that were analysed. These data suggest that GH has a direct effect on thyrocyte modulation through the GH-R.

185. P53 Oncogene Mutations in Thyroid Tumors: Evaluation of the Ability of SSCP to Detect Abnormalities.

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Single stranded conformational polymorphism analysis (SSCP) theoretically is a screening technique which may be capable of detecting mutations in p53 oncogene. A large number of samples can be analyzed rapidly and those few that are found to contain an abnormality can then be chosen for more laborious and expensive direct genomic sequencing. The purpose of the present study was to identify p53 mutations in human thyroid tumor samples and to determine the optimal conditions under which to perform SSCP with these tissues. Rather than employing customary radiolabelled primer techniques, we adapted SSCP to the ABI 373-672 Gene Scan System (Foster City, CA) by using fluorescently labelled primers flanking exon 8. Forward and reverse primers were uniquely labelled with specific fluorescent labels and SSCP was performed in 6.5 % polyacrylamide, 10 % glycerol and were run at 500 volts for 16 hours. Initially, the gels were run without strict temperature control and the temperature varied between 30-35 C. In addition, gels were run with strict temperature control at 15 C. None of 40 human thyroid tissues (papillary, follicular, lymphoma) obtained at surgery were found to have a p53 oncogene mutation by SSCP or direct sequencing. 3 of 7 papillary cancer cell lines (codon 273), 1 of 1 follicular cell lines (codon 273), as well as control breast cancer (codon 282) and an epidermal cell line (codon 273) had p53 oncogene mutations detected by SSCP when performed at 15 C, and confirmed by specific sequencing. SSCP performed at 30-35 C detected all mutations, but the ability to clearly discern abnormalities was markedly increased at 15 C.

We conclude that: (1) differentiated thyroid cancers obtained from surgery rarely possess p53 oncogene mutations; (2) by devising and incorporating the use of specific fluorescent labels, we were able to perform SSCP rapidly and effectively to detect p53 genomic mutations, thus avoiding the inherent problems of sensitivity and exposure to radioactivity; and (3) adaption of the ABI 373-672 Gene Scan System to allow performance of these gels at 15 C rather than at higher temperatures enhanced the sensitivity of the procedure. Further studies are warranted to examine the further utility and practicality of this non-radioactive fluorescent detection SSCP system.

186. METHIMAZOLE, 3-METHYL-2-THIOHYDANTOIN AND IODINE IN THYROID TISSUE

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Methimazole (MMI) therapy has been associated clinically with interference with radioactive iodine (RAI) therapeutic success. Moreover, Tuttle et al. (ATA 1993, abstract no.69) have demonstrated that propylthiouracil (PTU) therapy prior to RAI treatment is characterized by a higher failure rate (34%), even when PTU was discontinued 4 days before RAI. The aim of the present study was to extend our previous pharmacokinetic investigation of MMI and its metabolite, 3-methyl-2-thiohydantoin (MTH) (Aktuna et al., ATA 1993, abstract no. 30) to explore whether residual MMI was present in thyroidal tissues after MMI withdrawal 5 days prior to surgical thyroidectomy.

Methods: 11 goitre patients were given 40 mg MMI iv daily until surgery (group 1). In another 11 goitre patients, MMI-treatment was interrupted 5 days before surgery (group 2). MMI and its metabolite MTH was measured in thyroid tissue using our HPLC method (Aktuna et al., ETA 1994, abstract no. 79) and iodine determinations were done by a colorimetric method.

Results: The mean MMI concentration in thyroid tissue was 1286 (+/-608) ng/g in group 1, compared to 692 (+/-222) ng/g in group 2 ($p<0.05$). MTH could be detected in group 1 with a mean of 80 (+/-64) ng/g, while there were only minimal amounts of MTH demonstrable (20 +/-12 ng/g) in group 2 ($p<0.05$). The iodine content in group 1 was 89 (+/-66) µg/g, and rose to 221 (+/-117) µg/g ($p<0.05$) as early as 5 days after the completion of MMI treatment.

Conclusions: Even 5 days following the end of MMI treatment substantial amounts of MMI could be detected in thyroid tissue. Such residual MMI could account for the high failure rate of RAI treatment following pretreatment with a thionamide drug. MTH on the other hand, was barely demonstrable in thyroid tissue 5 days after withdrawal of MMI and was associated with restoration of iodide organification. Because of the rapid pharmacokinetics of dissappearance from thyroid tissues, the MMI metabolite, MTH, may be the preferred antithyroid agent prior to RAI therapy.

188. A LONGITUDINAL STUDY OF CHANGES IN BODY MASS INDEX AFTER I-131 TREATMENT FOR GRAVES' DISEASE.

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Many patients treated for Graves' disease complain of increases in body weight after treatment for their thyroid disorder. The current literature describes upward shifts in body mass index (BMI) and in gross body weight after treatment of Graves' disease when compared to estimated pre-morbid indices. The currently available studies do not clearly document the magnitude of short-term and long-term shifts in body weight in the post-hyperthyroid period.

We prospectively measured heights and subsequent body weights of 67 patients (14 male/53 female), ranging in age from 15-88 years (mean age = 48 years) treated for Graves' disease with I-131 doses from 5.2 to 25.7 mCi (mean dose = 8.0 mCi). Body weight was recorded at six monthly intervals for up to ten years; body mass index was calculated for each follow up period from the mean body weight recorded and the baseline height. Baseline BMI was defined as that obtained immediately prior to administration of I-131.

There was a significant ($p < 0.01$) increase in overall integrated interval BMI from baseline BMI. The greatest mean increase in BMI was to 28.4 from 25.8 ($p < 0.05$; $n = 40$) at the 13-18 months post I-131 treatment interval. This formed part of a significant ($p < 0.05$) trend of elevations in BMI observed from 13-46 months after I-131 treatment (BMI range 26.5-28.4; n range = 20-46).

Subgroups of patients who experienced no change in BMI versus patients with increases in BMI after treatment were further analyzed as outlined in the table below:

response of BMI to I-131 treatment	mean age (years)	mean I-131 dose (mCi)	mean baseline BMI	mean interval BMI
↑ in BMI	50	7.4	24.8	27.2
no ↑ in BMI	48	7.6	25.7	27.8
significance (p)	NS	NS	< 0.05	NS

The relative magnitude of increase in BMI after I-131 may be accounted for by the lower baseline BMI in the sub-group of patients experiencing significant weight gain. The present study suggests that non-overweight patients with Graves' disease will experience significant increases in BMI within 13-46 months of I-131 treatment. Patients in whom no significant changes in BMI will be observed will most likely be overweight or obese (BMI > 25) at the time of I-131 therapy.

194. **LOW - DOSE IODINE IN ENDEMIC GOITRE - a placebo - controlled, double blind trial.**

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In a controlled, double blind trial (Thyroid, 2, suppl 1, S-19, Aug 92), we recently reported on the comparison of the effects of iodine (I) 500 μg/d and L-T4 125 μg/d in patients (pts) with endemic goitre. Although nearly comparable results were obtained with both therapy regimens regarding thyroid (Thy) volume (vol), reversible I- induced Thy dysfunctions and autoimmune phenomena were observed in pts with endemic goitre. Aim of this randomized, double blind study was to compare the influence of low-dose I and placebo (P) on Thy parameters in pts with I deficiency goitre. 31 pts (16 f, median age 24 yrs, 69 kg, Thy vol 25.6 ml, TSH 0.98 mU/L, 47 μg I/24h urine) were administered 200 μg I/d whereas 29 pts (16 f, 24 yrs, 68 kg, 25.8 ml, 1.11 mU/L, 32 μg I/24h) received P for 12 months (mo) each. After termination of therapy, both groups were subject to a follow-up for another 6 mo. Thy sonography, TRH test (200 μg iv), determination of urinary I excretion /24h (Cer-arsenite method), thyroglobulin (Tg), microsomal and Tg antibodies (Ab, Elisa <250 U/L) were performed before as well as 3, 6, 9, 12, 15 and 18 mo after beginning of therapy. I administration engendered a marked decrease in Thy vol (3 mo 21.5 ml, 6 mo 18.55 ml, 9 mo 19.1 ml, 12 mo 17.6 ml; $p=0.0001$, Mann-Whitney U-test). In the P group, no increase of Thy vol was observed. At the end of the follow-up (18 mo), Thy vol in the I group did not change (15.7 ml, ns). Markedly elevated urinary I values were found in pts receiving I during (3 mo 127.75 μg, 6 mo 175 μg, 9 mo 204.75 μg, 12 mo 212.5 μg/24h; $p=0.0001$) as well as after therapy (15 mo 82.5 μg/24h, 18 mo 65 μg) compared to administration of P (3 mo 34 μg/24h, 6 mo 17.5 μg, 9 mo 40 μg, 12 mo 42 μg). Baseline (12 mo I 0.89 mU/L, P 1.09 mU/L) and delta TSH values remained stable in both groups throughout the observation period. In the I group, T4 increased (8.6 before vs 9.6 μg/dl at 6 mo, $p=0.002$) whereas Tg decreased (5.8 vs 2.3 ng/ml at 12 mo, $p=0.003$) contrasting with the P group where no changes were noted. High Ab titres (>2500 U/L) were present in 3/31 (9.7%) pts receiving I with I-induced hypo and hyperthyroidism having developed in 2 and 1, respectively. Fine needle biopsy revealed a marked lymphocytic infiltration in these 3 pts. After dropping I therapy, Thy dysfunctions ceased spontaneously and Ab titres decreased markedly. Follow-up of these 3 Ab+ve pts for 1 yr showed a further decrease of the Ab titres in 2/3 to upper normal values. In these 3 pts, Thy vol remained small (10 ml) with hypoechogenecity in sonography. Thus, low-dose I therapy successfully normalizes Thy vol and body I supplementation, nevertheless reversible I-induced Thy dysfunctions and autoimmune phenomena were observed in pts with endemic goitre.

196. TREATMENT OF DIFFERENTIATED THYROID CARCINOMA WITH A UNIFORM TREATMENT PROTOCOL; OUTCOME IN 658 PATIENTS OVER 26 YEARS. E G Wilmshurst, P Clifton-Bligh, L W Delbridge, G R Fulcher, I B Hales, A McElduff, A G Poole, T S Reeve, B G Robinson, J N Stiel, J C Wiseman. Royal North Shore Hospital, St Leonards, NSW, AUSTRALIA.

We report the results of treating a series of 658 patients with differentiated carcinoma of the thyroid over a 26 year period (1967-1992) using a uniform treatment protocol throughout. The standard treatment protocol consisted of total thyroidectomy with neck dissection if indicated, followed by a post-operative ^{131}I scan and an ablative dose of 6 GBq (150 mCi) ^{131}I if residual tissue was demonstrated. Thyroxine was given in a dose sufficient to suppress TSH. ^{131}I scans and measurement of serum thyroglobulin concentration were repeated at intervals with further 6 GBq ^{131}I doses administered when uptake of isotope was detected. Surgery was performed for recurrent palpable or surgically accessible tumour tissue. The patients were comprised of 133 males (papillary 102, follicular 31) and 525 females (papillary 387, follicular 138). Age at the time of surgery ranged from 1 to 88 years (mean 44 ± 16.51 SD) for the follicular group and from 5 to 89 years (mean 42 ± 15.90 SD) for the papillary group. Ten year survival rates from Kaplan-Meier curves were 94% in the papillary carcinoma group and 88% in the follicular carcinoma group of patients. Survival was not significantly different between male and female patients nor between papillary and follicular carcinomas. The most significant predictor of survival was age ($p<0.001$). Compared with the under 40 year age group, the risk of dying was increased by a factor of 29 (95% confidence interval 4-218) for the 40-70 year age group and by a factor of 270 (95% confidence interval 33-2198) for the over 70 year age group. The presence of metastases increased the risk of dying by a factor of 2.1 (95% confidence interval 1.1-4.4) and larger tumour size was associated with an increased risk of death ($p=0.004$). Complications of treatment included permanent hypoparathyroidism in 10 patients, and permanent vocal cord paralysis in one patient. Two patients died from acute myelocytic leukaemia and one patient died from lymphosarcoma of the parotid. The good overall survival rate and the relatively low incidence of treatment complications justifies an aggressive approach to the treatment of differentiated thyroid carcinoma.

198. **IS THE LONG-TERM ADMINISTRATION OF AMIODARONE REALLY DANGEROUS TO PATIENTS WITH ABNORMAL THYROID FUNCTION TESTS ?** LS Ward, MAB Teixeira, LC Oliveira, GA Fernandes and RMB Maciel. Departments of Medicine, University of Campinas School of Medical Sciences and Escola Paulista de Medicina, Campinas and São Paulo, Brazil.

Amiodarone, an iodine-containing drug frequently used in the treatment of cardiac arrhythmias, induces several changes in thyroid function tests and can cause hypo or hyperthyroidism; therefore, the current medical practice does not recommend its use when thyroid tests became abnormal during therapy. In this report we present the results of a long term use (Mo=4y) of Amiodarone (400 mg/day) in 97 patients, aged 32-69 years, with resistant or life-threatening cardiac arrhythmias caused by Chagas' disease, in whom was considered essential to continue Amiodarone to maintain sinus rhythm, since arrhythmias had been difficult to control with other drugs. All patients were euthyroid, but 14 had already goiter at the beginning of treatment. We examined them at 3 months-interval, measuring 24h ECG, TSH (sens: 0.05, range: 0.38-6.15 mU/mL), f T4, T4 and T3. The follow-up of 83 patients without goiter showed that 52/83 (62%) had always normal thyroid tests; 23/83 (28%), however, had elevated TSH on the first visit, that persisted in 15 during treatment, although some of them presented fluctuations between normal, elevated or low values. T4 and f T4 were normal in almost all of these patients, with very mild and transient abnormalities in few cases. 8/83 patients (10%) had low TSH in the first return, that normalized in 6, became elevated in 1 and persisted low in 2, with very mild and brief variations in T4 and f T4. In spite of these abnormal thyroid tests, all patients remained clinically euthyroid and the arrhythmias were adequately controlled by Amiodarone during the study. The follow-up of 14 patients with goiter showed that 7/14 (50%) had always normal thyroid tests; 4/14 (29%) had elevated TSH in the first visit, that normalized thereafter; 3/14 (21%) had low TSH initially, that persisted low in 2 for the rest of the study; also in this group T4 and f T4 presented mild and brief changes and all patients remained clinically euthyroid and without arrhythmias. We conclude that in patients with resistant or life-threatening arrhythmias, even in those with goiter, abnormalities in thyroid tests were not always associated with a higher risk to develop hyper or hypothyroidism and there is no reason to discontinue the treatment with Amiodarone.

199. **ELEVATED cAMP LEVELS GENERATE GROWTH INHIBITORY SIGNALS IN A THYROID ANAPLASTIC CARCINOMA CELL LINE (ARO).**

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Anaplastic thyroid carcinoma is a highly malignant neoplasm featured *in vivo* by a rapid growth rate and a very low grade of differentiation as assessed by morphological and molecular criteria.

In this study we evaluated the effects of a prolonged exposure, *in vitro*, to dibutyryl-cAMP (db-cAMP, 1mM) and/or retinoic acid (RA, 5 μM) of an anaplastic thyroid carcinoma cell line (ARO) cultured in RPMI 1640 plus 10% FCS for 10 days. We found a marked reduction (>44%) of cell proliferation, measured by [^{3}H]-thymidine uptake, after the first 72h of treatment, in the presence of db-cAMP but not of RA or RA+db-cAMP. In normal thyrocytes a rapid but transitory elevation of the intracellular cAMP levels usually follows TSH binding to the TSH receptor leading to an increase of the iodine uptake and of the cell growth. Our experiment, however, indicates that a prolonged exposure to cAMP produces inhibitory signals able to control proliferation of anaplastic thyroid carcinoma cells.

To define the molecular mechanism(s) activated by db-cAMP and able to inhibit tumor cell proliferation we analyzed cell cycle gene expression during the different treatments. By northern analysis the expression of the G1/S specific cell cycle kinase CDK2 was found relatively decreased (about 3-fold) after 6 days db-cAMP treatment. Moreover, immunofluorescence (IF) experiments indicated that, in presence of db-cAMP but not of RA or RA+db-cAMP, cyclin B1, the regulatory subunit of the mitosis promoting factor (MPF), was still well detectable in a large number of the cell population. However its distribution resulted prevalent in the citoplasm as occurs in premitotic cells. These results suggest that the effect of db-cAMP on ARO cells is specific and, at least in part directed to inhibit proliferation.

202. RELATIONSHIP OF SUBCLINICAL HYPOTHYROIDISM TO CARDIOVASCULAR RISK FACTORS AND DISEASE IN AN ELDERLY POPULATION[1]. Ladenson PW, Wilson MC, Gardin J, Krommal R, Kuller L, Tracy R, Burke G, Fried LP. Divisions of Endocrinology & Internal Medicine. Johns Hopkins University School of Medicine; Baltimore, MD 21287-4904.

Overt hypothyroidism (H) is associated with risk factors for atherosclerotic cardiovascular disease (CVD): hypercholesterolemia and diastolic hypertension (DBP). Subclinical hypothyroidism (SCH) has also been linked in some studies to increased LDL-cholesterol (LDL-C) and altered myocardial function. Although SCH and CVD are both common in the elderly, direct relationships between thyroid dysfunction and CVD have not been established. Therefore, we investigated thyroid function in 3,410 subjects over 65 years who have had extensive assessments to identify risk factors for, and clinical and subclinical evidence of ASVD. Subjects were screened by TSH assay; those with elevated TSH then had free T4 determined. Prevalences of SCH and H, respectively, were 17.1% and 2.5% in women (n=2063), and 13.7% and 1.5% in men (n=1347). Among euthyroid and SCH subjects, increasing TSH was related[2] to higher total C**, LDL-C**, triglycerides***, and factor 7*. However, when SCH subjects were stratified by TSH level (4.5-<6.0, 6.0-10.0, & >10 mU/L), higher LDL-cholesterol levels were only found for those with serum TSH>10 mU/L. There were no differences between euthyroid and SCH subjects in DBP, SPB, glucose, insulin, or BMI; arm-ankle BP or sonographic carotid thickness; echocardiographic LV mass, ejection fraction, or regional wall motion; or mitral peak flow velocities. Clinical histories of angina, MI, CHF, PVD, CVA, or TIA were no different between SCH (or H) versus euthyroid subjects. From these cross-sectional observations, we conclude that certain CVD risk factors are more common in this large cohort of elderly persons with SCH, particularly when it is severe; but a higher prevalence of existing CVD was not detected. Longitudinal follow-up to define the actual CVD risk associated with SCH is warranted.
[1]Supported by grants from National Heart, Lung, & Blood Institute and the Maryland American Heart Assoc; [2]By multiple logistic regression. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

212. **METHIMAZOLE, 3-METHYL-2-THIOHYDANTOIN AND IODINE IN THYROID TISSUE**
D. Aktuna, A. Berger, O. Lorenz, O. Eber.
Hospital Barmherzige Brüder, Bergstraße 27, A-8020 Graz, Austria, Europe.

Methimazole (MMI) therapy has been associated clinically with interference with radioactive iodine (RAI) therapeutic success. Moreover, Tuttle et al. (ATA 1993, abstract no.69) have demonstrated that propylthiouracil (PTU) therapy prior to RAI treatment is characterized by a higher failure rate (34%), even when PTU was discontinued 4 days before RAI. The aim of the present study was to extend our previous pharmacokinetic investigation of MMI and its metabolite, 3-methyl-2-thiohydantoin (MTH) (Aktuna et al., ATA 1993, abstract no. 30) to explore whether residual MMI was present in thyroidal tissues after MMI withdrawal 5 days prior to surgical thyroidectomy.
Methods: 11 goitre patients were given 40 mg MMI iv daily until surgery (group 1). In another 11 goitre patients, MMI-treatment was interrupted 5 days before surgery (group 2). MMI and its metabolite MTH was measured in thyroid tissue using our HPLC method (Aktuna et al., ETA 1994, abstract no. 79) and iodine determinations were done by a colorimetric method.
Results: The mean MMI concentration in thyroid tissue was 1286 (+/-608) ng/g in group 1, compared to 692 (+/-222) ng/g in group 2 ($p<0.05$). MTH could be detected in group 1 with a mean of 80 (+/-64) ng/g, while there were only minimal amounts of MTH demonstrable (20 +/-12 ng/g) in group 2 ($p<0.05$). The iodine content in group 1 was 89 (+/-66) µg/g, and rose to 221 (+/-117) µg/g ($p<0.05$) as early as 5 days after the completion of MMI treatment.
Conclusions: Even 5 days following the end of MMI treatment substantial amounts of MMI could be detected in thyroid tissue. Such residual MMI could account for the high failure rate of RAI treatment following pretreatment with a thionamide drug. MTH on the other hand, was barely demonstrable in thyroid tissue 5 days after withdrawal of MMI and was associated with restoration of iodide organification. Because of the rapid pharmacokinetics of dissappearance from thyroid tissues, the MMI metabolite, MTH, may be the preferred antithyroid agent prior to RAI therapy.

215. **CHANGES IN BODY WEIGHT, BODY COMPOSITION AND COLLAGEN RELATED PEPTIDES AFTER TREATMENT FOR THYREOTOXICOSIS**

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Subjects with thyreotoxicosis undergo wide changes in body composition after treatment and are at follow up frequently found to suffer from obesity. To examine short-term changes in body composition ten subjects, 4 men and 6 women (age: mean±SD, 40±18 years) were studied before and two months after treatment (surgery or radioiodine).

Methods: Body composition was determined from DEXA as well as from total body potassium determinations. Visceral adipose tissue was determined by computed tomography using a Philips Tomoscan 310. Five transectional scans were obtained at each examination. Anthropometric measurements were performed. All subjects were also studied in a newly constructed chamber for indirect calorimetry at our local laboratory. All blood samles were drawn after an overnight fast.

Results: Before treatment the subjects had a mean free T4 of 86±41 pmol/L and TSH of <0.1 mU/L. Two months after treatment the mean free T4 was 13±6 pmol/L and TSH within reference limits for a healthy population. Already after two months there was a significant increase in body weight with a mean of 3 kg (mean±SD: 67.1±13.3 kg; 70.0±11.5 kg, $p<0.05$). From Dexa examinations it could be shown that this increase was not associated with any significant change in adipose tissue mass (18.6±6.8 kg; 19.0±6.0 kg, ns) but with a significant increase in lean body mass (44.7±11.9 kg; 47.7±11.3 kg, $p<0.001$). After two months the mean decrease in 24 h energy expenditure (EE) was 760±360 kcal/24h. Compared to pretreatment concentrations we found a significant reduction after treatment in the concentration of the aminoterminal procollagen III peptide (1.45±0.44 kU/L; 0.66±0.16 kU/L). This reduction correlated with changes in total and free T3 ($r=0.86$, $p<0.05$).

Conclusion: Normalisation of thyroid hormone status after treatment for thyreotoxicosis is already after two months associated with a significant weight increase. The observed weight gain only seems to be associated with an increase in lean body mass and not with any significant change in adipose tissue mass. We also conclude that the aminoterminal procollagen III peptide might be a useful marker of thyroid hormon action.

218. **TSH Suppression: Daytime Values May Rise To Unacceptable Values At Night. V.J.Bernet, B.L.Solomon, T.S.Cranston and K.D.Burman, Walter Reed Army Medical Center, Washington,D.C.**

TSH levels are highest from 22:00 to 04:00 hrs and lowest from 08:00 to 14:00 hrs, and this circadian remains intact in hypothyroid patients receiving levo-thyroxine (L-T4) replacement therapy. L-T4 is used to suppress TSH and deter growth of goiters, thyroid nodules or cancer. We investigated whether nyctohemeral variations of TSH secretion persist in patients receiving either suppressive or replacement L-T4 therapy, and whether these levels would reflect a different impression of the adequacy of TSH suppression than was considered based on the daytime TSH levels alone. Patients were admitted to the metabolic ward and had serum sampled every 20 minutes for a 24 hour period beginning prior to their L-T4 dose at 08:00 on day one until 08:00 on the following day. Data were analyzed using descriptive procedures and Wilcoxen non-parametric procedure. The results below groups patients by their nadir TSH level between 08:00 - 16:00 hours:

Group per Minimum AM TSH (N)	Mean Minimum TSH AM*	Mean Maximum TSH PM**	Mean Percent Increase TSH	P Value ***
G1: <0.1 (5)	0.02 +/- 0.01	0.04 +/- 0.01	183%	0.031
G2: 0.1-4.0 (4)	0.21 +/- 0.05	0.52 +/- 0.17	237%	0.063
G3: >0.4 (3)	1.28 +/- 0.53	4.24 +/- 2.69	275%	0.125

TSH reported in uU/ml. * Mean TSH from 08:00 to 20:00; ** Mean TSH from 20:00 to 08:00 the next morning; *** P-value compares mean nadir to mean peak values.

The TSH circadian variations of patients A, B, C and D are of particular clinical interest. Patient A, who is on LT4 suppression for a thyroid nodule, had a nadir TSH of 0.13 uU/ml at 10:20 hrs which peaked at 00:40 hrs with a TSH of 0.39 uU/ml (166% change). Patient B, a hypothyroid patient, had a nadir TSH of 2.35 uU/ml at 13:20 and a peak of 9.62 uU/ml at 05:20 hrs (409% change). Patient C, who has thyroid cancer, had a minimum TSH of 0.19 at 14:40 hrs and a peak of 0.41 uU/ml at 01:20 hrs (216% change). Patient D, a hypothyroid patient, had a nadir TSH of 0.38 uU/ml at 13:20 hrs and a peak of 1.35 uU/ml at 01:40 hrs (400% change). In conclusion, clinical decisions based on TSH levels obtained during customary clinic hours, might not accurately reflect the peak TSH throughout the day. These findings indicate the need to investigate alternate dosing regimens of L-T4 to consistently suppress TSH secretion over the entire day.

219. INFLUENCE OF COMPENSATED RADIOIODINE THERAPY ON THYROID VOLUME AND INCIDENCE OF HYPOTHYROIDISM IN GRAVES' DISEASE. L. Hegedüs, B. Nygaard, M. Gervil, H. Hjalgrim, B.M. Hansen, B. Søe-Jensen, J.M. Hansen. Departments of Internal Medicine and Endocrinology and Ultrasound Herlev University Hospital, DK-2730 Herlev, Denmark. Department of Internal Medicine and Endocrinology, Odense University Hospital, DK-5000 Odense C, Denmark.

It was our objective to investigate the long term effect of radioactive iodine on thyroid function and ultrasonically determined size in patients with Graves' disease. We studied 358 consecutive patients (320 women) with Graves' disease selected for treatment from January 1972 through December 1989. Until January 1980 (241 patients) ^{131}I dose was calculated based on a clinical evaluation of thyroid gland volume. During the remaining period (117 patients) a precise ultrasonographic technique for thyroid volume determination was employed. Patients were followed for a minimum of 12 months (range 1-20 years, median 7 years) after an intended dose of 3.7 MBq/g thyroid tissue corrected to a 100% uptake of ^{131}I in 24 hours. The patients were investigated with thyroid function variables and ultrasonically determined thyroid volume before treatment as well as 3/4, 1½, 3, 6 and 12 months after treatment and then once a year. 211 patients were cured by one ^{131}I dose and 102 by two doses while the remaining 45 patients received additional doses (range 1-7, median 1 dose). Within one year 25% developed hypothyroidism and hereafter hypothyroidism developed at a constant rate of 3% per year independent of antithyroid pretreatment or whether thyroid size was calculated by ultrasound or clinically. The cumulative 15 year risk of hypothyroidism was 68% (95% confidence interval 57% to 78%). Initial median thyroid volume was 33 ml (range 9-106). At 12 months after the last ^{131}I dose median thyroid volume was reduced to 14 ml (6-36, $p < 0.00001$). The median reduction being 58% (0-80%), hereafter no further reduction occurred. Half of this reduction was observed within 3 months after the first ^{131}I dose. A significant reduction in thyroid volume was also noted in patients needing subsequent ^{131}I doses and in those developing hypothyroidism within the first year. Thyroid volume was normalized within one year in all patients. We conclude that ^{131}I normalizes thyroid volume in patients with Graves' disease. Hypothyroidism seems an inevitable end result of this treatment. The present study suggests that it will be impossible to modify ^{131}I therapy in a way to achieve both early control of hyperthyroidism and a low incidence of hypothyroidism.

222. PREVALENCE OF INCIDENTAL THYROID DISEASE IN A LOW IODINE INTAKE AREA BY ULTRASONOGRAPHY. F. Pedrinola, E. Tomimori, H. Cavaliere, N. Lima, M. Knobel. Thyroid Laboratory, Hospital das Clinicas, Univ São Paulo Med School, São Paulo, Brazil.

A survey of thyroid abnormalities was conducted in 547 consecutive apparently normal subjects (380 females and 167 males), aged 27-58 years in an urban area with relatively low iodine intake (urinary I excretion 156 μg I/g creatinine). Individuals with any previous thyroid disease or familial thyroid pathology were excluded. In 240 subjects high resolution ultrasonography of the thyroid was considered normal (43.9%). In 307 individuals abnormalities of the echo structure (33.2%) or thyroid nodular disease (22.9%) were detected by US. Seventeen patients (3.1%) had an enlarged thyroid volume (goiter) and 101 (18.4%) subjects had marked heterogeinity of the echo structure that was considered as suggestive of chronic thyroiditis. In 62 of these patients the serum anti TPO levels were positive by a sensitive RIA. In the remaining 38 subjects with presumptive chronic thyroiditis a positive anti TPO test was detected in 11 patients within 12 months of the first examination. Thyroid nodules either solid or predominantly cystic were present in 17.3% (n=66) of the studied female population and in 15.5% (n=26) of the males. In the later group seven patients had a relatively large nodule (diameter: 25 - 48 mm) that were not easily palpable at clinical examination. Pathological examination in these seven individuals (after surgery) indicated benign nodular goiter.

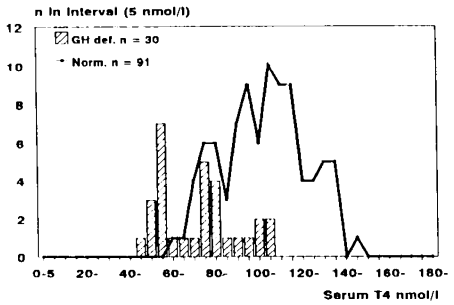
Thyroid function studies confirmed subclinical hypothyroidism in 27 patients (4.93%) all of them with elevated serum anti-TPO autoantibodies levels. It was concluded that the overall occurrence of thyroid disease is more common than suspected by clinical examination and that high resolution US of the thyroid is an important asset in the evaluation of thyroid disease.

223. SECONDARY THYROID FAILURE MAY BE UNDERDIAGNOSED AND UNDERTREATED IN GROWTH HORMONE (GH) DEFICIENT ADULTS.

Laurberg P, Hoeck HC, Jacobsen PE, Vestergaard P. Dept. Endocrinoi. Aalborg Hospital, DK-9000 Aalborg, Denmark.

Thyroid hormones and GH/IGF1 are major regulators of development and metabolism. Many processes are modulated in concert by the two systems, and alterations in one system may directly or indirectly affect the other. This interaction may make it difficult to evaluate which system is responsible for a certain modulation or defect in development and metabolism. The interplay may in several ways have consequences for the diagnosis and therapy of patients with thyroid and/or GH disorders.

In the present study we evaluated thyroid function in 30 adult patients (F/M 18/11, age 44.8 (2.4) mean (SE)) with GH deficiency diagnosed > 3 months after surgery for various types of pituitary adenomas. None were considered to have secondary hypothyroidism, and none received thyroxine supplementation. GH secretion was evaluated by insulin induced hypoglycaemia (max sGH < 5 ng/ml after blood glucose < 2.2 mM). The presence of secondary thyroid failure was evaluated at the time of testing by sT_4. None had elevated sTSH and none had TBG abnormalities. For comparison the distribution of sT_4 was evaluated in 91 normal healthy subjects. The distributions of sT_4 were markedly different with T_4 values from the patients clustering in the lower part of the normal distribution (T_4 83.5 (3.8) vs. 105.0 (2.2) nM in normal subjects (mean (SE), $P<0.01$).



Distribution of sT_4 in GH deficient and control subjects. In the GH deficient patients sT_4 had been reduced with 11%, corresponding to the average fall observed previously when GH is normalized in such patients.

Previous studies have shown that most patients operated on the pituitary are GH deficient, and that substitution with GH to adults leads to a fall in sT_4 of 10-15% due to enhancement of T_4 to T_3 deiodination. Such a decrease in sT_4 by normalisation of GH would bring sT_4 below the normal range in 11/30 of our patients (figure) - an effect well known from GH therapy in children.

Impaired T_4 deiodination to T_3 with a relatively high sT_4 in GH deficient patients may mask secondary hypothyroidism. Our data suggest that undiagnosed secondary hypothyroidism is relatively common in GH deficient adults .

228. IMMUNOHISTOCHEMICAL STUDY IN DIFFERENTIAL DIAGNOSIS OF THYROID TUMORS.

J.Hua, G.Yu, YY.Jiang, Chinese Great Wall Hospital, Beijing, China.

There are many histological classification for the malignant thyroid tumor. Sometime it is difficult to identify the histopathogenesis of tumor according to the morphologic. Nearly all malignant thyroid tumor traditionly designated as anaplastic small-cell carcinoma are either malignant lymphomas, small- cell variants of medullary carcinoma and related neuroendocrine carcinoma or undiffereated carcinoma. In practice this requires to use the immunohistochemicity in mamy cases. The record of 300 patients with maligant thyroid tumors treated in our hospital between 1972 and 1992 were reviewed. Nineteen patients with thyroid tumors, 3 epithelioid angioscarcoma (1%), 1 malignant lymphomas (0.3%), 10 undiffereated thyroid carcinoma (3.3%), 3 adenosquamous carcinoma of thyroid (1%), 2 teratoma of thyroid (0.6%), were studied. HB stained sections and immunohistogical reactions with monoclonal and polyclonal antibodies were evaluted. Epithelioid angioscarcoma has a very definte geographical favor. Only one case in Asia has been reported from Hong Kong in 1986. The tumor cell had a distinct epithelioid appearance. These cases were stained with a battary of antibodies to epithelial (Keratin), carcinoembrgonic antigen (CEA), meseachymal (Vimetnin) , endocine (Thyroglobulin, Calcitonin), FⅧ-RA and Schwann cell (S-100 protein) markers. The tumor cells were strongly positive for Keratin, Vimentin and FⅧ-RA. Stains for thyroglobulin, Calcitonin and S-100 protein were negative. The findings presented have support the existance of primary maligant vascular tumor in the thyroid. Anaplstic thyroid carcinoma (undiffereated carcinoma) can present differeat histologic pattern. Immunohistochemical identification of neuroendocrine tumor markers (neuron-specific enolase (NSE) , chromogranin-A (Chr-A) and S-100 protein)in paraffin embedden material form ten undiffereated thyroid carcinoma. Positive immunohistogical for Chr-A, NSE and S-100 protein were obtained in three cases (33%), but negitive for the Calcitonin. A lack of reactivity for Calcitonin indicates that these tumor are not derived from "C" cell. This is an new finding that neuroendocine tumor exist in a part of the undiffereated carcinoma of thyroid besides medullary thyroid carcinoma. In the blending of the squamous and glandular element for the adenosquamous carcinoma of the thyroid gland, thyroglobulin staining can identify that the squamous element is infiltrations or metastases. In conclusion, immunohistochemical technique represents an extremely helpful ancillary method in the histopathologic diagnosis of thyroid carcinoma. It may be provide an new concept for the thyroid tumor.

2:00 P.M. 27. MUTUAL ANTAGONISTIC INTERACTIONS BETWEEN THE cAMP AND PROTEIN KINASE C/TYROSINE KINASE PATHWAYS IN HUMAN THYROID CELL PROLIFERATION, DIFFERENTIATION, *c-jun* AND *c-fos* PROTO-ONCOGENE EXPRESSION. Z. Kraiem, O. Sadeh, M. Yosef, A. Aharon and R. Heinrich, Endocrine Research Unit, Carmel Medical Center, Haifa 34362, Israel.

Our aim has been to delineate the role of the major signal transduction pathways believed implicated in the control of thyroid function and growth: the cAMP-, tyrosine kinase- and protein kinase C (PKC)-mediated mechanisms. The experimental model used was our *in vitro* system of thyroid follicles of human origin cultured in suspension under serum-free conditions in which the follicular three-dimensional structure is retained. Exposure of human thyroid follicles for 1 hr to the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA), and to a lesser extent thyrotropin (TSH, 0.5mU/ml), induced *c-jun* and *c-fos* mRNA expression. The mRNA levels were markedly enhanced (almost sixfold over control) in the presence of cycloheximide, an inhibitor of protein synthesis. TSH combined with TPA, however, reduced the phorbol ester-induced *c-jun* and *c-fos* mRNA levels. TPA dose-dependently (10^{-11}-10^{-7}M) inhibited TSH-stimulated thyroid functions (cAMP formation, iodide uptake and organification and the hormonal end-product response: T_3 secretion). TPA also inhibited such forskolin- and 8-BrcAMP-stimulated effects, suggesting that the attenuation of the cAMP-dependent pathway occurs at steps both pre- and post- cAMP formation. The effects of TPA were mimicked by another PKC activator, phorbol 12, 13-dibutyrate, but not by a phorbol ester that fails to activate PKC, 4α-phorbol 12, 13-didecanoate, and were reversed by staurosporine, a PKC inhibitor. The TPA actions seem therefore to be PKC-mediated. EGF exhibited a dose-dependent (0.02-8nM) restraining influence on the above TSH-stimulated differentiated functions, except for cAMP formation which was enhanced. EGF also blunted such forskolin- and 8-BrcAMP- induced response parameters, suggesting inhibition at a post-cAMP locus. Regarding cell proliferation, during the initial stages of culture (days 2-3), TPA dose-dependently (10^{-11}-10^{-7}M) attenuated cell proliferation but subsequently the same doses of TPA stimulated cell multiplication. The TPA-mitogenic and anti-mitogenic effects could not be mimicked by 4α-phorbol-12, 13-didecanoate and were reversed by staurosporine, thus indicating a PKC-mediated pathway for such TPA actions. EGF, on the other hand, only enhanced cell proliferation at a late stage (coincident with the TPA-mitogenic effect). TSH (0.5mU/ml) inhibited both the mitogenic and anti-mitogenic actions of TPA as well as the cell-proliferative influence of EGF. In conclusion, the data demonstrate mutual antagonistic interactions between the signal transduction pathways: the PKC and EGF (tyrosine kinase) pathways seem to inhibit cAMP-mediated human thyroid cell differentiation, whereas cAMP attenuates PKC-mediated thyroid cell mitogenesis and anti-mitogenesis as well as tyrosine kinase- mediated cell proliferation. The results may be related to the induction of *c-jun* and *c-fos* mRNA expression by TPA and repression by TSH.

2:15 P.M. 46. INHIBITING IODIDE UPTAKE IN RAT THYROID CELLS USING CHLORIDE CHANNEL BLOCKERS; PROBES FOR THE STUDY OF IODIDE TRANSPORT. A. Fanelli, W. K. Berlin and E. F. Grollman, National Institutes of Health, Bethesda, Maryland.

This study was initiated to search for compounds that specifically would inhibit iodide transport and could be used to identify, isolate or study the mechanism of NaI transport in thyroid. It was shown previously that stilbene disulfonates, compounds that inhibit chloride/bicarbonate exchange in red blood cells, stimulate iodide accumulation in rat thyroid cells (half-maximal stimulation is 50μM with 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid). Recently a group of compounds that are derivatives of diphenylamine-2-carboxylate (DPC) were found to be potent inhibitors of chloride channels in epithelia of various tissues. We made three derivatives reported to the most active inhibitors of chloride channels to determine whether they either blocked or stimulated iodide transport. FRTL-5 cells were used in the study as these cells demonstrate properties of iodide entry described in intact thyroid tissue, *i.e.* iodide entry depends on external sodium, requires ATP, and is inhibited by complex anions such as thiocyanate or perchlorate. Using these cells, 22μM 2-cyclooctylamino-5-nitro-benzoic acid and 17μM 5-nitro-2-(3-phenylpropyld 17μM 5-nitro-2-(3-phenylpropylamino)-benzoic acid decrease sodium- dependent iodide accumulation in FRTL-5 cells to 50% of that seen in the control. This effect is immediate, reversible, and independent of the chloride concentration used in the incubation medium. A double-reciprocal plot of 1 over the iodide accumulation in the cells *vs.* 1 over the iodide concentration in the medium was consistent with a competitive effect of these compounds with iodide. The parental compound DPC was inactive, and 2-(3-chlorophenylamino)-benzoic acid was 10-fold less effective as an inhibitor of iodide accumulation. Although stilbene disulfonate enhancement of iodide uptake in FRTL-5 cells is dependent on sodium and independent of the chloride concentration in the medium, the effect is non- competitive with iodide. *In summary*, derivatives of DPC, blockers of epithelial chloride channels, inhibit NaI transport in FRTL-5 cells, and because of their ease of synthesis and modification, may be useful new probes for the study of the NaI symporter.

2:30 P.M. 91. NOVEL EFFECTORS OF RAS IN THYROID CELLS. E. Kupperman*, S. Ching*, N. Al-Alawi*, T. Tominaga*, M. White[Δ], M. Wigler[Δ], J.R. Feramisco*[ΘΦ] and J.L. Meinkoth*[Θ]. Departments of Medicine*, Cancer Center[Θ], and Pharmacology[Φ], University of California at San Diego, La Jolla, California 92093. Cold Spring Harbor Laboratories[Δ], Cold Spring Harbor, New York 11724.

There is a large body of experimental evidence indicating that Ras functions downstream of tyrosine kinase receptors. Thyrotropin (TSH) is the primary regulator of thyroid follicular cells, and binds to the G protein coupled TSH receptor, whose activation leads to increases in cAMP levels and to the activation of the cyclic AMP dependent protein kinase. We have previously shown that injection of an inhibitory Ras protein or inhibitory anti-Ras antibodies significantly reduced TSH stimulated DNA synthesis, demonstrating that Ras is required for the full mitogenic response to TSH in Wistar rat thyroid (WRT) cells. Since TSH signaling employs Ras we are currently investigating the role of known downstream effectors of Ras in TSH-stimulated mitogenesis. Injection of anti-Raf antibodies did not reduce TSH-stimulated DNA synthesis in WRT cells, although it did reduce serum stimulated DNA synthesis in fibroblasts. Similarly, TSH treatment does not increase MAP kinase activity. These data suggest that Ras may be interacting with a novel effector in thyroid cells. To further address this issue, WRT cells were transfected with either an oncogenic Ras expression vector (val12 Ras) or a vector encoding an oncogenic Ras mutant which no longer binds to Raf (val12 G37 Ras). Both oncogenic Ras and the val12 G37 Ras mutant stimulated colony formation in the absence of TSH treatment in WRT cells. The ability of the mutant Ras, although unable to interact with Raf, to stimulate cellular proliferation suggests that Ras couples to effectors other than Raf in WRT cells. Future experiment will attempt to identify the downstream effectors of Ras in thyroid cells.

2:45 P.M. 144. **CHOLERA TOXIN A1 SUBUNIT GENE ELIMINATES LIGAND-STIMULATED INOSITOL PHOSPATE PRODUCTION IN STABLY TRANSFECTED RAT FRTL-5 THYROID CELLS.** G. Laglia, M. Saji, M.A.Zeiger, P. Caturegli, M.A.Levine, and L.D.Kohn National Institutes of Health, Bethesda and Johns Hopkins University, Baltimore, Maryland.

TSH stimulates the thyroid follicular cell through binding to a cell surface receptor that is coupled via G proteins Gs and Gq to stimulation of adenylate cyclase (AC) and phospholipase C (PLC), respectively. We created a permanently transfected FRTL-5 cell line (TGCT8) in which the thyroglobulin gene promoter (TG) directs expression of the cholera toxin A_1 subunit (CT). CT catalyzes ADP-ribosylation of Gsα, resulting in persistent activation of Gsα. The activated Gsα causes constitutive stimulation of AC and increased levels of intracellular cAMP. Because G protein-linked signal pathways exhibit "cross-talk", we compared TGCT8 cells to FRTL5 cells transfected with neomycin-resistance gene (TGCT4) to determine whether constitutively activated Gsα and AC influences the PLC pathway.

PLC activity was assessed by measuring production of inositol phosphates (IP) by TGCT4 and TGCT8 cells that had been preincubated with [^{3}H]myo-inositol for 2 days. Baseline values for [^{3}H]IP were similar for TGCT4 and TGCT8 cells. Incubation of TGCT4 cells with 10^{-8}M TSH, 300 μM ATP, and 100 μM norepinephrine stimulated a 2.5-, 8.1- and 3.4-fold increase, respectively, in [^{3}H]IP production over control. By contrast, there was no response to any of these ligands in TGCT8. There was no significant difference in [^{125}I]TSH binding or levels of immunoactive α subunits for Gq or G_{11} between TGCT4 and TGCT8. Finally, treatment of TGCT4 cells with either 100 ng/ml CT or 50 μM forskolin for 4 days duplicated the loss of ligand-stimulated [^{3}H]IP synthesis seen in TGCT8 cells.

These data strongly suggest that activated Gsα inhibits PLC activity through a post-receptor mechanism, specifically through changes in cAMP levels. Further study of this model will elucidate our understanding of the exact mechanism responsible for this interaction.

8:00 A.M. 57. **ANALYSIS OF THE T-CELL ANTIGEN RECEPTOR V-GENE REPERTOIRE IN LYMPHOCYTES INFILTRATING THE PRETIBIAL LESIONS OF PATIENTS WITH GRAVES' OPHTHALMOPATHY AND PRETIBIAL DERMOPATHY.** A E. Heufelder and R. S. Bahn*. Molecular Thyroid Research Unit, Medizinische Klinik, Klinikum Innenstadt, Ludwig-Maximilians-Universität, München, Germany, and Division of Endocrinology*, Department of Internal Medicine, Mayo Clinic/Foundation, Rochester, MN, USA.

Activated T-lymphocytes within the retroorbital and pretibial tissue are thought to play an important role in the pathogenesis of the extrathyroidal manifestations associated with Graves' disease. Recently, we have demonstrated marked restriction of the retroorbital T-cell antigen receptor (TcR) V gene repertoire in untreated patients with severe Graves' ophthalmopathy (GO) of short duration. To date, it is unknown whether T-cells infiltrating the pretibial lesions of patients with GO and pretibial dermopathy (PTD) represent a primary immune response in which a limited number of T-cell clones are activated against specific antigenic epitopes. To characterize the T-cells present in the pretibial skin of patients with PTD at the molecular level, we examined the TcR V gene repertoire in pretibial tissue biopsies. Tissue was obtained from three untreated patients with clinically marked, inflammatory PTD presenting early in the clinical course of their disease. Each of these patients also had severe GO. In addition, specimens derived from the pretibial skin of five patients with longstanding, clinically inactive PTD were examined. Control tissue specimens included biopsies derived from pretibial skin of three normal individuals and three patients with inflammatory skin lesions unrelated to PTD. RNA extracted from pretibial tissue and peripheral blood lymphocytes (PBL) was reverse transcribed and amplified using the polymerase chain reaction and 22 Vα and 24 Vβ gene family-specific oligonucleotide primers. The resulting TcR Vα and Vβ transcripts were verified by Southern hybridization analysis using digoxigenin-labeled, TcR C-region-specific, internal oligonucleotide probes. Compared with matched PBL, the pretibial TcR Vα and Vβ gene repertoire expressed was heterogeneous, but revealed limited diversity of V gene usage in all three patients with severe PTD of short duration. Marked restriction and similarity of TcR V gene usage was demonstrated both in the retroorbital and pretibial tissue, but not PBL, obtained from a single patient with severe inflammatory GO and PTD. In contrast, greater diversity of the TcR Vβ gene repertoire and absence of TcR Vα gene restriction was noted in two patients with longstanding PTD. No TcR constant and variable region transcripts were amplified in three patients with PTD of long duration and in any of the normal individuals. Further, TcR V gene usage was unrestricted in pretibial specimens obtained from patients with non-PTD inflammatory skin lesions and in PBL samples obtained both from patients and control subjects. Our data suggest that pretibial TcR V gene usage may be variable, but is markedly restricted during the early stages of PTD. With increasing disease duration, greater diversity of the TcR V gene repertoire appears to develop, and oligoclonality of the T-cell response may be lost. Careful selection of patients with early stages of GO and PTD will be mandatory when further dissecting TcR usage and antigen specificity of T-lymphocytes infiltrating the extrathyroidal manifestations of patients with Graves' disease.

[Supported by grants from Deutsche Forschungsgemeinschaft, Bonn, Germany (He 1485/3-1 and He 1485/5-1)].

8:15 A.M. 109. **CTLA-4 GENE POLYMORPHISM ASSOCIATED WITH GRAVES' DISEASE.** T. Yanagawa and L.J. DeGroot. Thyroid Study Unit, The University of Chicago, Chicago IL.

Multiple genetic factors are believed to influence the autoimmunity evident in Graves' disease (GD), but the factors are largely unknown, except for sex (female disease preponderance) and the role of HLA genes on chromosome 6. To understand mechanisms underlying the development of GD, a search for non-HLA linked genes is crucial. The magnitude of the task has been reduced by application of the polymerase chain reaction (PCR), particularly through amplification of short tandemly repeated segments of DNA called microsatellites. Using this method, we tested several candidate genes that are directly related to autoimmunity or encode thyroid antigens, including the CTLA-4 gene on chromosome 2q33. CTLA-4 is co-expressed with CD28 on activated T cells and cooperatively regulates T cell activation by binding to B7 on antigen-presenting cells. One hundred and twenty eight unrelated patients with GD (25 males) and 84 unrelated controls were included in this study. PCR was used to amplify DNA containing the (AT)n repeat in the 3' untranslated region of exon 3 of the CTLA-4 gene. The 5' end of the forward primer was labeled with 32P. Amplified products were resolved on 5 % sequencing gels and detected by autoradiography. Nineteen alleles were observed with sizes ranging from 88 to 130 base pairs. The phenotype frequencies (%) of each allele in GD patients and controls are shown below, except for rare alleles. The assignments for larger sizes have not been completed.

Allele size in base pairs	88	104	106	108	110	112	114	116-120	122-130
GD (n=128)	52.3*	5.5	50.8**	13.3	6.3	5.5	8.6	15.6	18.8
Control (n=84)	66.7	4.8	28.6	11.9	8.3	3.6	7.1	13.1	17.9

*) P=0.038, Pc=NS
**) P=0.0014, Pc<0.03, RR=2.58

Allele 106 was significantly more prevalent in patients with GD than controls, and the association persisted after correction for comparisons made. To reduce possible heterogeneity, the patients were subdivided with respect to sex and HLA. (DR3 is a susceptibility gene and DR7 is weakly protective). The phenotype frequency of the 106 band was higher in DR7(+)DR3(-) female patients (71.4%, n=14), but not significantly different from other groups (Total male patients;56%, n=25. DR3(+)DR7(-) females;46.2%, n=39. DR3(-)DR7(-) females;45.8%, n=48. DR3(+)DR7(+) females;50%, n=2). These results suggest that an allele of CTLA-4 gene or a closely associated gene helps confers susceptibility to GD. This association may be important in female patients who have also a protective HLA.

8:30 A.M. 139. EPITOPIC "FINGERPRINTS" OF THYROID PEROXIDASE AUTOANTIBODIES IN HASHIMOTO'S THYROIDITIS: EVIDENCE FOR CONSERVATION OVER TIME AND IN FAMILIES. J.C. Jaume, S.M. McLachlan, C.L. Burek, W.H. Hoffman, N. Rose and B. Rapoport, Thyroid Molecular Biology Unit, VAMC, Univ. of California, San Francisco and Johns Hopkins Univ., Baltimore MD.

Autoantibodies to thyroid-peroxidase (TPO) in patients' sera are polyclonal. Previously, we generated a panel of human monoclonal IgG-class TPO autoantibodies expressed as recombinant F(ab). These F(ab) interact with an immunodominant region on TPO comprising conformational epitopes in two closely associated domains (A and B). Competition studies between 4 selected F(ab) and serum autoantibodies for binding to ^{125}I-TPO have permitted the epitopic "fingerprinting" of the polyclonal TPO autoantibodies in individual patients. In the present study, we addressed the important unanswered question of whether or not the TPO autoantibody fingerprint changes over time. For this purpose, we studied 9 children with Hashimoto's disease from whom sera were available on 2 or 3 occasions over 15 years. When used as a pool, the 4 TPO-specific F(ab) inhibited TPO antibody binding by the patients' sera by 81 $\pm$ 7 % (mean $\pm$ SE) indicating that the serum autoantibodies were largely directed to the TPO immunodominant region. Fingerprinting (preferential recognition of domains) was determined from the ratio of inhibition by domain A F(ab) to inhibition by domain B F(ab). In sera obtained from the patients ~ 15 years previously, 4/9 interacted preferentially with the B domain (A/B ratio <0.75), 4/9 recognized both A + B domains (>0.75 to <1.3) and 1/9 preferentially recognized the A domain (A/B >1.3). The same epitopic profile was observed 2 - 3 years later in the 7 patients whose sera were available. After another 12 years, sera was available from all 9 patients. Three patients had become TPO autoantibody negative. Remarkably, of the 6 TPO autoantibody positive patients, 5/6 still had retained the same epitopic profile observed 15 years previously. We also determined the TPO autoantibody fingerprint in the sera of the families of two of these patients. TPO domain recognition in the proband closely resembled that in TPO antibody relatives (family #1, domain A + B, father and 1 sibling; family #2, domain B, father and 2 siblings). In conclusion, TPO autoantibody epitopic fingerprints in Hashimoto's thyroiditis patients and their families appear to be conserved, suggesting that the production of autoantibodies to particular TPO autoantibody epitopes is inherited.

8:45 A.M. 141. HUMAN THYROID PEROXIDASE AUTOANTIBODIES OF LAMBDA LIGHT CHAIN TYPE CLONED BY PHAGE DISPLAY: IMMUNOGLOBULIN V GENE USAGE. S.Portolano and S.M.McLachlan, Thyroid Molecular Biology Unit, VA Medical Center and University of California, San Francisco.

Autoantibodies to thyroid peroxidase (TPO), a hallmark of thyroid autoimmune disease, can be of kappa or lambda light chain type. Using combinatorial libraries of immunoglobulin heavy (H) and light (L) chain genes we have previously obtained a comprehensive panel of kappa human TPO autoantibodies. We have now begun to assess the lambda L chain repertoire of TPO autoantibodies. B cell cDNA, reverse transcribed from mRNA isolated from Graves' thyroid tissue, was used as template in the PCR to amplify immunoglobulin H and lambda L chain genes. PCR products were ligated into the pComb3 vector to obtain an IgG1/lambda library. Because the H chain genes were linked with the gene encoding a viral coat protein, the phage particles express on their surface immunoglobulins in the form of Fab. The library was enriched for TPO-specific Fab by 3 rounds of incubation with immobilized recombinant TPO ("biopanning"). After the third round, binding was confirmed using ^{125}I-labeled TPO and 24 positive clones were isolated. Sequencing the variable regions (V) revealed Fab with 4 different combinations of VH and VL genes:-

Clone	VH family	Germline	D region	VL family	Germline
WR1.103	VH3	har	a	VL3	III.1
WR1.104	VH1	hv1L1	b	VL2	DPL11
WR1.107	VH1	hv1L1	b	VL3	III.1
WR1.112	VH4	4.21	c	VL3	III.1

Remarkably, the VH genes encoding for these TPO Fabs are also used by other autoantibodies (rheumatoid factors and cold agglutinins). In addition, hv1L1 encodes the H chain of several kappa TPO Fab, although with different diversity (D) segments. The lambda III.1 gene encoding 3 TPO Fab also encodes a TPO Fab previously cloned from a different patient. In summary, these are the first data on a series of human TPO autoantibodies with lambda L chains. The data show a) VH genes encoding TPO autoantibodies are related to those encoding for other autoantibodies; b) TPO autoantibody VH and VLambda gene usage appears to be restricted.

10:30 A.M. 14. MOLECULAR CLONING AND FUNCTIONAL ANALYSIS OF THE THYROROPIN RECEPTOR FROM NON-THYROID TISSUE. T. Endo, K. Ohta, K. Haraguchi and T. Onaya, Third Department of Internal Medicine, University of Yamanashi Medical School, Tamaho, Yamanashi 409-38, Japan.

Whether or not thyrotropin receptor (TSH-R) is expressed and functions even in non-thyroid tissues is particularly important for the pathogenesis of extrathyroidal manifestations such as ophthalmopathy and dermopathy in Graves' disease. We previously demonstrated the existence of TSH-R immunoreactivity in rat retro-orbital and adipose tissues. However, expression level of TSH-R in non-thyroid tissues and its function remain to be clarified.

By Northern blot analysis, we found that rat adipose tissue highly expressed TSH-R mRNA, the level of which was almost comparable to that of the thyroid. In order to analyze the function of TSH-R from non-thyroid tissues, we then screened rat adipose tissue λgt11 cDNA library for TSH-R using ^{32}P-rat thyroid TSH-R cDNA as a probe. Among 10^6 plaques, we obtained four positive clones. Sequencing of these cDNAs has revealed that two of them (Fα and Fβ) contained the initiation and the termination codons. Comparison of Fα with thyroid TSH-R cDNA has revealed that Fα was almost identical to that of the thyroid except that 1041th and 1277th nucleotides were changed from A to G, and from C to T, respectively. In contrast, we found that Fβ contained unidentified 21 nucleotides between 467th and 468th residues of the thyroid TSH-R cDNA, so they encode additional 7 amino acids, CHRFSCR. However, when we prepared mRNA from the adipose tissue and transcribed into cDNA, we failed to amplify Fβ type of TSH-R cDNA by polymerase chain reaction, suggesting that there exists a very small amount of Fβ mRNA in the tissue. We then ligated Fα or Fβ cDNAs into pSG5, transfected them with pSV2-neo into CHO-K1 cells. TSH sitmulated cAMP formation in CHO-Fα cells, the manner of which was very similar to that in CHO cells transfected with thyroid TSH-R cDNA (CHO-thyroid TSH-R cells). In contrast, no increase of cAMP was observed in CHO-Fβ cells. IgG from patients with Graves' disease (n=4) stimulated cAMP formation only in CHO-Fα cells (1288% ~ 4582%). In addition, CHO-Fα cells and CHO-thyroid TSH-R cells possessed similar ^{125}I-TSH binding activity.

These results indicate that the adipose tissue expresses extremely high levels of TSH-R, the function of which is indistinguishable from that of the thyroid, and suggest that TSH-R autoantibody plays a role an important role in the pathogenesis of extrathyroidal manifestations in Graves' disease.

10:45 A.M. 55. **A GENOMIC TSH RECEPTOR POINT MUTATION IS HIGHLY ASSOCIATED WITH AUTOIMMUNE THYROID DISEASE IN FEMALES. R.M. Cuddihy, C.M. Dutton, and R.S. Bahn. Division of Endocrinology, Mayo Clinic and Foundation, Rochester, MN.**

We have described previously a genomic point mutation in the first position of codon 52 (C→A) of the human TSH receptor (TSH-r) gene that results in a threonine for proline amino acid substitution in the predicted peptide (JCEM 78: 256-260, 1994). In order to determine the potential clinical significance of this mutation, we tested a population of female patients with Graves' disease (n = 42), Hashimoto's thyroiditis (n = 21), and normal female controls (n = 58) for the presence of this mutation, as well as for the presence of HLA class II genes of the DRB1*03 (DR3) and DRB3 (DRw52) groups. TSH-r gene mutation screening was performed using Aci 1 restriction enzyme digestions of a 240 bp segment of PCR-amplified genomic DNA in the region of the mutation. All mutations found on screening were verified by direct DNA sequencing. HLA screening was accomplished using PCR with sequence specific and appropriate control primers. Multivariate analysis of the data using regression analysis (Spearman Rank Order Correlations) indicates that the TSH-r gene codon 52 mutation is highly associated with the presence of Graves' disease ($p = 0.010$) or Hashimoto's thyroiditis ($p < 0.010$) in females. As expected, we found a strong, independent, positive correlation with the DR3 haplotype and Graves disease ($p = < 0.001$). Hashimoto's thyroiditis was also associated with DR3 haplotypes ($p = 0.010$) in this population. No disease association with DRB3 status was found. We conclude that the codon 52 mutation is strongly associated with autoimmune thyroid disease in females. It is possible that the substitution of threonine (a neutral and polar amino acid) for proline (a neutral and hydrophobic amino acid) would result in a conformational change in the extracellular portion of the TSH-r. Such a change could alter the immunogenicity or function of the protein.

11:00 A.M. **88.** **NOVEL MUTATIONS OF TSH-RECEPTOR GENE IN THYROID HYPERFUNCTIONING ADENOMAS.**

S. Pannain, A. Porcellini, I. Ciullo, G. Amabile, V.E. Avvedimento, G.F. Fenzi. CEOS-CNR, Dipartimento di Biologia e Patologia Molecolare e Cellulare; Cattedra di Endocrinologia, II Facoltà di Medicina, via S. Pansini 5, 80131 Naples, Italy; Dipartimento di Medicina Sperimentale, Facoltà di Medicina di Catanzaro, 3 via T. Campanella, Catanzaro, Italy

Hyperfunctioning thyroid adenomas are clonal neoplasms with the intrinsic capacity of growing and differentiate independently of thyroid-stimulating hormone (TSH). We analysed the RNA encoding thyrotropin receptor of 11 adenomas obtained by fine needle aspiration biopsy (FNAB) and found 7 mutants in three aminoacids (residues 631-632-633) located in the six transmembrane domain of the receptor, adjacent to the third intracitoplasmic loop, where other mutations have been found (J. Parma et al. Nature 1993; 365: 649-651). These mutations were somatic and present in one allele. To test the biological effects we inserted these mutations in wild type receptor. We transiently transfected mouse 3T3 fibroblasts with plasmid constructs expressing wild-type (TSH-R WT) or mutated receptor at residues 632 (TSH-R M632) and 631 (TSH-R M631) respectively. pSV-LacZ DNA and CRE-CAT were included in the transfection. LacZ activity was used to normalise for differences in transfection efficiency. The activation of CRE driven transcription represents a very specific assay of cAMP dependent response (M. Hagiwara et al. Mol. Cell. Biol. 1993 13:4852-9). Basal and stimulated (TSH 1 mU/ml or forskolin 25uM) CAT activity was measured and the values, expressed as % of conversion from non acetylated to acetylated chloramfenicol, are shown below:

Control	basal 0.5±0.1	+TSH 0.4±0.1	+FSK 7.0±0.5
TSH-R WT	basal 2.0±0.5	+TSH 11±1	+FSK 6.0±0.7
TSH-R M632	basal 5.5±1	+TSH 10±1.21	+FSK 6.5±0.6

We have also data indicating that mutation 631 (Phe->Cys) produced the same biological effects. Transcription of CRE in 3T3 cells was induced only by forskolin since these cells did not express TSH-R. The expression of TSH-R WT conferred TSH induction of the CRE promoter. The expression of the mutated receptor increased significantly the basal cAMP dependent transcription.

Our data suggest that these mutations have a biological effect on cAMP-PKA transduction pathway. The increased basal expression of cAMP-induced transcription may be the ultimate cause of hyperfunctioning thyroid adenomas.

11:15 A.M. **89.** **FUNCTIONAL CHARACTERIZATION OF TWO NEW SOMATIC MUTATIONS IN THE THYROTROPIN RECEPTOR IN HYPERFUNCTIONING THYROID ADENOMAS**

R. Paschke, M. Tonacchera, J. Dumont, G. Vassart
Service de Génétique Médicale and IRIBHN Université Libre de Bruxelles, Bruxelles, Belgique

Recently two somatic mutations of the Thyrotropin Receptor (TSHr) in hyperfunctioning thyroid adenomas have been described. These TSHr mutations confer constitutive activation of adenylyl cyclase when tested by transfection in COS cells. We have identified 2 new mutations from hyperfunctioning thyroid adenomas. At position 623 Alanine was changed to Valine and at position 632 Threonine was changed to Isoleucine. When transiently expressed in COS cells both mutations caused an increase in the constitutive activation of adenylyl cyclase as compared to wild type receptors. Stimulation with increasing doses of TSH (0.03, 0.3, 1, 10, 100 mU/ml) showed a lower EC 50 for the cAMP response for 632I as compared to 623V. The inositol phosphate response was increased in comparison to the wild type with 10 and 100 mU/ml for both mutations. These differences were not due to an increased expression of mutated TSHr in comparison to the wild type as evidenced by binding of labeled TSH to the transfected COS cells. Activating mutations in the TSH receptor can therefore differ with respect to their response to TSH. Contrary to the situation with the alpha 1b adrenergic receptor, several but not all amino acid substitutions at position 623 result in constitutive adenylyl cyclase stimulation and hyperthyroidism. Together with the constitutively activating mutation in the LH receptor, which is homologous to position 633 in the TSHr and further mutations at position 631 of the TSHr as evidenced in sporadic toxic thyroid hyperplasia and other toxic adenomas, the mutation at position 632 demonstrates the importance of this segment of transmembrane helix VI in the activation of the TSHr.

11:30 A.M. **122.** **HUMAN TSH RECEPTOR VARIANT 1.3 mRNA IN HUMAN EXTRAOCULAR MUSCLES.**

M. Nakashima, D. L. Kendler*, J. Rootman* and P. Graves. Departments of Medicine, Mt. Sinai School of Medicine, NY, NY, and University of British Columbia*, Vancouver, BC.

Controversy exists as to the expression of the human TSH receptor (hTSHR) by muscle cells and fibroblasts. Since the hTSHR is the major antigen in Graves' disease, the presence of potentially cross-reacting antigen in non-thyroidal tissues is of major import. Extraocular muscle (EOM) biopsies from patients with either Graves' orbitopathy or non-autoimmune ophthalmic disease were examined for the expression of human TSH receptor (hTSHR) mRNA and its variants by RT-PCR. Using a forward oligonucleotide primer comprising nucleotides 106-123 of the hTSHR-ecd and a reverse primer complementary to nucleotides 697-714, present in the unique 3' region of the hTSHR 1.3 kd variant (v1.3) (BBRC 1992, 187:1135), we were able to detect appropriately sized (609 bp) PCR fragments in 2 of 6 apparently normal EOM samples and in all 3 EOM samples from patients with Graves' orbitopathy. Similar fragments were seen with thyroid control cDNA but not in a normal dermal fibroblast line nor in fetal brain and liver. These data were confirmed by Southern blot analysis using an internal oligonucleotide probe complementary to nucleotides 526-548 of the normal hTSHR. Further control studies using amplimers to detect human thyroglobulin cDNA were negative in all samples except normal thyroid controls. Using amplimers for the transmembrane (TM) region of the hTSHR, an appropriately sized fragment was seen in a wide variety of these tissue samples. However, these fragments were subsequently shown to be secondary to minor contamination of the tissue mRNA samples with genomic DNA. Since the TM region is non-intronic, the same size PCR fragments accumulate secondary to the presence of either mRNA or genomic DNA. In contrast, genomic DNA could not have influenced the generation of PCR fragments specific for hTSHRv1.3 mRNA since the DNA encoding this mRNA region contains several intronic sequences. These data indicate that variant forms of the hTSHR mRNA are expressed in human muscle cells, in particular EOM, and may be involved in their autoimmune dysfunction.

11:45 A.M. **132.** **Congenital non-autoimmune hyperthyroidism caused by a mutation in the thyrotropin (TSH) receptor gene**

P. Kopp, J. van Sande, J. Parma, L. Duprez, K. Zuppinger, J.L. Jameson, G. Vassart.
Department of Internal Medicine and Laboratory of Endocrinology (P.K.), Clinic of Pediatrics (K.Z.), Inselspital, University of Berne, Switzerland; Center for Endocrinology, Northwestern University, Chicago, USA (P.K., J.L.J.); Institut de Recherche Interdisciplinaire, Faculty of Medicine, University of Brussels, Belgium (J.S., J.P., L.D., G.V.)

Congenital hyperthyroidism is usually a transient disease caused by maternal-to-fetal transfer of thyroid-stimulating antibodies from a mother with a history of autoimmune thyroid disease. In rare cases, neonates with persistent non-autoimmune hyperthyroidism of unknown etiology have been observed. We describe a patient with congenital hyperthyroidism, growth retardation and attention deficit, but without evidence of autoimmunity. Thyroidectomy was performed at $8^{8/12}$ years of age because of rapid growth of a multinodular goiter and recalcitrant hyperthyroidism. Although only a few grams of thyroid tissue remained after surgery, radioiodine treatment was required to control hyperthyroidism.

The TSH receptor gene was examined for mutations by direct sequencing of DNA from leukocytes, nodular and non-nodular thyroid tissue. DNA-sequencing revealed a heterozygous mutation in the TSH receptor gene resulting in an amino acid substitution (Phe^{631} [TTC] → Leu [CTC]) in the sixth transmembrane segment of the receptor. The mutation, not present in the parents or his sister, was detected in all analyzed tissues, consistent with a new germ line mutation. In two other patients with hyperfunctioning thyroid adenomas, an identical amino acid substitution caused by a transversion (Phe^{631} [TTC] → Leu [TTA]) was found in the neoplastic tissue, but not in the adjacent normal tissue.

Binding of TSH to the mutated receptor was similar to the wild type receptor. Transfection of the mutant receptor revealed a marked increase in constitutive activity leading to stimulation of adenylyl cyclase in the absence of TSH.

The identified de novo germ line mutation in the TSH receptor gene is the underlying defect in this patient with congenital goiter and hyperthyroidism whereas somatic mutations at this locus lead to the development of hyperfunctioning thyroid adenomas.

3. REGULATION OF MOTILITY AND ADHESION OF FOLLICULAR AND PAPILLARY THYROID CANCER CELLS: A POSSIBLE MECHANISM FOR INHIBITION OF INVASION BY TRANSFORMING GROWTH FACTOR-ß1. Th.Hölting, Q.Y.Duh, A.E.Siperstein, Ch. Herfarth and O.H.Clark, Surgical Departments of the Universities of Heidelberg, Germany and San Francisco, CA.

Introduction: Genetic aberrations affecting growth factors or their receptors are found frequently in the development of thyroid cancer. We have reported, that transforming growth factor ß1 (TGF ß1) inhibits invasion and growth of follicular (FTC) and papillary thyroid cancer cells (PTC) *in vitro*. Therefore, we investigated whether TGF ß1 affects tumor cell motility and adhesion in 3 FTC cell lines from 1 patient (FTC133-primary; FTC236-lymph node- and FTC238-lung metastasis) and 2 PTC cell lines from 2 patients (PTC-UC1, PTC-UC3- primary tumors). **Methods:** Tumor cells were grown in serum-free medium. Using the MTT-assay, we studied the effect of TGF ß1 on attachment of tumor cells to major components of the extracellular matrix (ECM), collagen IV, collagen I, laminin, fibronectin and Matrigel and on chemotactic migration (penetration of 8 um pore polycarbonate membranes versus ECM). **Results:** Adhesion of all FTC and PTC was increased when wells were coated with collagen IV (FTC133 by 48%; PTC-UC1 by 39% ($p<0.01$)) and fibronectin (FTC133 by 30%; PTC-UC1 by 24% ($p<0.02$)). Laminin, collagen I and Matrigel did not significantly affect adhesion. TGF ß1 (10 ng/ml) increased adhesion of FTC133 to uncoated wells by 14%, to collagen IV by another 21% and to fibronectin by another 17% (PTC-UC1: 12%, 18% and 23%, respectively; $p<0.01$). Collagen IV and fibronectin also stimulated chemotactic migration of tumor cells. After 8 hours, 28% more FTC133- and PTC-UC1 cells had migrated through the membrane towards collagen IV ($p<0.02$). Fibronectin stimulated tumor cell motility by 22%, respectively 34% ($p<0.03$). Again, collagen I, laminin and Matrigel had no significant effect. TGF ß1 stimulated chemotactic migration of FTC133 by 39% (collagen IV) and 33% (laminin) and that of PTC-UC1 by 36% (collagen IV) and 42% (laminin) ($p<0.03$). **Conclusions:** We could previously demonstrate that TGF ß1 reduces invasion and protease activity of follicular and papillary thyroid cancer cells. The present study suggests that a possible mechanism may be its influence on tumor cell motility and adhesion.

4. DOUBLE POINT MUTATIONS IN THE PROMOTER REGION OF THE THYROGLOBULIN GENE IN A PATIENT WITH CONGENITAL GOITER.

A point mutation in the coding region of the thyroglobulin (TG) gene that resulted in alternative splicing has been reported in two cases of congenital goiter. We describe the third patient with congenital goiter who suffered from point mutations in the promoter region of the TG gene. A 43-year-old female patient was operated 18 years ago because of thyroid struma that she noticed from the childhood. However, since her remaining thyroid gland continued growing, she visited Dokkyo University Hospital again. Free T3 and TSH were within the normal limit though free T4 was low. Autoantibodies against the thyroid (TBII, TGHA, MCHA) were not detected. Radiological studies using [123]I did not show any defects in iodide uptake and organification. Her serum TG level was below the detection limit. Histological examination of her re-operated thyroid tissue demonstrated that colloid substance inside the follicles was remarkably reduced. A part of the removed tissue was homogenized in PBS and total protein, TG, T4, and T3 in the supernatant were measured. TG (1.38% of total protein) was reduced compared to an adenoma tissue (19.91%). T4/TG (3.35) was almost the same as that of the adenoma (3.09). T3/TG (0.30) was a half of that of the adenoma (0.74). Analysis by native-PAGE and Western blot demonstrated that TG in this patient was reduced (<1/10) compared to the adenoma. We then quantitated mRNA's by RT-PCR using probes from 592 (exon 5) to 1184 (exon 9) for TG and from 917 (exon 9) to 1414 (exon 10) for TSH receptor (TSHR). TG mRNA level was reduced although TSHR mRNA level was the same as that in the adenoma patient and a normal subject. The Northern blot analysis also showed that the TG mRNA level was reduced, but the size of the TG mRNA was the same as that of the normal TG. Since expression of the TG gene was reduced in this patient we sequenced the promoter region of the TG gene from -279 to +100 by the PCR direct sequencing method. Double point mutations (TA to AT) at -174 and -173 in the promoter region of the TG gene were detected. These mutations were within the region homologous to LBP (lipopolysaccharide binding protein)-1-RS (responsive sequence). In summary, we reported a new TG mutation in the promoter region of the TG gene in a patient with congenital goiter which resulted reduced expression of the TG gene.

8. INCREASED HEPATIC INNER RING DEIODINATING TYPE III ACTIVITY AFTER PARTIAL FEED RESTRICTION IN THE RAT AND THE CHICKEN.

V.M. Darras, E. Dewil, M. Cokelaere*, S. Arnout, E.R. Kühn and E. Decuypere. Leuven Poultry Research Group, KUL, 3000 Leuven, Belgium and * KULAK, 8500 Kortrijk, Belgium.

Complete fasting is known to decrease both plasma 3,3',5-triiodothyronine (T_3) and thyroxine (T_4) levels as well as hepatic outer ring deiodinating type I (ORD-I) activity in rats. The effects of less severe feed restriction have, however, been investigated less thoroughly. We therefore submitted rats and chickens to a partial feed restriction and measured plasma T_3 and T_4 as well as the *in vitro* hepatic ORD-I and inner ring deiodinating type III (IRD-III) activity. Adult male rats were fed during 4 weeks respectively 45 or 60% of the normal *ad libitum* (*ad lib*) feed intake, resulting in respectively 30 and 27% body weight reduction compared to the *ad lib* fed group. Plasma T_3 levels in both restricted groups were decreased but plasma T_4 levels remained unchanged as well as the hepatic ORD-I activity. The hepatic IRD-III activity showed, however, a more than 4-fold increase. Young growing chickens submitted for 4 or 5 weeks to a feed restriction to 40% of the *ad lib* intake, showed a decrease in plasma T_3 compared to the control group while plasma T_4 was increased. The hepatic ORD-I activity was unchanged while IRD-III activity was increased 4-fold in one experiment and 10-fold in another one. The body weight reduction after 4 weeks of treatment was ±40% compared to the *ad lib* fed group. We also studied the effects of short-term feed restriction on peripheral thyroid hormone metabolism in young chickens. Samples were taken after 1, 3 and 7 days of restriction to 60% of the *ad lib* intake. Here too, the treatment resulted in a decrease in plasma T_3 and an increase in hepatic IRD-III activity, while plasma T_4 and hepatic ORD-I were unaffected. We can conclude that the increase in hepatic IRD-III activity found in rats and chickens after partial feed restriction may contribute to the decrease in circulating T_3 levels. Studies are in progress to compare the effect of a short-term partial feed restriction *versus* complete fasting on hepatic deiodinating activity in both species.

10. REGIONAL ANESTHESIA FOR THYROIDECTOMY AND PARATHYROIDECTOMY. R. Kulkarni, L.E. Braverman, and N. Patwardhan, Depts. Anesthesiol, Medicine, and Surgery, Univ. of Mass. Med. Ctr., Worcester, MA

Regional anesthesia for thyroid/parathyroid surgery has rarely been employed. It may be preferred in patients (pts) with cardiac or pulmonary disease. Between October, 1993 and April, 1994, 9 pts with cold nodules, 2 pts with Graves' disease, and 3 pts with hyperparathyroidism were operated on under regional anesthesia. The thyroid procedures were: total thyroidectomy (TX) (3), near total TX (4), subtotal TX (2) and 2 lobectomies. Bilateral superficial cervical plexus block was carried out by injecting 20-25 ml of Bupivacaine (0.375 to 0.5%) with epinephrine (1:200,000) along the posterior border of the sternocleidomastoid muscles followed by bilateral deep cervical plexus block with the same anesthetic at C2, C3, and C4 transverse processes bilaterally. One pt received only a superficial plexus block because of abnormal coagulation studies. Supplemental sedatives included intravenous midazolam, fentanyl, propofol, or ketamine or supplemental nitrous oxide by mask in amounts necessary to keep the patient comfortable. Using incentive spirometry, Forced Vital Capacity (FVC) was measured before and after the block and in the recovery room. Oxygen was administered via face mask or nasal canula. Eleven pts tolerated the procedure well with supplemental sedation (six remained awake and communicative and five remained sedated), 2 pts required volatile anesthetic via mask, and one pt required intubation secondary to vigorous coughing. None of the pts exhibited respiratory or cardiac complications intraoperatively. Five pts required local anesthesia at the surgical site with 1% Lidocaine + 0.25% Bupivacaine (0.5 to 14 ml). Surgical planes were easy to dissect with minimal bleeding. All pts were fully awake at the end of the procedure and transferred to the stretcher independently. Length of stay in the recovery room ranged from 60 to 90 min. Upon arrival in the recovery room, 7 pts had no pain and seven had minimal discomfort at the incision site. Nine pts reported pain upon swallowing. None had hoarseness or post-operative bleeding. Only one pt had mild nausea. Two pts had minimal bilateral apical pneumothorax which did not require intervention. Most pts were ready for discharge the same day but chose to stay overnight. One pt who had a near total TX was discharged home the same day. FVC measured in 7 pts before and after bilateral deep cervical plexus block did not change. Conclusions: Regional anesthesia decreased post-operative incisional and nasopharyngeal pain, had a lower incidence of nausea and faster recovery, and was well received by the pts compared to patients receiving general anesthesia. Since pts could be discharged from the hospital on the same day, thyroid/parathyroid surgery might be considered for day surgery.

18. PREPARATION AND CHARACTERIZATION OF MONOCLONAL ANTI-THYROTROPIN RECEPTOR ANTIBODIES (TSH-R Ab) OBTAINED FROM PERIPHERAL LYMPHOCYTES OF HYPOTHYROID PATIENTS WITH PRIMARY MYXEDMA

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Anti-thyrotropin receptor antibodies (TSH-R Ab), detected in sera from some patients with primary myxedema, are considered to induce hypothyroidism. These are termed "blocking type" TSH-R Ab (TSH-R BAb), since they inhibit adenylate cyclase stimulation by TSH on thyrocytes or nonthyroidal cells transfected with TSH-R cDNA.

We prepared monoclonal TSH-R BAb and characterized them. Peripheral lymphocytes from three patients with primary hypothyroidism having potent TSH-R BAb were transformed by EB virus and the culture supernatants were screened by thyrotropin binding inhibitor immunoglobulin (TBII) assay. Twenty positive and 7 negative lymphocyte clones were obtained; their monoclonality was confirmed by Southern blot analysis, using an immunoglobulin JH probe. These monoclonal antibodies were then tested for TSH-R BAb activity. TSH-R BAb activity was 24.1%-58.5% (normal range <24%) in all 20 TBII positive clones and was also detected in 2 of 7 TBII-negative clones. ELISA assay showed that the immunoglobulin isotypes of these clones with TBII and/or TSH-R BAb activity were IgG in 8 and IgM in 14. Another ELISA assay and Southern blot analysis of the light chains revealed that 13 clones had κ chains, while the light chains could not be determined in the other 9 clones. To summarize: 1) we obtained 22 clones that produced monoclonal TSH-R BAb, including 8 IgG type clones. 2) The clones exhibited dominant usage of the κ chain. 3) Although all TBII clones had TSH-R BAb activity, their TBII and TSH-R BAb activities were not significantly correlated each other, and two TSH-R BAb clones did not show TBII activity.

21. THE SELENIUM ANALOG OF PROPYLTHIOURACIL; MEASUREMENT OF ITS INHIBITORY EFFECT ON TYPE I 5'-DEIODINASE AND OF ITS ANTITHYROID ACTIVITY. Alvin Taurog, Martha L. Dorris, Lynn J. Guziec, and Frank S. Guziec, Jr. Univ. of Texas Southwestern Medical Center, Dallas, TX 75235 and New Mexico State Univ., Las Cruces, NM 88003.

Inhibition of type I iodothyronine deiodinase (ID-1) by 6-propyl-2-thiouracil (PTU) was originally attributed to formation of a mixed disulfide between PTU and a cysteine residue at the active site. However, it has recently been demonstrated that ID-1 contains selenocysteine, rather than cysteine, at the active site (Berry and Larsen, Endoc. Rev. 12:207, 1992). It seemed possible, therefore, that the selenium analog of PTU (PSeU) might be a more active inhibitor of ID-1 than PTU itself, as formation of the Se-Se bond with the enzyme would be expected to occur more readily than formation of the Se-S bond. We developed a procedure for the synthesis of PSeU and compared PTU and PSeU for inhibition of ID-1, and also for antithyroid activity. The incubation system for measurement of ID-1 activity contained 0.5μM ^{125}I-rT_3, 5mM dithiothreitol, 2mM EDTA, and 25μg/ml rat liver microsomal protein in phosphate buffer, pH 7.2. After 20 min of incubation at 37°C, organic ^{125}I was precipitated by addition of serum and cold trichloroacetic acid. ^{125}I-iodide production was measured by counting an aliquot of the supernatant, with correction for a blank. PTU and PSeU were tested at concentrations of 0.1, 0.3, 1, and 3μM in 4 separate experiments. Both were potent inhibitors of ID-1, and we observed no essential difference in potency between the two drugs. Our results differ from those of Visser et al., who reported (BBRC 189:13621, 1992) that PSeU was about twice as potent as PTU as an inhibitor of ID-1. We also compared PTU and PSeU for antithyroid activity, in vitro and in vivo. As an inhibitor of TPO-catalyzed iodination of BSA, PSeU was almost as active as PTU. However, when injected into rats in the dose range 0.1-1μmol/100g bw, PSeU had little or no effect on thyroidal organic ^{125}I formation. PTU, on the other hand, at 0.3μmol/100g bw reduced organic ^{125}I formation to 7% of the control. Based on our previous studies with the selenium analog of MMI (submitted), the very low antithyroid activity of PSeU in vivo is likely due to low concentration of the drug by the thyroid. PTU and PSeU were also compared for inhibition of TPO-catalyzed guaiacol oxidation. Very little difference in potency between the two drugs was observed. Our results differ from those of Aboul-Enein et al., who reported that PSeU is about 5 times more active than PTU in this assay (J. Enz. Inhib. 7:147, 1993). Our previous results with the selenide analog of MMI, together with the results of this study, lead us to conclude that replacement of the sulfur in PTU or MMI with selenium, has relatively little effect on inhibition of ID-1 or of TPO-catalyzed reactions in vitro, but greatly lowers antithyroid activity in vivo. Supported by Boots Pharmaceuticals.

32. INTERLEUKIN-6 REGULATES TYPE 1 5'DEIODINASE IN RAT LIVER CELLS

P H Davies, M C Sheppard and J A Franklyn; Department of Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham, B15 2TH, United Kingdom.

Type 1 5'deiodinase enzyme (5'DI) is essential for the peripheral conversion of T4 to T3 and is therefore a major determinant of tissue thyroid hormone action. Decreased 5'DI activity is implicated in the characteristic changes in circulating thyroid hormones seen in non-thyroidal illnesses (NTI). Recently it has been proposed that inflammatory cytokines in NTI may influence 5'DI expression. We have studied the effects of recombinant murine interleukin-6 (mIL-6) upon 5'DI expression in rat Φ_1 hepatoma cells, measuring enzyme activity by conversion assay and mRNA by Northern blot analysis. Φ_1 cells were grown to confluence in MEM/10% fetal calf serum/1% essential (EAA) and non-essential amino acids (NEAA) before being washed and maintained in MEM/1%EAA+NEAA supplemented with insulin/transferrin/sodium selenite for 24 hours. Medium was substituted with the addition of mIL-6 2.5, 5, or 10μg/l or diluent and cells incubated for 48 hours. In duplicate experiments either total RNA was extracted and subjected to Northern blot analysis with a cRNA probe for 5'DI (courtesy of Prof. Larsen, Boston), or cells were scraped into ice cold PBS and pelleted before homogenisation with a Teflon pestle; homogenates were centrifuged and supernatants assayed for 5'DI activity in the presence of [^{125}I]rT3 and 10mM DTT. There was a dose dependent increase in 5'DI activity with mIL-6 treatment over control: control 100 ± 11%; 2.5μg/l 203 ± 11%**; 5μg/l 254 ± 24%**; 10μg/l 272 ± 25%** (mean ± SEM; n=6; $p<0.01^{**}$). 5'DI mRNA abundance (signal relative to 18s rRNA) was not significantly changed by mIL-6: control 100 ± 15%; 2.5μg/l 80 ± 12%; 5μg/l 100 ± 11%; 10μg/l 68 ± 9% (n=6; mean ± SEM; $p>0.05$=NS). Our data suggest that mIL-6 stimulates 5'DI activity in the absence of an effect upon pretranslational expression in Φ_1 cells. This finding is contrary to the hypothesis that IL-6 is responsible for the decrease in 5'DI activity in NTI. The precise regulatory role of IL-6 and other inflammatory cytokines remains to be explored.

40. INTEGRIN EXPRESSION IN HUMAN THYROID CELL LINES AND EFFECT OF N-*ras* OVEREXPRESSION. M. Vitale, M. Illario, A. Casamassima, V. Bassi, S. De Riu, C. Sandomenico, G. Rossi and GF. Fenzi, Dep. Biologia e Patologia Cellulare e Molecolare, Dep. Endocrinologia e Oncologia Molecolare e Clinica. Università Federico II, Naples, Italy.

In the integrin superfamily, the very late activation antigens (VLA, β1 integrins) are those most involved in cell-extracellular matrix interaction. This group of 6 αβ1 heterodimers (α1β1 to α6β1) is widely distributed on the cell surface and their expression is tissue type related. We have previously shown that in normal thyroid glands the majority of cells express only α3β1; a minor subset, much more represented in multinodular goiter, besides α3β1 also expresses α1β1, α5β1 and α6β1 while α2β1 is only occasionally expressed and α4β1 is never expressed (J.Clin.Endocrinol.Metab.76:1575-79). In benign and malignant thyroid tumors all β1 integrins were expressed when assessed by flow cytofluorometry (manuscript in preparation). In this study we assessed by flow cytofluorometry and specific monoclonal antibodies, the β1 integrin expression in some thyroid carcinoma cell lines (TPC1, NPA, WRO) and in a cell line obtained by SV40 infection of fetal thyroid cells (TAD-2, kindly donated by T. Davies). In the 3 tumor cell lines all α subunits were expressed and the β1 integrin profile was comparable with that observed in thyroid tumor specimens. α4β1 was also detectable with a variable intensity (TPC1=3.1, NPA=10.7, WRO=3.3, control antibody=1). In TAD-2 cells all α subunits were expressed with the exception of α4. A TAD-2 mutant cell line has been generated by transfecting a plasmid carrying the N-*ras* p21 encoding domain and the neomicin selectable marker. In G418 resistant clones (TAD*ras*) cell shape was more rounded and proliferation rate was higher than in wild type TAD-2. No changes of integrin expression were observed, α4 was not expressed and as in TAD-2, TAD*ras* were not able to growth in soft agar neither in nude mouse.

Conclusions: All subunits of β1 family of integrins are expressed by thyroid tumor cell lines TPC1, NPA and WRO. Immortalized fetal thyroid cell line TAD-2 and N-*ras* transfected TAD*ras* did not express α4 neither were able to growth in soft agar or in nude mouse. These results support the idea that *ras* overexpression dose not induce malignant phenotype in human thyroid cells.

41. RETINOIC ACID AND PROINFLAMMATORY CYTOKINES INDUCE UP-REGULATION OF CELLULAR ICAM-1 MOLECULE AND PARALLEL SHEDDING OF SOLUBLE ICAM-1 FORM IN HUMAN THYROID CELL LINES.

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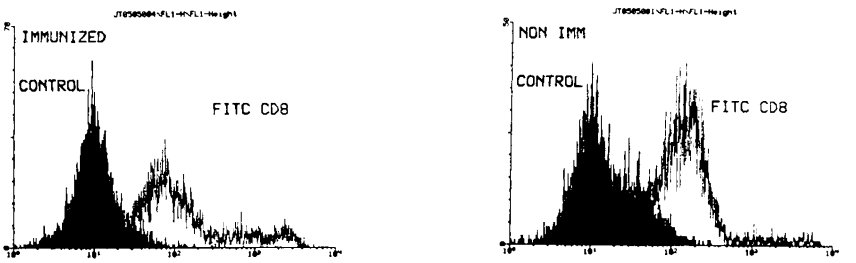
Recently, we have demonstrated, using flow cytometry and Northern blot analysis, that Retinoic Acid (RA) alone and in combined treatment with proinflammatory cytokines as IFN-γ and TNF-α induce up-regulation of surface Intercellular adhesion molecule-1 (cICAM-1) and specific mRNA levels in human thyroid carcinoma cell lines. The aim of our study was to investigate shedding of soluble ICAM-1 molecule (sICAM-1) by RA and/or proinflammatory cytokines as IFN-γ and TNF-α. Two different human thyroid papillary carcinoma cell lines (TPC-1 and NPA) and an anaplastic cell line (ARO) were cultured in RPMI 1640/ 10% FCS/ L-Glutamine 2 mM with RA 10 μM or IFN-γ 200 U/ml or TNF-α 200 U/ml alone and in combined treatment. At 72 h we investigated cICAM-1 molecule with flow cytometry analysis and shedding of soluble molecule in the spent medium with a sICAM ELISA test Kit (Bender System). The correlation between cICAM and sICAM was studied with linear regression analysis.
RESULTS: At 72 h RA, IFN-γ and TNF-α induced a cICAM-1 increase than control. cICAM-1 expression was higher in cell cultures treated with RA and each cytokine in combined treatment compared to each agent alone. sICAM-1 was detected in spent media of all investigated lines, release of sICAM-1 correlated with cellular expression (NPA $R=0.97$, $P<0.001$; ARO $R=0.92$, $P<0.01$; TPC-1 $R=0.96$, $P<0.001$).
CONCLUSIONS: RA or proinflammatory cytokines alone and in combined treatment induce ICAM-1 up-regulation as surface and soluble molecule in human thyroid cell line. Then, increase of cICAM could be balanced by increasing shedding of molecule functionally active and able to reduce or suppress interactions between immune system and tumoral cells.

43. **PHENOTYPIC CHARACTERIZATION OF THYROGLOBULIN-SPECIFIC T CELL LINES DERIVED FROM THYROIDITIS-PRONE BB/WOR RATS.** E. M. Allen, J.N. Thupari, Baltimore VAMC and University of Maryland Medical Center.

NB line LT-prone BB/Wor rats develop spontaneous autoimmune lymphocytic thyroiditis (LT) and anti-thyroglobulin (Tg) antibodies with nearly 100% incidence by 120 days of age. Therefore, it is reasonable to assume these animals have autoreactive T lymphocytes that can be isolated and characterized. Forty day old NB line BB/Wor rats were immunized with 0.5 mg rat Tg in complete Freund's adjuvant. T lymphocytes were isolated from the spleen and maintained in longterm culture by intermittent feeding with rat Tg in the presence of 10% Il-2. The lymphocyte response to rat Tg was assessed in proliferation assay by measuring ^3H thymidine uptake. Maximal T cell activation occurred with 0.25 ug / ml Tg (SI = 3.4). Using similar conditions, splenic T cells were also isolated from 110 day old *nonimmunized* LT-prone rats and maintained in longterm culture. This cell line also showed maximal T cell activation with 0.25 ug / ml Tg (SI = 3.2). These animals' sera had positive anti-Tg antibodies (ELISA), but histologically, their thyroids had minimal lymphocytic infiltration. Neutralization data showed that T cell responses were attenuated by serial dilutions of murine monoclonal anti-Tg antibody (Ig G_1) ($p < 0.05$ vs controls). FACS analysis of both cell lines demonstrated CD_8 lymphocyte predominance (immunized: CD_8 85%, nonimmunized: CD_8 83%).



Conclusion - These data suggest that Tg - specific T cell lines from LT-prone BB/Wor rats are CD_8 predominant. These cells may have role in the pathogenesis of LT.

45. INHIBITION OF TSH-STIMULATED IODIDE ORGANIFICATION IN VITRO FOLLOWING DIACYLGLYCEROL KINASE INHIBITION. J. Ginsberg, W. Matowe and K. Chen, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada.

Although phorbol esters inhibit TSH-stimulated iodide organification (TSH-IO) in vitro, phorbol-mediated protein kinase C (PKC) activation differs from physiological activation by endogenous diacylglycerol (DG). To examine the role of endogenous PKC activation, we determined the effects of the DG kinase inhibitor, R59022 on TSH-IO in porcine thyroid cells. Inhibition of DG kinase would lead to an accumulation of DG resulting in enhanced PKC activation. Following 45 min. exposure, TSH-IO was inhibited by 34% ($p<.01$) and 38% ($p<.001$), respectively when 30μM and 50μM R59022 were utilized. No effect on basal IO was observed. Similar results were seen following long-term exposure (12 hr.) to identical concentrations of R59022. To ensure that PKC activation occurred as a consequence of R59022 exposure, PKC isoforms were measured by Western blotting and relative densitometry following R59022 exposure (50μM, 45 min.). Only α and ζ PKC isoforms were detected. Compared to controls, membrane associated α- and ζ-PKC isoforms increased by 17% and 19%, respectively ($p<.05$). To determine whether R59022-mediated inhibition of TSH-IO was due to PKC activation, we studied the effects of pre-incubation with the PKC inhibitor, chelerythrine. In the presence of R59022 50μM alone, TSH-IO was inhibited by 90.4%. However, with R59022 and chelerythrine, TSH-IO recovered by 535% to 51.5% of control values ($p<.001$).

These data show that in porcine thyroid cells: 1. Inhibition of TSH-IO occurs following short and long-term exposure to the DG kinase inhibitor R59022, 2. α and ζ-PKC activation occurs following R59022 exposure, 3. The PKC inhibitor, chelerythrine, causes partial recovery of TSH-IO in the presence of R59022. These results indicate that DG-stimulated α and ζ-PKC acts as negative modulators of TSH-IO in vitro.

56. EARLY CELLULAR EVENTS IN THE THYROID AFTER EXPOSURE TO IODINE. N. Bagchi, T.R. Brown, P. Anand and R.S. Sundick. Departments of Internal Medicine and Immunology and Microbiology, Wayne State University, Detroit, MI 48201

Iodine is known to exacerbate autoimmune thyroiditis and also cause thyroid cell injury. We have studied the relationship between these effects since the release of thyroid antigens following injury may be causal in the induction of autoimmune thyroiditis. Obese strain (OS) chickens, which develop thyroiditis spontaneously, and normal strain chickens were placed from hatching on an iodine depletion regimen. Thyroxine was added to the diet to maintain euthyroidism. Three week old chickens were injected ip with 10-1000 μg NaI at 24h intervals for up to 7 days, then examined for thyroid histology. Iodine caused thyroid infiltration only in OS chickens. Significant infiltration was seen 24h after NaI injection, and was maximal from 72h to at least 7 days. The incidence of infiltration was maximal at 20 μg iodine and did not increase with higher dosage. The infilitrating cells were mononuclear. Polymorphonuclear cells were not observed. Upon immunohistologic analysis with specific monoclonal antibodies, the infiltrate appeared to consist of CD8, CD4, B cells and macrophages in the ratio 40:20:22:17. Thyroid follicles were intact with no evidence of cellular damage by light microscopy. Electron microscopy, however, revealed extensive subcellular changes at 250 μg iodine in both OS and normal strains. These changes were minimal at 20 μg iodine. Summary: 1) iodine causes acute infiltration only in the genetically susceptible strain. 2) The infiltration appeared to be immune mediated. 3) The infiltration occurred even when thyroid cell injury was minimal. We conclude that thyroid cell injury may not necessarily precede the development of autoimmune thyroiditis. Supported by a grant from Boots Pharmaceuticals and NIH grant DK 44473.

61. THE KINETICS OF RAT TYPE III IODOTHYRONINE DEIODINASE PRESENT IN THE PLACENTA IS QUITE DIFFERENT FROM THAT IN THE CEREBRAL CORTEX. K. Mori, K. Yoshida, H. Fukazawa, Y. Kiso, N. Sayama, K. Kikuchi, Y. Aizawa and K. Abe, Tohoku University School of Medicine, Sendai, Japan.

Type III iodothyronine deiodinase (5-D) in the placenta is a phospholipid-requiring enzyme and independent of the thyroid state whereas that in the brain is increased in hyperthyroidism and decreased in hypothyroidism. The kinetics of 5-D in the placenta and brain and the role of phospholipids in the enzyme activity was determined in the rat. Pregnant Sprague-Dawley rats were given either vehicle (control; C) or T_4 (15 μg/100g bw/day; hyperthyroid; H) from the 14th day to the 21st day of gestation. Mitochondrial-microsomal fractions of the placenta and brain were prepared and used as the source of T_4 5-D. Placental T_4 5-D activity in the hyperthyroid group was increased when determined at 13 nM T_4 whereas it was not significantly different from the control value when assayed at 1.3 μM T_4. In contrast, T_4 5-D in the brain was significantly increased in the hyperthyroid group regardless of the substrate concentration. Hyperthyroid rats had decreased Km for placental 5-D and increased Vmax for brain 5-D. Then we performed CM-Sephadex chromatography of solubilized placental and brain microsomes to determine whether phospholipids caused the reduction in the Km of placental 5-D in hyperthyroid rats. T_3 5-D activity was undetectable in both tissues unless protein-containing fractions were combined with phospholipid-containing fractions. Kinetic studies revealed that phospholipids had no effect on both Km and Vmax for placental T_3 5-D. These data indicate that: 1) both placental and brain 5-D activities were increased in hyperthyroidism; 2) the amount of brain 5-D molecule is probably increased in hyperthyroidism; 3) changes that affect the affinity of placental 5-D for substrate are caused in hyperthyroidism; 4) changes in the phospholipid composition in hyperthyroidism seem to have nothing to do with such changes in placental 5-D; 5) brain 5-D activity is dependent on phospholipids as well as placental 5-D. The mechanism responsible for the difference in the kinetics between placental and brain 5-D remains to be elucidated.

	T_4 5-D					Placental T_3 5-D			
	Placenta		Brain		Protein	C		H	
	C	H	C	H	Phospholipid	C	H	C	H
Km (nM)	60.6	39.2	87.5	102.2	Km (nM)	1.45	1.50	1.18	1.15
Vmax (pmol/mg protein•h)	17.4	15.6	3.5	9.3	Vmax (pmol/μg protein•h)	31.9	31.1	26.7	31.9

65. **EXPRESSION OF THE THYROTROPIN-RECEPTOR IN ORBITAL TISSUES FROM PATIENTS WITH GRAVES' DISEASE.** L. Tallstedt[1], A. Janson[2], C. Marcus[2], O. Törring[3] and M. Brönnegård[2]. Department of Ophthalmology[1] and Pediatrics[2], Huddinge University Hospital and Department of Endocrinology[3], Karolinska Hospital, Stockholm, Sweden.

The expression of the thyrotropin receptor (TSHR) in orbital fat and extraocular muscle from patients with Graves' ophthalmopathy and normals was studied by Western blot and solution hybridization analysis, using TSHR antibodies and a human radiolabeled TSHR probe. TSHR mRNA was detected in all samples analyzed and there were no significant differences in orbital fat or extraocular muscle from patients with Graves' ophthalmopathy compared to controls. Similar TSHR mRNA expression levels were detected in thyroid tissue. Reverse transcription polymerase chain reaction analysis (RT-PCR) confirmed the presence of TSHR mRNA. Furthermore, the TSHR protein was examined by Western blot analysis, and the TSHR was detected in cell membrane preparations from extraocular muscle as well as orbital fat and thyroid tissue, using a serum with high levels of TSHR antibodies. These results provide further evidence for the presence of the TSH receptor in orbital tissue.

66. STUDIES TOWARD AN IMMUNOASSAY FOR THYROTROPIN RECEPTOR ANTIBODIES (TRAb): PRODUCTION OF ANTIBODIES TO THE TRAb EPITOPE

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Current laboratory methods for measuring TRAb, a diagnostic marker of autoimmune hyperthyroidism (Graves' disease), are based on in vitro bioassay or radioreceptor assay. We investigated the possibility of producing a more robust immunoassay based on antibodies to the TRAb epitope. Monoclonal antibodies were raised against the TSH receptor-binding $F(ab)_2$ fragment of IgG from the serum of a patient with Graves' disease. An antibody, D 10, inhibited the TRAb-stimulated release of cAMP from human thyroid cells, whilst having no effect on TSH stimulated cAMP release. Antibody D 10 did not bind TSH, indicating it was not recognizing an internal image anti-TSH anti-idiotypic antibody population of TRAb. The binding of a group of 15 Graves' patients' serum Ig, in an IRMA assay based on D 10, correlated significantly with their ability to inhibit TSH binding to its receptor (TBII activity), ($r = -0.88$; $p < 0.001$) but not with TSI activity (cAMP release). Polystyrene beads coated with D10 stripped the sera of 9 Graves' patients of TBII activity ($p = 0.033$). Antibody D 10 would therefore appear to be recognizing a 'TBII idiotype' of TRAb.

Mice immunized with commercial rabbit anti-hTSH antibodies (anti-hTSH and anti-hTSH(beta)) were found to possess TRAb activity (as anti-anti-TSH) in the form of both TSI and TBII. The sera of mice challenged with hTSH(beta) showed significant binding to bTSH and Graves' $F(ab)_2$ fragments in an ELISA system, whereas sera from control mice did not. Thus immunization with anti-hTSH(beta) has produced, presumably by the auto-anti-idiotypic immune response, both anti-TSH and anti-TRAb binding activity. It should be possible to produce, using this immunization procedure, a monoclonal antibody which similarly mimics the TSH receptor and which is capable of recognizing the 'TSI idiotype' of TRAb.

68. TSH STIMULATES ^{3}H-CHOLINE INCORPORATION INTO SPHINGOMYELIN OF AGED BUT NOT YOUNG FRTL-5 RAT THYROID CELLS.

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We have recently reported that TSH stimulates 3-fold the rate of $^{32}P_i$ incorporation into sphingomyelin of aged FRTL-5 cells while the corresponding rate for young cells, with or without TSH, was undetectable. Neutral spingomyelinase, which hydrolyzes sphingomyelin and gangliosides to ceramide, has recently been shown to be activated by TNF-α and other cytokines. Ceramide mediates many of the cytotoxic effects of TNF-α. Because the cytotoxic effects of TNF-α on FRTL-5 cells increases with passage number and TSH concentration, we hypothesized that the sphingomyelin concentration is passage number- and TSH-dependent. Young (<20 passages) and aged (>40 passages) FRTL-5 cells were grown to near confluency in 75 cm^2 flasks with standard TSH-containing (6H) medium. Fresh media containing 0 to 2 U/L TSH was added 24 h prior to adding 1.0 mCi/ml ^{3}H-choline. Media were replaced with the corresponding fresh, serum-free media 48 h later. This is sufficient time for ^{3}H-choline to have reached a steady-state distribution with unlabeled choline and its metabolic precursors and products which include sphingomyelin. After a 3 h incubation, plasma membrane lipids were extracted and separated by TLC. In aged cells, the ^{3}H-sphingomyelin levels (cpm/μg DNA) increased in a TSH dose-dependent manner to a maximum at 0.5 U/L TSH which was 2 times the TSH-free level ($p < 0.01$). On the other hand, TSH did not increase the ^{3}H-sphingomyelin levels in young cells. This TSH-induced increase in sphingomyelin levels in aged cells coupled with our previous observation of TSH-facilitated $^{32}P_i$ incorporation by this phospholipid in aged cells only is consistent with a parallel, TSH- and passage number-dependent, enhancement of sphingomyelinase activity. We conclude that TSH increases spingomyelin levels in aged but not young FRTL-5 cells. This may contribute to the TSH-enhanced cytotoxicity of TNF-α in aged cells by enhancement of ceramide production.

70. A POINT MUTATION OF THE Gs-α GENE IN THYROID TISSUE FROM PATIENTS WITH GRAVES' DISEASE (GD) : CORRELATION WITH THE LEVEL OF EXPRESSION OF Gsα-PROTEINS. V.Gorelov, N.Barteneva#, K.Dumon , D.Palm , H.-D.Röher, P.Goretzki, B.E.Wenzel#
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There is conclusive evidence in a subset of endocrine tumors for an involvement of an agonist related pathway; i.e. hormones, neurotransmitters; at the level of the Gs-protein transducer. Point mutations occur at two specific sites in the Gsα- gene, namely c.c.201 and 227, which leads to an impaired intrinsic GTPase activity of this protein. It is believed that mutations in the Gsα- gene fullfill the criteria of oncogenic mutations.

The aim of this study was i) to evaluate the level of expression of the Gsα- proteins in thyroid tissue from GD patients, ii) to examine the possible relation between alterations in the level of expression of Gsα and the presence of point mutations in c.c. 201 and 227.The overexpression of Gsα-proteins in GD-tissue was assessed by immunoblotting with two anti-peptide antibodies which we re mid-region or C-terminal specific. In order to define point mutations in the Gsα-gene,we applied the two step-RFLP of PCR-amplified fragments of the Gsα- gene (1).

In 5/8 GD-tissues an overexpression of the Gsα- proteins was demonstrated. In 2/8 cases the expression level was as high as 200% of the control which comprised 9 pooled normal thyroid tissues. None of the GD-tissues revealed any mutation in c.227. One of the highest Gsα -expressing tissues, however, showed a mutation in c. 201 of the Gsα -gene. Our ongoing studies will show, if the overexpression of Gsα- proteins correlates with adenylatcyclase activities.

1) Goretzky et al. Exp. Clin. Endocrinol. 101:54-59 (1993)

Supported by DFG (Go 356/3-1) and DFG/SFB/B6.

74. LOCALIZATION OF HLA-DR ANTIGEN AND HEAT SHOCK PROTEIN 70 AND CD44 ANTIGEN EXPRESSION IN UPPER EYE LIDS FROM PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY.

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Lid retraction and lid edema are common and early manifestations of thyroid-associated ophthalmopathy (TAO). We previously reported electron microscopical evidence of subcellular changes and fiber damage of Müller's muscle in patients with TAO who had lid retraction. However, its relation to the autoimmune process in Graves' disease is still unclear. In the present study, to investigate the localization of HLA-DR antigen and heat shock protein 70 (HSP-70) and CD44 antigen expression in upper eye lids from patients with TAO, we have carried out immunohistochemical study using anti-HLA-DR, anti-HSP-70 and Anti-CD44 monoclonal antibodies. Smooth muscle specific α-actin and two negative monoclonal antibodies were used as controls. Upper eye lids were obtained at surgery from 8 patients with TAO and one control subject, and frozen sections were incubated with these monoclonal antibodies and then labelled using a streptavidin-biotin-peroxidase detection system. HLA-DR antigen was detected in interstitial cells of upper eye lid tissue from patients with TAO, but not the control subject. HSP-70 and CD44 were detected in Müller's muscle in both patients with TAO and the control subject. Two control monoclonal antibodies gave no positive staining. Despite lack of control specimens so far examined, the expression of HSP-70 on Müller's muscle suggests that these muscle cells are under stress due to metabolic or immunologic process in Graves' disease. Since CD44 is a hyaluronic acid receptor and acts as an adhesion molecule and may influence various immunological reactions, the expression of CD44 in Müller's muscle may play a part in the development of eye lid edema and dysfunction in TAO. These results support the notion that the Müller's muscle is the main target of the upper lid inflammation of TAO. We are presently testing additional patients with TAO and control subjects to further investigate a role of the expression of these antigens in the development of TAO.

76. **Prostaglandin E_2-Mediated Shape Changes in Orbital Fibroblasts From Patients With Graves' Ophthalmopathy** H-S Wang, MG Hogg and TJ Smith. Molecular and Cellular Medicine, Departments of Medicine and Biochemistry, Albany Medical College and the Department of Veterans Affairs Medical Center, Albany, NY 12208.

Orbital fibroblasts from patients with Graves' ophthalmopathy exhibit phenotypic attributes that set them apart from other fibroblasts. We have recently reported that orbital fibroblasts, when treated with PGE_2, undergo a dramatic change in morphology characterized by the development of prominent cellular processes and a stellate appearance. We now begin to characterize that effect. Orbital fibroblasts were used to inoculate glass coverslips or plastic culture dishes. Cells were allowed to attach for at least 24 hr. before treatment. The concentration threshold of the PGE_2 effect on cell morphology was 10 nM, was dose-dependent and near-maximal at 0.5 μM. 16,16-dimethyl PGE_2, a prostanoid agonist, could also elicit an effect however thromboxane, PGI_2, $PGF_{2\alpha}$, 17-phenyl trinor PGE_2, and 11-deoxy, 16,16-dimethyl PGE_2 all failed to mimic the action of PGE_2 on shape change. 8-Br-cAMP (1 mM) and forskolin (5 μM) caused shape-changes indistinguishable from those of PGE_2. Addition of actinomycin D (2 μg/ml) or cycloheximide (10 μg/ml) failed to attenuate the effect of PGE_2, which evolved over 6-8 hr. After 24 hr. of treatment with the prostanoid, the cellular morphology had begun to revert to that of untreated cells. Immunofluorescent staining with phalloidin revealed an absence of actin stress fibers in the cells undergoing shape change. Dermal fibroblasts failed to manifest these effects of PGE_2 which apparently represent a site-selective vulnerability of orbital fibroblasts to the action of this prostanoid. The established roles of these molecules in tissue inflammation make our observations of potential importance in understanding the pathogenesis of Graves' ophthalmopathy.

78. COCULTURE OF PERIPHERAL BLOOD MONONUCLEAR CELLS WITH ALLOGENEIC HUMAN EXTRAOCULAR AND SKELETAL MUSCLE CELLS: POSSIBLE RELEVANCE TO THYROID ORBITOPATHY. D.L. Kendler, P. Dolman, C. Cordeiro and J. Rootman, University of British Columbia, Vancouver BC, Canada.

Extraocular muscle (EM) cells are specifically affected by cellular and/or humoral elements in thyroid orbitopathy (TO). We separated peripheral blood mononuclear cells from TO patients with active disease (n = 9) and from healthy controls (C) (n = 9). Human fetal skeletal muscle (SM) and EM primary cell cultures were established. Cocultures of 1.25 x 10^3 stimulating cells with 4.0 x 10^5 PBMC were pulse-labelled with tritiated thymidine and harvested at 3 days and 7 days. Stimulation indices were calculated as (counts per minute for stimulated PBMC) / (counts per minute for unstimulated PBMC). Each TO PBMC had C PBMC run in the same experiment and Con A was used as positive control for both TO PBMC and C PBMC. Stimulation index (SI) for TO with EM at 3 days was 21.5 $\pm$ 16.6, significantly greater than for C with EM: 7.0 $\pm$ 4.0 ($p = .02$). Lesser stimulation was seen in TO with SM: SI = 16.6 $\pm$ 17.7, C with SM SI = 5.4 $\pm$ 2.7 ($p = .08$). At 7 days, stimulation was greatest in EM with TO SI = 29.7 $\pm$ 36.0 (vs EM with C SI = 9.1 $\pm$ 5.6, $p = .16$). Lesser stimulation was seen in SM with TO 15.3 $\pm$ 20.5 (vs SM with C SI = 6.2 $\pm$ 2.2, $p = .3$). Eight of 9 TO with EM and 1 of 9 C with EM had 3 day SI greater than 10; 5 of 9 TO with SM and 0 of 9 C with SM had 3 day SI greater than 10. Con A stimulation at 3 days with TO PBMC was 51.2 $\pm$ 53.7 and 52.0 $\pm$ 43.8 with C PBMC. We found significantly higher stimulation of most orbitopathy patient PBMC with allogeneic EM as compared to controls and to SM stimulators. This stimulation may be due to processed antigen shed from stimulating cells or from non-MHC mediated stimulation of cell proliferation and may be relevant to the pathogenesis of TO.

79. **ENZYME ACTIVITY AND BLOOD LYMPHOCYTES IN GRAVES' DISEASE.** Marisa C. Werner, Joao H. Romaldini, Luiz F. C. Rosa and Rui Curi. Department of Endocrinology, HSPE-IAMSPE and Institute of Biomedical Sciences, University of Sao Paulo, S.P., Brazil.

Lymphocytes utilize glucose and glutamine at high rates and these metabolites seem to be important for their function. In order to evaluate the metabolism of these cells in patients with Graves' disease (GD) and possible changes caused by treatment with methimazole (MMI) we studied the maximum activities of Hexokinase (HR), citrate syntase (CS) and glucose-6-phosphate dehydrogenase (G6PDh) in peripheral blood lymphocytes of three groups of GD patients: untreated hyperthyroidism (group I; n=8), Graves' hyperthyroidism receiving MMI (group II; n=6), euthyroid patients after treatment with MMI (group III; n=8) and compared with normal subjects (group C; n=8). The relationship between lymphocytes proliferation and enzyme activities were assessed by ^{3}H-thymidine incorporation in 48h-cultured normal cells stimulated with concavalin-A or LPS and exposed to T3 (10^{-8} M, 10^{-11} M), T4 (10^{-8} M, 10^{-11} M), MMI (10^{-3} M, 10^{-5} M) and combinations of MMI plus T3 and MMI plus T4. The activities of HK, CS, G6PDh and glutaminase (GT) were assayed in 24h-cultured lymphocytes before and after T3 (10^{-8} M), T4 (10^{-8} M) and MMI (10^{-3} M) addition. The enzyme activities were measured by spectrophotometry (expressed in nmol / min / mg protein). The results showed a significant ($P < 0.001$) increase in HK (47%) and CS (24%) activities in group I. Treatment with MMI was related to normalization of HK and CS activities in group II and III and a reduction in G6PDh activity in group II (55%, $P < 0.02$ vs. group I and group III). *In vitro* enzyme experiments showed an increase in HK (24%, $P < 0.01$) and CS (23%, $P < 0.01$) but reduction in G6PDh (63%, $P < 0.001$) and GT (25%, $P < 0.01$) activities after addition of T3. Treatment with MMI also reduced G6PDh (71%, $P < 0.001$) and GT (31%, $P < 0.01$) but did not change HK and CS activities. A decrease in the proliferative response of normal lymphocytes was observed after addition of T3, T4 and MMI in a dose dependent way. The inhibition was greater (83%, $P < 0.001$) in the presence of T3 (10^{-11} M) plus MMI (10^{-3} M). In conclusion, an increase in glucose metabolism and a reduction in glutamine metabolism was observed in the presence of elevated concentration of T3 and also of MMI. The decrease of glutamine utilization may be related to the reduction in proliferative response of lymphocytes. This could be one of the mechanisms involved in the immune suppression of MMI in GD.

87. CHARACTERISTICS OF THE IMMUNE RESPONSE TO THYROID PEROXIDASE IN PATIENTS WITH POST-PARTUM THYROIDITIS. J. Janssen, P. Arscott, R. Smallridge and J.R. Baker, Jr. University of Michigan Medical School, Ann Arbor, MI. and Walter Reed Army Institute of Research, Washington, D. C.

Post-partum thyroiditis (PPT) is a common disorder of great clinical importance. However, the immune pathogenesis of this disorder and its relationship to other autoimmune thyroid diseases is not clear. Thyroid peroxidase (TPO) autoantibodies are seen in a majority of patients with PPT and can be helpful in the diagnosis of PPT but do not appear valuable in determining the potential for developing hypothyroidism in these individuals. To clarify the role of the immune response to TPO in this disorder, patients were evaluated for autoantibodies to TPO and several localized autoantibody epitopes within this molecule. Fifty-three patients with PPT were evaluated at the time of their diagnosis and agreed to donate serum for the study. The patients were then followed over five years to determine the outcome of their disease. 49 of 53 patients had anti-microsomal antibodies in titer of 1:400 or greater at the time of diagnosis while only 7 of 53 had antibodies to thyroglobulin (both by agglutination assay) indicating the stronger association of microsomal antibodies with this disorder. Patients who became hypothyroid had higher median titers of microsomal antibodies (see table) and the four patients with titers greater than 1:25,600 all demonstrated hypothyroidism. Reactivity to two localized binding sites in TPO also correlated with the development of hypothyroidism as 8 of 14 patients with antibodies to amino acids 708-725 and 9 of 13 patients with antibodies to amino acids 592-613 became hypothyroid. The frequencies of antibodies to these epitopes were similar to those we have previously described for Hashimoto's thyroiditis (and distinct from the frequencies in Graves' Disease). These findings demonstrate similar immune responses to TPO in PPT and Hashimoto's thyroiditis, and suggest that the development of hypothyroidism correlates with more intense immune responses to TPO.

Clinical Status and Patient Number (#)	Median Mic. Antibody Titer	Antibodies to AA 708-725	Antibodies to AA 592-613
Overall (53)	1: 1,600	14	13
Euthyroid (18)	1: 400	4	3
Hyperthyroid (7)	1: 1,600	2	1
Hypothyroid (18)	1: 6,400	7	6
Both Hyper and Hypo (10)	1: 1,600	1	3

93. ROLE OF GRB2 IN TSH SIGNAL TRANSDUCTION. D. Wofford*, J.R. Feramisco and J. L. Meinkoth, Departments of Biology*, Pharmacology and Medicine, Cancer Center, University of California, San Diego, La Jolla CA 92093

Previous work in our laboratory had implicated Ras downstream from the thyrotropin (TSH) receptor in growth signaling pathways in Wistar rat thyrocytes (WRTs). In order to explore the mechanism through which activation of the TSH receptor invokes Ras function, experiments were conducted to assess the function of GRB2 in TSH-stimulated DNA synthesis. Proteins comprised of GST fused to either wild type GRB2 or to GRB2 proteins containing a mutation in the SH2 (S90-L) or amino terminal SH3 (P49-L) domain were injected into quiescent WRT cells which were subsequently stimulated with TSH. Injection of the S90-L (SH2 point mutant) protein significantly reduced TSH-stimulated DNA synthesis. In contrast, injection of the P49-L (SH3 point mutant) or wild type GRB2 proteins did not reduce TSH-stimulated DNA synthesis. These results support a role for GRB2 in TSH signaling. Since SH2 domains bind specifically to tyrosine-phosphorylated proteins, the effects of TSH on tyrosine phosphorylation was assessed. TSH treatment increased tyrosine phosphorylation of several cellular proteins and decreased tyrosine phosphorylation of an aproximately 66KDa protein which associates with GRB2 in WRT cells.

96. RELATIONSHIP BETWEEN EYE MUSCLE AUTOANTIBODIES AND SEVERITY OF THYROID-ASSOCIATED OPHTHALMOPATHY. A. Boucher, F. Ertug, C. Corriveau, P. Gauvin, H. Beauregard, R. Comtois, Notre-Dame Hospital, Montreal, Canada.

Thyroid-associated ophthalmopathy (TAO), a known complication of autoimmune thyroid disorders, has an unpredictable outcome. The aim of the study was to determine the relationship between antibodies against a 64 kDa eye muscle antigen present in the sera of TAO patients, and the severity of the eye disease, as assessed by a clinical score and extraocular muscle ultrasounds. Thirty-eight patients with recently diagnosed autoimmune thyroid disorders (Hashimoto and Graves) had a clinical neuroophthalmological exam grading anatomic, functional or inflammatory abnormalities according to ATA recommendations (1992) which allowed a diagnosis of TAO and the determination of a clinical score of severity maximal of 60. In 10 patients, the transversal diameter of each extraocular muscle was determined in both eyes using the ultrasounds. Determination of eye muscle antibodies was done with pig eye muscle (PEM) membranes, run on a 7% SDS-PAGE, transferred on a nitrocellulose sheet and incubated with the various sera in a 1/25 dilution. In the positive sera for antibodies against a 64 kDa PEM protein, titers were considered to be the highest dilution at which a band could be observed (1/100, 1/500, 1/1000, 1/2000, 1/4000, 1/6000, 1/8000, 1/12,000). The prevalence of eye muscle antibodies in our population of recently diagnosed TAO was 29/38 (76.3%) which is similar to what was reported previously. In the 10 patients who had the eye muscle ultrasounds, we found a significantly higher sum of all muscles transversal diameters than in a normal population (44.7 ± 7.7vs 29.3 ± 2.5, $t = 6.3$, $p < 0.0001$). A positive correlation was also observed between the sums of the transversal diameters measured by ultrasounds in our TAO patients and titers of their anti eye muscle antibodies ($r = 0.801$, $p < 0.005$). However, although a significant correlation between the proptosis and the clinical score was observed ($r = 0.417$, $p < 0.05$), the titers of antibodies did not seem to be related to the score nor the proptosis. We conclude that the titers of antibodies against eye muscle in the sera of patients with autoimmune thyroid disorders reflect the early eye muscle abnormalities.

103. **COMPARATIVE STUDIES OF HUMAN THYROID XENOGRAFTS FROM GRAVES' DISEASE IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE AND NIH-BEIGE-NUDE-XID (NIH-3) MICE.** T. Mukuta, G. Arreaza, M. Nishikawa, N. Yoshikawa, E. Resetkova, R. Volpé. Endocrinology Research Laboratory, Department of Medicine, The Wellesley Hospital, University of Toronto, Toronto, Ontario, M4Y1J3 Canada.

To compare the effect of human thyroid xenografts from Graves' disease in SCID mice and triple immunodeficient NIH-3 mice, thyroid tissues from 6 patients with Graves' disease (GD) were xenografted to SCID mice and NIH-3 mice; in addition, peripheral blood mononuclear cells (PBMC) from 12 patients with GD were intraperitoneally engrafted to separate SCID mice (SCID-PB) and NIH-3 mice (NIH-PB). Human IgG was detected in all mice engrafted with GD thyroid tissues and some mice engrafted with PBMC peaking at 6-10 wks after xenografting. The maximum human IgG levels showed 500±150 (Mean ± SE) mg/L in NIH-3 xenografted with thyroid tissues (NIH-TH) similar to 640±230 mg/L in SCID xenografted thyroid tissues (SCID-TH), 1200±250 mg/L in NIH-PB similar to 1000±280 mg/L in SCID-PB. The change in human IgG in sera of NIH-TH was similar to that in SCID-TH. Thyroperoxidase-antibodies (TPO-Ab) were also detected in some mice with GD thyroid grafts and a few mice injected with PBMC peaking at 4-6 wks after xenografting. The maximum TPO-Ab levels showed 18.3±7.3 U/mL in NIH-TH similar to 14.8±6.6 U/mL in SCID mice. Moreover, though thyroglobulin antibodies (Tg-Ab) were detected in some mice with GD thyroid grafts, there were no significant difference in the maximum Tg-Ab levels in both mice (9.3±7.1 vs 18.3±6.3 U/mL). After 8 wks following xenografting into mice, the expression of intercellular adhesion molecule-1 (ICAM-1) on thyroid epithelial cells (TEC) of xenografted thyroid tissues decreased significantly in both SCID mice and NIH-3 mice (from 43.4% to 35.9% in NIH-3, $P<0.05$, from 43.4% to 32.5% in SCID, $P<0.05$). However, the expression of HLA-DR on TEC did not change in NIH-3 mice (from 11.5% to 10.8%, NS), while the expression of HLA-DR decreased significantly in SCID mice (from 11.5% to 4.2%, $P<0.02$). We conclude that not only SCID mice but also NIH-3 mice might be helpful as animal models with xenografted thyroid tissues to elucidate the pathogenesis of autoimmune thyroid disease. NIH-3 mice are superior to SCID mice in terms of maintaining the expression of HLA-DR on TEC in GD thyroid grafts as high as that before xenografting; thus might be due to the lack of NK cells in NIH-3 mice.

106. **Does thyroidectomy, RAI therapy or antithyroid drug treatment alter reactivity of patients T cells to epitopes of thyrotropin receptor in autoimmune thyroid diseases? M. Soliman, E. Kaplan and A. Abdel-Latif. Thyroid Study Unit, The University of Chicago and Mansoura University Hospital, Egypt.**

In Graves' disease (GD), TSH receptor (TSHR) is believed to be the major target of an autoimmune response. Protein antigens are processed to peptide fragments and presented by class I & II molecules. Accordingly, T cells specific for TSHR will be directed against immunogenic peptides derived from TSHR. To determine whether the human T cell repertoire responding to proposed immunogenic peptides of TSHR changes as a result of treatment, we tested the reactivity of PBMC and TSHR-specific T cell lines to rec h TSHR extracellular domain (TSHR-ECD) and a battery of synthetic peptides encompassing the entire TSHR-ECD before and after thyroidectomy, RAI treatment, and during the course of propylthiouracil (PTU) therapy for patients with GD and HT. Reactivity of 18 patients (14 GD and 4 HT) preoperatively was heterogenous and spanned the entire ECD. However, the reactivity was significantly different from controls for peptide regions 44-75, 132-176, 227-263 and residues 195-210, 272-291, 301-320 and 357-376 ($p<0.05$). 6-8 weeks after subtotal thyroidectomy, the number of patients PBMC positive to any peptides decreased, as did the average number of positive responses. There was a further decrease in positive responses at 3-6 months for some peptides. Before RAI, 5/18 Graves' patients showed a response to an average of 8 peptides. The number of responders increased to 7, 6-8 weeks after RAI, and was the same at 3-6 months. The reactivity of PBMC from 8 patients with GD who were euthyroid on PTU therapy was confined for peptide region 145-176 only. Reactivity of T cell lines in different groups reflected a pattern similar to PBMC following treatment. While the mean S.I.s of T cell lines in response to individual peptides were higher after RAI, they tended to be lower after thyroidectomy. **Conclusions** ❶ The difference in the number of peptides recognized by patients PBMC before RAI, and surgery, may reflect long-term therapy with PTU in the former (39±40 months) versus the shorter time in the later (20±28). Support for this observation comes from the fact that patients maintained euthyroid exclusively on PTU therapy recognized the least number of epitopes. ❷ The observed remission of the immunologic process after thyroidectomy may be a consequence of reduction of thyroid gland mass. On the other hand, the apparent increase of T cell reactivity after RAI could be explained by release of thyroid antigens or damage to Ts lymphocytes by RAI ❸ Thyroid hormone levels seem unlikely to be directly involved in the effects of surgery or RAI on the T cell response since these changes continued despite the maintenance of thyroid hormone in the normal range.

			6-8 weeks		3-6 months	
	Pre-op	Pre-RAI	Pos-op	Pos-RAI	Pos-op	Pos-RAI
# of patients positive to any peptide	11/18	5/18	4/18	7/18	4/18	7/18
Average of peptides recognized	4.8±2.1	8±8.7	3.6±1.5	6.2±5.8	2±0.6	4.5±2.9
FTI	11.9±8	12.9±5	6.3± 4	10.4±4.3	7.8±2	7.6±4.2
TSH	0.4±1	0.2±0.3	23±43	0.1±0.2	14±23	5.3±15

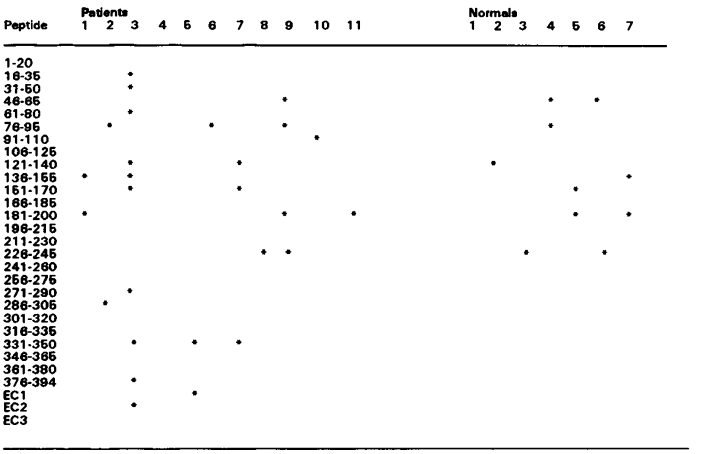
116. T LYMPHOCYTE REACTIVITY TO EYE MUSCLE PROTEINS AND THEIR PREDICTED PEPTIDES IN PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY. J. Kiljanski, C. Stolarski, D. Scalise. V. Nebes, M. Hayes and J. R. Wall. Thyroid Center, Allegheny-Singer Research Institute, Pittsburgh, PA.

While the possible role of antibodies reactive with eye muscle (EM) antigens in thyroid-associated ophthalmopathy (TAO)has been well studied the initial event in the development of the eye disorder is likely to be T lymphocyte recognition of short linear peptides which have been processed and presented to the T cell receptor. There is considerable evidence that the EM is the main target of this orbital inflammatory reaction although the nature of the specific antigens is unknown. We have studied the role of T cell reactivity against 64 kDa proteins as well as other potential antigens in solublized EM membranes. We performed T cell epitopic analysis of the recombinant 64 kDa protein $_1$D, cloned from a thyroid expression library by M. Ludgate et al., using the "T-Sites" software program which analyzes its primary sequence, identifying those AA with the greatest amphipathicity scores. Short peptides containing predicted sequences were synthesized for use as antigens in a lymphocyte transformation assay. Ten peptides, each 15 AA residues in length comprising 8 predicted fragments (P3-P12) and two negative control fragments (P2, P3), were tested. Peripheral blood lymphocytes from patients with TAO, Graves' hyperthyroidism (GH) without eye disease and Hashimoto's thyroiditis (HT) and, as controls, patients with non autoimmune thyroid disorders and normal subjects were cultured with $_1$D peptides and, as controls, phytohemaglutinin (PHA), tetanus toxoid and a reactive peptide fragment of tetanus toxoid. Results were expressed as stimulation indices (SI) and a positive test taken as SI > mean + 2 SD for normals. No patient or normal subject showed reactivity to $_1$D peptides P5 or P9. Whilst positive transformation tests to one or more of P3, P4, P7, P8, P11 or P12 was observed in 30% of patients with TAO, 20% of patients with GH without eye disease and 25% of those with HT a similar proportion of normals reacted to these peptides and overall there was no significant reactivity to any peptide for any group of patients. We performed similar assays with full length pig EM proteins separated by SDS-PAGE and isoelectric focusing and electroeluted from gels. We demonstrated positive transformation responses to a purified 64 kDa protein in 25% of patients with TAO but in no normal subject. In conclusion while we have failed to show T cell reactivity to predicted T cell epitopes of the 64 kDa molecule $_1$D in patients with TAO, suggesting that this protein is not closely associated with ophthalmopathy, more promising results have been obtained using a full length 64 kDa EM protein shown, in our other studies, to be a prominent target for serum antibodies in patients with TAO.

119. **REGION SPECIFIC T CELL INTOLERANCE TO TSH RECEPTOR EXTRACELLULAR DOMAIN.** A.Martin, J.C. Morris[1], S. Yeung and T. F. Davies. Department of Medicine, Mount Sinai School of Medicine, New York, NY, and Division of Endocrinology[1], Mayo Clinic, Rochester, MN.

To explore the major T-cell epitopes of the human TSH receptor (hTSHR) in patients with Graves' disease (n=11) and normals (n=7) we have tested their peripheral blood mononuclear cells (PBMC) against overlapping synthetic peptides of the hTSHR extracellular domain (ecd) and the three extracellular loops of the transmembrane region (n=29). Peptide lengths varied from 12-21 amino acids (aa) and after purification by HPLC were checked for purity by aa composition analysis and mass spectrometry. PBMC (2 x 10^6 cells per well) were incubated in triplicate with 50 ug/ml of peptide for 6 days and labeled with ^{3}H-TdR for 18 h and harvested. Data were analyzed by ANOVA (Tukey protected t-test) and significant stimulation compared to baseline.

The mean number of epitopes recognized by patients was 2.5±0.8 (SI range 1.8-7.6) and by normals 1.4±0.3 (SI range 1.6-3.3) ($p<0.05$ for stimulation). While patients reacted to epitopes distributed over the entire extracellular domain, the data show that normals did not react to the membrane proximal region of the ecd or to any of the 3 extracellular loops. Both Graves' disease patients and normals, therefore, have hTSHR reactive T-cells in their peripheral blood but the membrane proximal region of the TSHR-ecd (sequence 240-394) appears to be immunologically tolerized in normals.



121. INTRATHYROIDAL T CELL RECEPTOR GENE EXPRESSION IN MURINE AUTOIMMUNE THYROIDITIS INDUCED BY TRANSFER OF MOUSE THYROGLOBULIN-ACTIVATED LYMPHOCYTES. M. Nakashima, N. Matsuoka and Y. M. Kong*, Department of Medicine, Mount Sinai School of Medicine, New York, NY, and Department of Immunology and Microbiology*, Wayne State University School of Medicine, Detroit, MI.

Transferring lymphocytes from mouse thyroglobulin (mTg)-immunized CBA/J ($H\text{-}2^k$) mice, activated in vitro with mTg, initiates experimental autoimmune thyroiditis (EAT) in syngeneic recipents. Since the intrathyroidal population in EAT comprises primarily $CD4^+$ and $CD8^+$ T lymphocytes, we analyzed the T cell receptor (TcR) V gene families used by the intrathyroidal lymphocytic infiltrate of such mice. This analysis was achieved by using the RT-PCR technique with oligonucleotides detecting 17 mouse TcR Vβ gene families. Thyroiditis was detected histologically and also demonstrated by the presence of constant region RT-PCR fragment accumulation. Although the activated lymphocytes had a heterogeneous TcR repertoire prior to transfer, the responding CBA/J mice ($n = 7$, $>40\%$ infiltration) demonstrated an intrathyroidal T cell population which was markedly oligoclonal in 5 of the 7 mice. The prominent TcR V gene families displayed included Vβ 1, 7 & 8 in all specimens and Vβ 2, 4, 13 & 16 in selected samples. A similar but more restricted oligoclonal response was seen previously in our studies of EAT after either mouse or human Tg immunization. There are a number of possible mechanisms for such oligoclonality. These include a major response of antigen-specific T cells expanded by in vitro activation with the additional accumulation of selected bystander T cells involved in antigen-specific T cell activation or self-recognition. Earlier data have excluded a role for Vβ 8 T cells in the transfer of thyroiditis in CBA/J mice, yet Vβ 8 T cells accumulated intrathyroidally in all recipients. We conclude that T cells responding to a limited number of autoepitopes, as well as bystander T cells, have integral roles in the development of autoimmune thyroiditis.

134. ETa AND ETb ENDOTHELIN RECEPTOR MESSENGER RNAs ARE EXPRESSED IN THE RAT THYROID GLAND AND REGULATED IN GOITER FORMATION AND IODIDE-INDUCED INVOLUTION. D. Toussaint-Demylle, J. Weiss, D.M. Maiter, J-F. Denef, and I.M. Colin. Center for Endocrinology and Molecular Medicine (JW, IMC), Northwestern University, Chicago, IL, U.S.A. and Histology (DT-D, J-FD), Diabetology and Nutrition Research Units (DMM), University of Louvain, Brussels, Belgium.

We previously reported that endothelin-1 (ET-1) is synthesized in the normal rat thyroid gland and that its glandular peptide and mRNA levels are increased in hyperplastic and involuted glands. The ET-1 actions are mediated by at least 2 different receptors: ETa and ETb. The binding of ET-1 to ETa elicits vasoconstriction, whereas vasodilatation is induced after binding to ETb. Since the ultimate response of ET-1 (vasoconstriction or vasodilatation) on a given vascular bed is modulated through differential expression of both receptors, we investigated the regulation of their respective genes in a model of goiter formation and involution.

After demonstration of ETa and ETb expression in the rat thyroid gland by RT-PCR, and sequencing of their products, changes in their mRNA levels were assessed by a semi quantitative RT-PCR assay, using the ribosomal protein L19 mRNA as internal reference. To induce thyroid hyperplasia, male Wistar rats were fed a low iodine diet (LID) supplemented with 0.25% thiouracil for 10 days, followed by LID alone for two additional days. Involution was induced after 12 days by refeeding a normal iodine diet and an i.p. injection of 100 µg iodide. Animals were sacrificed at 2 (H.2d) and 12 days hyperplasia (H.12d), and 12 (I.12h) and 24 hours (I.24h) after iodide administration. At each time point, 4 samples were analyzed.

After 2 days of goitrogen diet, ETb mRNA levels increased significantly (2 fold vs. control) with no change in ETa mRNA levels. After 12 days hyperplasia, both mRNAs reached a peak (ETb: 4 fold and ETa: 3 fold vs. control). Iodide administration induced a sharp decline of ETb and ETa gene expression that was more pronounced for ETb than for ETa (ETb; I.12h: -46%, I.24h: -45% vs H.12d, ETa; I.12h: -34%, I.24h: -38% vs. H.12d). Thyroglobulin mRNA was used as control. Its expression was unmodified among the different experimental groups.

In conclusion, the genes encoding the ETa and ETb endothelin receptors are expressed in the rat thyroid gland and are dynamically regulated in goiter formation and involution. These findings further expand the role of endothelins in the regulation of thyroid cell function and in the remodeling of the vascular bed.

136. EVIDENCE FOR NITRIC OXIDE ACTIVITY IN THE RAT THYROID GLAND. I.M. Colin, E. Nava, D. Toussaint-Demylle, J-F. Denef, D.M. Maiter, T.F. Lüscher, and J.L. Jameson. Center for Endocrinology and Molecular Medicine (IMC, JLJ), Northwestern University, Chicago, IL, U.S.A.; Histology (DT-D, J-FD), Diabetology and Nutrition Research Units (DMM), University of Louvain, Brussels, Belgium, and Cardiology, (EN, TFL), Inselspital, University of Berne, Switzerland.

The mechanisms that mediate changes in thyroid vasculature during goiter formation and involution involve complex interactions between growth factors, neuropeptides, prostaglandins, interleukins and the sympathetic nervous system. Recent evidence suggests that, in many systems, the endothelium layer may release vasoactive substances that interact with each other to regulate local vascular tone. Nitric oxide (NO) is an endothelial-derived relaxing factor, generated by the NO synthases (NOS), a family of isoenzymes widely expressed in mammalian cells. The present study was designed to identify, in the thyroid gland, the constitutive *c*(NOS) and inducible *i*(NOS) isoforms of NOS and to analyze potential modifications of their respective activity in a rat model of thyroid hyperplasia and iodide-induced involution.

Expression of the two isoforms was detected by RT-PCR. After sequencing, the amplification products were shown to correspond to the respective published sequences. Activity of both enzymes was assessed by measuring the conversion of L-[U-^{14}C] arginine into [U-^{14}C] citrulline. Control thyroid glands displayed detectable levels of NOS activity. In goitrous rats, fed a goitrogen diet for 12 days, the activity of *i*NOS was 3 times higher than in controls. After 24 hours of iodide administration, the activity of this enzyme returned to control levels. The increased *i*NOS activity in the thyroid of goitrous rats was completely blocked when N^{ω}-nitro-L-arginine methyl ester (NAME), a potent competitive inhibitor of NOS, was administered in drinking water (1mg/ml). *c*NOS activity was unmodified by the goitrogen treatment as well as in iodide-induced involution, but was abolished in presence of NAME.

In summary, we show in this study that 1) the *c*NOS and *i*NOS genes are expressed in the rat thyroid gland; 2) both enzymes are functional since detectable levels of *c*NOS and *i*NOS activity were observed in the normal gland; 3) the activity of the inducible isoform is increased in goiter, suggesting that greater amounts of NO are produced in hyperplastic glands; 4) the activity of both isoforms was inhibited when a NOS inhibitor was administered.

In conclusion, these data demonstrate that NO is produced in the thyroid gland and that its production is regulated in goiter formation and involution. They suggest a potential role for this factor in trophic modifications occurring in these conditions.

143. STRUCTURE AND FUNCTION OF THE TSH RECEPTOR EXTRACELLULAR REGION EXPRESSED UNDER THE INFLUENCE OF DIFFERENT BACULOVIRUS VECTOR PROMOTERS. G.D.Chazenbalk and B.Rapoport, Thyroid Molecular Biology Unit, VAMC, UC San Francisco, California.

Expression of large amounts of functional, extracellular TSH receptor (ETSHR) is vital for understanding the structure-function of the TSH receptor. Several laboratories, including our own, have achieved high levels of ETSHR expression in bacteria. However this non-glycosylated ETSHR does not bind TSH or TSHR auto-antibodies. Even with the baculovirus system, which does generate a glycosylated protein, many laboratories have experienced great difficulty in generating large amounts of functional ETSHR protein. All studies have used baculovirus vectors with the very late polyhedrin promoter which drives protein expression when insect cell N-glycosylation efficiency has declined. Therefore, we expressed the ETSHR in the baculovirus system using a vector, pAcMP3, which utilizes an earlier promoter. A six histidine tag was introduced at the carboxyl terminus of the ETSHR to allow affinity purification. ETSHR expression was assessed by ^{35}S-methionine incorporation. As expected, maximal ETSHR expression in Sf9 insect cells infected with ETSHR-pAcMP3 occurred earlier (1 vs 2 days) than with ETSHR-pVL1393, a vector containing the standard polyhedrin promoter. On SDS-PAGE, the pAcMP3-derived ETSHR (~68kD) was larger than the pVL1393-derived protein (~62kD). Enzymatic deglycosylation of both radiolabeled proteins with endoglycosidase F revealed this size difference to be due to the greater carbohydrate content of the ETSHR generated with the pAcMP3 vector. We, therefore, focused on the ETSHR-pAcMP3. Despite its greater carbohydrate content, most of the ETSHR remained within the insoluble fraction of the cells and this was the only fraction in which ETSHR protein was visible by Coomassie blue staining. Only radiolabeled ETSHR could be detected in the culture medium and in the soluble, cytosolic fraction of the insect cells. Nevertheless, preliminary data indicate that there was sufficient soluble ETSHR in the cytosol to specifically bind radiolabeled TSH and to inhibit TSHR autoantibody binding in a dose-dependent manner. In conclusion, the present data indicate that a baculovirus vector with a promoter earlier than the conventional polyhedrin promoter generates a more highly glycosylated ETSH. Even though the amount of ETSHR generated by this system is very small, it, nevertheless, appears to be functional.

147. PREFERENTIAL DEVELOPMENT OF BLOCKING ANTIBODIES TO TSH RECEPTOR AFTER ABLATIVE ^{131}I THERAPY IN GRAVES' DISEASE. M. K. Gupta, R. Beham, M. Gliga, M. Secic, G. Kosmorsky, J. Perl, A. Licata, L. Kohse, B. Hoogwerf, and C. Faiman, The Cleveland Clinic Foundation, Cleveland, OH 44195

Ablative ^{131}I has become the therapy of choice for Graves' hyperthyroidism in our Institution. However, its effect on TSH receptor antibodies (TRAb(s)) and on ophthalmopathy (GO) remains unclear. We have prospectively measured blocking (TBAb), stimulating (TSI), and TSH binding inhibitory (TBII) activities of TRAb(s) at the time of diagnosis (Pre), at 2 months post (Post 1), and at six months post (Post 2) ^{131}I therapy in 18 patients with Graves' disease. Also at the same time, GO was assessed by MRI and ophthalmic exam (OE) in these patients. Twelve patients had no detectable GO and 6 had only mild GO at the time of diagnosis. There was no statistically significant change observed in GO post therapy as assessed by both MRI or OE. Both TBAb and TBII showed a statistically significant increase at 2 months and 6 months post treatment with a concomitant decrease (not statistically significant) in TSI levels. The data are summarized below.

	Median			P Value* for	
Antibody Activity	Pre	Post 1	Post 2	Post 1	Post 2
TBAb (%)	48	80	89	0.10	0.01
TBII (U/L)	20	70	51	0.02	<0.001
TSI (% cAMP)	387	355	251	0.04	0.3

There was a significant correlation between pretreatment TBAb and TBII activities ($r = 0.56$, $p = 0.01$) but not between TBAb and TSI ($r = 0.24$, $p = 0.3$). Also the changes in TBAb post therapy were correlated with the changes in TBII (Post 1, $r = 0.51$, $p = 0.03$; Post 2 $r = 0.61$, $p = 0.01$). Our data indicate that ablative ^{131}I therapy causes preferential development of blocking antibodies which is reflected in an increase in TBII. Also blocking antibodies may cause the decrease in TSI activity as observed post therapy by their ability to inhibit TSI detection in FRTL-5 cell bioassay.

148. THYROID HORMONE PLASMA MEMBRANE TRANSPORT: STRUCTURAL HOMOLOGY AND BINDING SITE INTERACTIONS WITH AMINO ACIDS AND BENZODIAZEPINES. M. Lakshmanan, M. McCourt, Y. Liu, and L. Kragie, Dept of Medicine, MetroHealth Med Ctr, Cleveland, OH, Electron Diffraction Dept, Medical Foundation of Buffalo, Buffalo, NY, and ADARC, McLean Hospital, Belmont, MA.

Thyroid hormone cellular uptake is inhibited in the presence of amino acids and benzodiazepines (BZ). Previously it has been shown that the structure activity relationship (SAR) of BZ inhibition correlates strongly with halogen substitution of the nonfused phenyl ring and indicates that this ring is required for activity. A SAR series of thyromimetic inhibitors to the HepG2 iodothyronine transporter further demonstrates the critical importance of the amino group of the alanine side chain, it's L stereo configuration, and the size of the substituents of the inner and outer phenyl rings. In a mouse neuroblast model it has been shown that thyroid hormone transport is inhibited by system-L amino acids. The effect of twenty amino acids on T_3 and T_4 transport was quantified by determining their Ki's. All were in the mM range. Using both the BZ and the thyromimetic SAR, along with computer assisted molecular modeling techniques, the important chemical structural components were determined for the transporter interaction site. By superimposing structures from more active inhibitors, excluding residues from poor inhibitors, and incorporating molecular electrostatic potential data, we developed a five point model of conformational similarity to the endogenous transporter ligand, L-T_3. The alkyl substitution at the N1 of the BZ ring seems to simulate the alanine side chain of T_3, and the electronegative halogen and oxygen atoms of substituents at R3/R7/R2'/R4' of BZ form a pyramidal pharmacophore that seems to correspond with the 3-I/5-I/3'-I/4'-OH substituents of T_3 respectively. These points, suggesting a tilted crossbow formation, may be sites for ligand interaction with the iodothyronine transporter.

150. **DIVERGENT CAMP RESPONSES TO HCG IN DIFFERENT CELL LINES EXPRESSING THE RECOMBINANT HUMAN TSH RECEPTOR (hTSHr).** S. Poertl, M. Broecker*, J. Hammer*, K. Mann+ and R. Hoermann+, Medical Department II, Klinikum Grosshadern, University of Munich, 81377 Munich, *Clinic of Internal Medicine, University of Bochum, 44789 Bochum and +Dep. of Endocrinology , University of Essen, 45122 Essen, Germany.

Hyperthyroidism has been observed frequently in association with trophoblastic tumors or pregnancy and is thought to be mediated by hCG, which is present in high serum concentrations in such patients. In vitro studies, however, have so far yielded conflicting data with respect to the thyrotropic potency of hCG. We have therefore undertaken the present studies to assess the in vitro thyrotropic activity of hCG and its variant asialo-hCG in different cell lines expressing the hTSHr. Purified hCG (~12,000 IU/mg) was obtained by ion exchange and gel chromatography from crude urinary hCG (Ayerst), and asialo-hCG by digestion of purified hCG with neuraminidase. Two CHO cell clones, IP09 and IP26, transfected with the hTSHr were kindly provided by Dr. Vassart, Brussels, Belgium. In addition, a human thyroid carcinoma cell line (HTC, donated by Dr. Goretzki, Duesseldorf, Germany), that expressed the recombinant, but lacked the endogenous TSH receptor, was used. ^{125}I-bTSH binding to the transfected cells was studied, in the presence and absence of unlabeled bTSH, hCG or asialo-hCG, at 4° C for 24h in modified Hank's buffer without NaCl supplemented with 278 mM sucrose and 0.25% BSA. cAMP release in response to bTSH, hCG or asialo-hCG was measured by a commercially available RIA after incubating the cells at 37° C for 2 h. Specific binding of ^{125}I-bTSH was 26% to IP09, 7% to IP26, 6% to HTC-TSHr and <1% to the different wild type cells. The receptor number per cell delineated by Scatchard plotting was 34,000, 2,000, 4,000, in IP09, IP26 and HTC-TSHr cells, respectively. In all transfected cells, hCG showed little activity to inhibit ^{125}I-bTSH binding, whereas asialo-hCG was much more potent in this respect (ID50 10 - 20 mg/L). With respect to cAMP response to hCG and asialo-hCG, on the other hand, marked differences were observed among the different cells. IP09 cells were markedly stimulated by both hCG and asialo-hCG (1 to 100 mg/L), IP26 cells showed little, if any, response to hCG, but were significantly stimulated by asialo-hCG, whereas HTC-TSHr cells were completely unresponsive to both hCG and asialo-hCG. Maximum stimulation by asialo-hCG relative to bTSH was 44% in IP09, but less than 6% in IP26 cells. In conclusion, when hTSHr is expressed in different cell clones its functional activation by weak thyroid stimulators such as hCG and asialo-hCG may vary considerably. The variation observed seems to be related to receptor density as well as postreceptor mechanisms. Thus, the physiological meaning of hCG response by cells of non-human origin expressing supraphysiological number of hTSHr per cell remains questionable.

151. PRIMARY HORMONOGENIC SITES AS CONSERVED AUTOEPITOPES IN MURINE AUTOIMMUNE THYROIDITIS: ROLE OF IODINATION. Y.M. Kong, D.J. McCormick°, Q. Wan, R.W. Motte*, B.E. Fuller, A.A. Giraldo* and C.S. David°, Wayne State Univ. School of Medicine and *St. John Hospital, Detroit, MI, and °Mayo Clinic, Rochester, MN.

Several synthetic peptides correlating with a.a. sequences on human thyroglobulin (hTg) have been found to induce moderate thyroiditis or activate mouse Tg (mTg)-primed T cells to transfer disease in mice susceptible to experimental autoimmune thyroiditis (EAT). These reports support our earlier hypothesis that conserved epitopes and those unique to mTg contribute to the total thyroiditogenicity of mTg. One peptide, a 12mer, contained tyrosine corresponding to the hormonogenic site at position 2553; it was not thyroiditogenic unless the tyrosine had been substituted with thyroxine (T4) (Hutchings et al., J.Exp.Med. 175:869, 1992). However, noniodinated, thyronine (T0)-containing peptide was not compared. We therefore posed two questions: 1) if other primary hormonogenic sites are likewise immunogenic, and 2) if iodination was requisite for this site and those at 5 and 2567 to be an autoepitope. We derivatized three pairs of 12mer peptides (1-12, 2549-2560, 2559-2570), with T4 or T0 at positions 5, 2553 or 2567 respectively, using N^{α}-FMOC protection chemistry on Rink polystyrene resin. The peptides were tested for their capacity to stimulate mTg-primed cells in vitro (5-20 μg/ml) and to immunize mice (50 μg with adjuvant). Of the three T4-containing peptides, only hT4(5) and hT4(2553) served as stimulus for mTg-primed T cells in vitro, with hT4(2553) being the stronger. hT4(2567) and hT0(2567) were deficient in immunogenicity, neither stimulating mTg-primed T cells in vitro nor immunizing mice to produce primed T cells or antibodies. On the other hand, hT0(2553), similar to hT4(2553), activated mTg-primed T cells to transfer thyroiditis and were immunogenic for mice, producing antibodies and primed T cells which can be expanded with peptide in vitro to transfer EAT. By comparing T4- and T0-containing peptides in parallel, our data show that antigenicity of the conserved hormonogenic sites is intrinsic, dependent more on their a.a. composition than saturation of the iodination sites. Iodination may enhance antigenicity, but our data suggest that it is not required for a hormonogenic site to be an autoepitope. (Supported by NIH DK 45960 and St. John Hospital)

152. DYNAMICS OF T4 AND T3 DEIODINATION IN MAN - DOMINANT ROLE OF EXTRAHEPATIC METABOLISM. S.J. Eng, J.S. LoPresti, H. Liang and J.T. Nicoloff, USC School of Medicine, Los Angeles, CA

The influence of liver and other "rapidly equilibrating" tissues in regulating T_4 and T_3 activation and disposal has been a subject of substantial controversy. Taking advantage of the observation that all injected thyronines initially distribute to the liver, we devised a method of determining the relative rates of deiodination of labeled thyronines in both the "rapid" (hepatic) and "slow equilibrating" tissue pools *in vivo* in man. This method employs the simultaneous intravenous injection of a purified ^{125}I labeled thyronine (T_4, T_3, rT_3) and Na ^{131}I (serving as a reference source for the ^{125}I generation). The deiodination of the thyronine is then assessed by counting $^{125}I/^{131}I$ in serially collected timed urine samples. Using this method, we observed that both ^{125}I T_4 and ^{125}I T_3 underwent minimal (<10%) deiodinative disposal in the initial 3 hrs (hepatic phase), which was modestly reduced by PTU administration (100mg/hr X 9 hrs). In contrast, ^{125}I rT_3 underwent prompt deiodination during this same period (>95%) and was markedly inhibited by PTU administration. The rate of deiodinative disposal for T_4 and T_3 was unaltered until 18 hrs when the rate of deiodination progressively increased, reflecting metabolism in slow tissue pools. At this time and subsequently, PTU (100mgm/hr X 6) significantly inhibited and T_3 (25μg TID X 2 D, pre-injection) accelerated ^{125}I T_3 deiodination. In contrast, no alteration in rT_3 deiodinative disposal was seen after 12 hrs, indicating the absence of slow pool metabolism. Conclusions: 1) Hepatic type I, 5'D is not important in direct deiodinative metabolism of either T_4 or T_3; 2) Entry of T_4 and T_3 into slow equilibrating tissues is very delayed (>18 hrs); 3) Both T_4 and T_3 must undergo obligatory transformation to other metabolites (ie, rT_3, sulfate conjugation, side-chain deamination) before serving as substrates for hepatic 5'D; 4) This latter observation is consistent with the low Vmax/Km values for T_4 and T_3 determined *in vitro*; 5) Some unknown slow equilibrating tissue site(s) appears to be primarily responsible for peripheral T_4 to T_3 conversion and regulation of circulating T_3 levels in man.

156. IMAGING OF CALCIUM FLUXES IN INDIVIDUAL THYROID CELLS. R.W. Lash, C.A. Zimmerman, C.W. Balke, P.S. Shacklock, Divisions of Endocrinology and Cardiology, Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland.

Signal transduction in thyroid cells is primarily mediated by the cAMP and phosphoinositol (PI) pathways. In thyroid cells, activation of the P_1 purinergic receptor is linked to PI hydrolysis, inositol-(1,4,5)-triphosphate (IP_3) generation, and increases in intracellular calcium levels. It has been proposed that the local release of purinergic agonists in the thyroid may modulate the effects of TSH and thyroid stimulating immunoglobulins (TSI) on thyroid function. In this study, we imaged intracellular calcium fluxes in individual rat thyroid FRTL-5 cells after treatment with the purinergic agonist ATP, as well as TSH and TSI. Nonconfluent monolayer cultures of FRTL-5 cells were loaded with Fura 2-AM, and perfused at room temperature with a buffered saline solution containing 0.4 mM $CaCl_2$. Cell fluorescence was stimulated with alternating UV wavelengths of 340 and 380 nm and photomicroscopic images were captured at 10 second intervals. Data on ATP treated cells were collected on individual cells in groups of five, and repeated in triplicate. The fluorescence of individual cells over time was analyzed as both pseudocolor images and line plots. The resting levels of intracellular calcium were 30 to 50 nM before and after treatment with ATP. Exposure of cells to ATP (100 μM) resulted in a rapid (30 second) 50-fold increase in intracellular calcium concentrations to greater than 2000 nM. This increase was sustained throughout the 6.5 minute treatment period. Preliminary studies were also carried out with TSH and TSI. Both ligands increased intracellular calcium concentrations in a peak and plateau pattern consistent with mobilization of IP_3 sensitive intracellular calcium stores followed by the entry of extracellular calcium. In conclusion, we have demonstrated increases in the intracellular calcium concentrations of individual thyroid cells in response to ligands which activate the PI pathway, including ATP, TSH, and TSI. This technique can be used to study the role of PI hydrolysis in thyroid function, and may be useful diagnostically in Graves' disease.

157. THYROTROPIN (TSH) REGULATES THYROID GROWTH FACTORS IN HYPOPHYSECTOMIZED (HYPOX) RATS. G.P. Becks*, M. Forsyth**, A. Logan** and D.J. Hill*, *Department of Medicine, St. Joseph's Health Centre and the Lawson Research Institute, University of Western Ontario, London, Canada and **Department of Clinical Chemistry, The University of Birmingham, Birmingham, UK.

We recently reported on alterations in the expression of transforming growth factor beta ($TGF\beta_1$), basic fibroblast growth factor (basic FGF), insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs) in normal and hypertrophic/hyperplastic rat thyroids, confirming their likely autocrine/paracrine roles in thyroid growth and function. Further studies were undertaken to elucidate regulation of the thyroid growth factor axis in vivo and in vitro by TSH and low-iodine diet (LID), and vis-à-vis other possible growth factor interactions, utilizing hypox male Fischer rats and primary cultures of sheep thyroid follicles. Morphological point counting studies of H and E-stained paraffin sections revealed a 2 to 5-fold increase in thyroid epithelial/colloid surface area, and thyroid/bodyweight (BW) ratio (mg/100 g) in intact and sham-hypox rats on LID after 1 and 2 weeks (wks) vs. controls. Similar results were seen in hypox-LID-bTSH (100 mIU/100 gBW sc daily X 1 or 2 wks) - treated rats compared to hypox, hypox-LID or hypox-TSH rats. Immunocytochemical (ICC) staining for basic FGF and IGF-I was prominent over the cytoplasm of hypertrophic/hyperplastic epithelial sections as above at 1 or 2 wks, while slight or patchy staining was apparent in controls and in all other hypox groups. $TGF\beta_1$ ICC was prominent over the epithelial cytoplasm, perivascularly and in adjacent C-cells in normal and sham-hypox controls, which increased and additionally localized to the nuclei in hyperplastic epithelium following LID, a potentially novel observation. No increase in intensity of $TGF\beta_1$ staining was evident in hypox-LID-TSH rats. $TGF\beta_1$ staining decreased in all other hypox rats as well. Quantification of mRNA by ribonuclease protection assay and scanning densitometry using cyclophilin as an internal control, showed no rapid increase in TGFß expression in hypox rat thyroids with LID although this was seen after 1 wk in intact and sham-hypox animals. However, $TGF\beta_1$ mRNA did increase in 2 wk hypox-LID and hypox-LID-TSH rats. In vitro, $hTGF\beta_1$ inhibited TSH-stimulated iodine uptake and organification in primary cultures of sheep thyroid follicles (ED_{50} 1ng/ml) and increased secretion of IGFBP-2, -3 and -5 into conditioned media as assessed by ligand blotting with ^{125}I-IGF-II. These effects were prevented by $TGF\beta_1$ neutralizing antisera. These data underscore the central importance of the pituitary to normal and hyperplastic thyroid morphology and growth and support our hypothesis of a hierarchical interdependence of TSH, low-iodine status and local autocrine/paracrine growth factors. While increased autocrine expression of basic FGF and IGF-I were positively correlated with TSH-induced epithelial hyperplasia, the role and regulation of $TGF\beta_1$ are more complex, including probable inhibitory effects on function and growth involving additional intracrine or paracrine mechanisms of actions.

164. THYROID PEROXIDASE (TPO) ACTIVITIES AND TPO mRNA LEVELS IN THE PRESENCE OF PROPYLTHIOURACIL AND METHIMAZOLE IN CULTURED PORCINE THYROID FOLLICLES. M. Sugawara, H. Murai, PE. Cizdziel, VA Medical Center, West Los Angeles, University of California Los Angeles, School of Medicine, Los Angeles, CA. and Life Technology Inc., Germantown, MD.

Propylthiouracil (PTU) and methimazole (MMI) inhibit the action of thyroid peroxidase (TPO). We examined whether PTU or MMI inhibits cellular TPO activity and TPO mRNA levels in cultured porcine thyroid follicles. Porcine thyroid follicles were cultured in 6-well culture plates in the presence of 1 mU/ml bovine TSH. From the sixth day of culture, follicles were exposed to 0, 10 μM, and 100 μM PTU or MMI for 5 days. TPO activity was measured in the 100,000 x g pellet of the thyroid sonicate by the guaiacol oxidation method. TPO mRNA levels were measured by the Northern blot using pig TPO probe (PF6) provided by Dr. Rapoport. The quantitive analysis of the Northern blot was done using LYNX Image Analyzer. PTU and MMI at 10 μM increased TPO activity by 10 to 50% from the baseline values in four different experiments. MMI at 100 μM constantly inhibited TPO activity by 50%, however, PTU at 100 μM did not inhibit TPO activity. Exposure to 10 μM PTU and 10 μM MMI caused 2-fold and 1.5-fold increase in TPO mRNA levels, respectively. PTU at 100 μM and MMI at 100 μM decreased TPO mRNA by 50% and 14%, respectively. Conclusions. 1. PTU or MMI at the concentration of 10 μM increases both cellular TPO activity and TPO mRNA level. 2. A high concentration of PTU or MMI inhibits TPO mRNA; however, TPO activity does not always reflect TPO mRNA levels.

166. MELITTIN, A MEMBRANE ACTIVE PEPTIDE, INCREASES IODIDE EFFLUX INDEPENDENTLY OF PHOSPHOLIPASES C AND A_2 IN FRTL-5 CELLS. R.C. Smallridge, X-D. Wang, and I.D. Gist, Walter Reed Army Institute of Research, Washington, DC.

Melittin, a membrane active cytolytic peptide from bee venom, activates phospholipase (PLC) and phospholipase A_2 (PLA_2) in some tissues. As iodide efflux (IE) requires activation of both PLs by several agonists (ATP, TSH, norepinephrine), we investigated the effects of melittin on phospholipases and IE in FRTL-5 cells. Cytosolic calcium ($[Ca^{2+}]_i$) was measured with Indo-1, PLA_2 activity by $[^3H]$ arachidonic acid (AA) release, and iodide transport by ^{125}I uptake or release. Melittin (0.1-1.0 μM) dose-dependently increased $[Ca^{2+}]_i$ by mobilizing from intracellular stores. At 0.3 μM the melittin effect on increasing $[Ca^{2+}]_i$, $[^3H]AA$, and IE mimicked that of 5 μM ATP. Melittin also inhibited iodide uptake by ~45%. A PLC inhibitor, U-73122, {1-[6-[[17ß-3-methoxyestra-1,3,5(10)trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione} (Upjohn Co.) abolished the $[Ca^{2+}]_i$ increase. PLA_2 activation depended on both Ca^{2+} and protein kinase C (PKC), as partial inhibition of $[^3H]AA$ release occurred with TMB-8, absence of extracellular Ca^{2+}, a PKC inhibitor (bisindolylmaleimide, GF 109203X) and PKC down regulation (confirmed by Western analysis of PKC isoforms). U-73122 abolished $[^3H]AA$ release, confirming a PLC requirement for melittin activated PLA_2. The aminosteroid PLA_2 inhibitor U-26384, N-[3-(dimethylamino) propyl-3-methoxy-N-methylestra 2,5(10)-dien-17ß-amine, prevented $[^3H]AA$ release without altering the $[Ca^{2+}]_i$ increase. Melittin (0.1-1.0 μM) increased iodide efflux dose dependently. Neither U-73122 nor U-26384 reduced melittin induced IE, whereas both abolished ATP induced iodide efflux. Conclusions: (1) Melittin activates two second messenger pathways in a highly regulated, inhibitable manner, (2) Melittin enhances iodide efflux via a pathway independent of the phospholipases required for receptor stimulated IE, (3) The effect on iodide uptake suggests Na^+/K^+ ATPase inhibition. These studies highlight the complex nature of iodide transport in FRTL-5 cells.

171. BOTH 3' AND 5' REGULATORY ELEMENTS ARE INVOLVED IN THE TISSUE-SPECIFIC HORMONAL REGULATION OF EGF mRNA. L.G. Sheflin, E.M. Brooks and S.W. Spaulding, Buffalo VAMC and SUNYAB, Buffalo NY, 14215

Androgen receptors have been demonstrated in the thyroid gland, and androgen treatment can block the action of some goitrogens and carcinogens in the mouse thyroid. Epidermal growth factor (EGF) is an autocrine thyroid gland regulator whose mRNA precursor levels increase when the circulating level of thyroid hormone is raised in the mouse. EGF is also responsive to testosterone in some tissues, so it seemed of importance to determine whether testosterone also regulated EGF transcription in the thyroid. Potential thyroid and androgen response elements identified in the 5' regulatory region of the EGF gene probably play a role in the increased transcription of EGF in the submaxillary gland (SMG) of both sexes when thyroxine or testosterone is administered. In the male thyroid, however, EGF protein only responds to increased circulating levels of thyroid hormone, but does not respond to the administration of testosterone. Since it was possible that the lack of response in the male simply reflected the fact that high basal levels of testosterone had already caused a maximal response, we treated 10 week old female mice with testosterone propionate 200 ug QOD SQ in sesame oil for a week and measured EGF mRNA in total RNA by RT-PCR, using primers unique for mature EGF and primers unique for the 3' UTR to measure poly-A tail length. The level of mature EGF mRNA rose 3-fold ($p<0.05$) and poly-A tail length increased 5-fold (from 20 to 100 A's) in the SMG, but there was no response in the thyroid. In contrast, thyroid hormone increased mature EGF message 2-3-fold in both SMG and thyroid, but the increase in poly-A tail length was only evident in the SMG. Thus the mouse EGF gene contains 5' regulatory elements which are involved in enhanced transcription in some tissues, but the EGF transcript also contains 3' regulatory elements involved in alternative sites of polyadenylation and altered poly-A tail lengths, which also can play a role in tissue-specific hormone responsiveness. We have identified several regulatory elements in the 3' UTR of the EGF gene which can alter the steady-state levels of EGF message, including alternate sites of polyadenylation, five copies of the ATTTA pentamer involved in message stability and two cytoplasmic polyadenylation signals (TTTTTAT). Tissue-specific factors are involved in both the post-transcriptional as well as the 5' regulatory elements to determine the hormone responsiveness of this autocrine factor.

175. **DELINEATION OF AMINO ACID RESIDUES WITHIN hTSHr 256-275 THAT PARTICIPATE IN HORMONE BINDING.** W.P. Bryant, E.R. Bergert, and J.C. Morris, Endocrine Research Unit, Mayo Clinic & Foundation, Rochester, MN 55905.

The amino acid sequence 256-275 of the human TSH receptor extracellular domain (hTSHr-ECD) has previously been shown to participate in a high affinity TSH binding site by a synthetic peptide approach (J. Biol. Chem., 268:10900, 1993) as well as site directed mutagenesis (Endocrine J., 40:363, 1993). To further investigate this binding site, we synthesized a series of peptides with alanine substitutions for each residue, as well as peptides containing truncations or deletions of the native sequence. The peptides were synthesized using Fmoc chemistry and purified by HPLC. The molecular weight and amino acid composition of each was confirmed by mass spectroscopy and automated amino acid analysis. Each peptide was tested for its ability to inhibit ^{125}I-bTSH binding to porcine thyroid membrane preparations, and the concentration at which 50% inhibition of binding occurred was calculated (EC_{50}). Alanine substitution at residues Tyr^{258}, Cys^{262}, Cys^{263}, Phe^{265}, Lys^{266}, Asn^{267}, Lys^{269}, Lys^{270}, and Arg^{272} all resulted in statistically significant decrease in activity when compared to the native sequence ($p<0.05$). Alanine substitution of the remaining residues did not alter activity. As shown below, these residues lie within one of the most highly conserved regions of the glycoprotein hormone receptor extracellular domains.

```
hLHr   251  LTYPSHCCAFRNLPTKEQNF  270
            |.|||||||:|
hTSHr  256  LSYPSHCCAFKNQKKIRGIL  275
            |.|||||||| | ::  : |
hFSHr  269  LTYPSHCCAFANWRRQISEL  288
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We conclude that nine specific amino acids within the sequence 256-275 of hTSHr, (Y---CC-FKN-KK-R), participate in the interaction of the hTSHr-ECD with TSH. This may represent a binding site in which the non-conserved residues are involved in the binding of the β-subunit, and the conserved residues are involved in the binding of the α-subunit or a region of the β-subunit that is common to all glycoprotein hormones.

180. INVOLVEMENT OF THYROID-SPECIFIC TRANSCRIPTION FACTORS IN HORMONE- AND SERUM-DEPENDENT REGULATION OF THE THYROGLOBULIN GENE EXPRESSION IN FRTL-5 CELLS. F. Kambe and H. Seo. Department of Endocrinology and Metabolism, Research Institute of Environmental Medicine, Nagoya University, Nagoya, JAPAN

Recently it has been shown that some tissue specific transcription factors are involved not only in the differentiation and maintenance of cell-type specific function but also in hormonal regulation of certain genes. However, involvement of thyroid-specific transcription factors in hormonal regulation of thyroglobulin (TG) gene is not well understood. This study aimed to clarify the contribution of the thyroid-specific transcription factors (TTF-1, TTF-2 and Pax-8) to the hormone- and serum-dependent regulation of TG gene. Rat TG promoter (-178 to -3) previously shown to be a minimum sequence required for tissue-specific expression of TG gene was fused to a reporter gene, luciferase. This construct was transfected into FRTL-5 cells 4 days after culturing in the basal medium (0.5 % calf serum with no supplement of TSH and insulin). TSH addition (1mU/ml) for 2 days resulted in 2.0-fold induction of the luciferase activity. Insulin (10 μg/ml) increased the activity by 2.7-fold and 5 % serum by 2.3-fold. The endogenous TG mRNA level was also increased by these additives. These results indicate that the minimum TG promoter confers responsiveness of TG gene to TSH, insulin and serum. Since the binding sites for TTF-1, TTF-2 and Pax-8 are present in the minimum promoter, their binding activity to the promoter sequence was determined by electrophoretic mobility shift assay (EMSA). The whole cell extracts from the FRTL-5 cells cultured in the basal medium containing TSH, insulin or 5 % serum for 3 days were used for the assay. It was noted that the amount of protein in the cell extract was increased significantly by these additives. However, the DNA content was not altered. When the binding activity per unit DNA was examined, TSH, insulin or serum markedly increased the binding of all three factors. This observation suggests that these three transcription factors are involved in the hormone- and serum-dependent induction of TG gene transcription. The levels of TTF-1 and Pax-8 mRNA determined by Northern blot analysis were neither affected by insulin or serum, while both levels were decreased by TSH. The binding activities of TTF-1 and Pax-8 had, thus, no correlation with their mRNA levels. Taken together, the present results suggest that TSH, insulin and serum increase the binding activities of thyroid-transcription factors, TTF-1, TTF-2 and Pax-8, to the TG promoter presumably through the modification at a posttranslational level to induce the TG gene transcription.

189. CHANGES OF SERUM SULFATED IODOTHYRONINES AND THE SEVERITY OF LIVER CIRRHOSIS. W.S. Huang, T.H. Yung, S.W. Kuo, H.S. Tang, W.L. Chen, S.Y. Wu. Departments of Nuclear Medicine and Internal Medicine, Tri-Service General Hospital, Taipei, R.O.C.; Nuclear Medicine Service, VA Medical Center, Long Beach, CA.

The liver plays an important role in peripheral metabolism of thyroid hormones. Abnormalities in thyroid function tests are very common in patients with liver disorders but may vary with the severity of the disease. There is considerable evidence that hepatic type I monodeiodinase activity (MD-I) is reduced in cirrhosis. Recent studies have shown that sulfation pathways are accentuated in states where tissue MD-I is decreased, eg. in fetuses or in selenium-deficient rats. The present study was carried out to correlate the severity of liver cirrhosis and the changes in serum levels of thyroxine sulfate (T_4S), T_3S and rT_3S. Thirty age-matched healthy subjects and 45 cirrhotic patients were recruited. The latter was further divided into 3 subgroups according to the Child's classification. Serum levels of sulfated iodothyronines and other thyroid hormone parameters were also measured by RIA. The results are shown in the table below:

	(n)	T3	T4	FT4	rT3	TSH	TBG	T4S	T3S	rT3S
NL	30	102.1± 3.3	7.6± 0.3	1.29± 0.04	22.8± 2.2	1.3± 0.1	31.7± 1.0	<1	2.7± 0.4	7.0± 1.1
A	15	105.0± 7.2	8.8± 0.6	1.28± 0.08	24.9± 3.1	1.0± 0.1	32.8± 2.2	<1	4.6± 0.6	12.0±** 0.9
B	15	70.9±*** 6.2	6.5±** 0.4	0.98±* 0.05	28.4±* 3.0	1.5± 0.3	25.0±** 1.5	1.1± 0.03	6.4±* 0.6	18.1±** 1.3
C	15	41.3±** 4.4	5.0±* 0.5	0.81± 0.06	41.1±* 8.2	1.1± 0.3	19.3±* 1.3	4.2± 0.7	14.8±*** 0.7	32.1±** 3.6

Units: ng/dl for T3, rT3, T3S, T4S and FT4, ug/dl for T4, mU/L for TSH. Child's class A, B, and C represent the severity of liver cirrhosis. *p<0.05, **p<0.01 and ***p<0.005 cf. adjacent group.

The results show that serum T3S and rT3S levels are increased relative to the severity of liver cirrhosis and may be due to a proportional reduction of hepatic MD-I activity.

190. THE MECHANISM OF PEROXIDASE-CATALYZED COUPLING AND ITS STIMULATION BY LOW CONCENTRATIONS OF FREE DIIODOTYROSINE. Daniel R. Doerge, Martha L. Dorris and Alvin Taurog. National Center for Toxicological Research, Jefferson, AR 72079 and University of Texas Southwestern Medical Center, Dallas, TX 75235.

Two mechanisms for peroxidase-catalyzed coupling have been proposed: a radical mechanism (Taurog, *Rec. Prog. Horm. Res.* **26**: 189, 1970), and an ionic mechanism (Gavaret *et al. J. Biol. Chem.* **256**: 9167, 1981). Evidence is presented here favoring the radical mechanism. Single turnover experiments were performed using horseradish peroxidase (HRP). HRP was used because, unlike TPO and lactoperoxidase (LPO), the spectral properties of its compound I and II forms are readily distinguishable. This made it possible to correlate the kinetics and stoichiometry of T_4 + T_3 formation with spectral data. Human goiter thyroglobulin (Tg) was iodinated with $^{125}I_3^-$ to produce iodinated Tg (~28 atoms I/molecule) in which almost all of the ^{125}I was present as DIT and MIT residues and only a small percentage as T_4. HRP compound I (HRP-I) was prepared by addition of equimolar H_2O_2 to native HRP. Incubation of 2 μM preformed HRP-I with 2 μM ^{125}I-Tg for 10-15 min at pH 7.0 in the presence of 1 μM free DIT (a known stimulator of peroxidase-catalyzed coupling) yielded about 0.8 residue T_4 and 0.2 residue T_3 per molecule of Tg. This represents the theoretical maximum for iodothyronine formation, demonstrating remarkably efficient use of the oxidizing equivalents in HRP-I for coupling. These results indicate that 1) only hormonogenic residues in Tg were oxidized, and 2) oxidation of 2 hormonogenic residues occurred within the same molecule of Tg. The time course for formation of T_4 + T_3 was biphasic. During a rapid initial phase (< 1 min). HRP-I was completely converted to HRP-II, coincident with the formation of about 0.65 residues T_4 + T_3. During a slower second phase (10-15 min), HRP-II was completely reduced to native HRP, with formation of about 0.35 residues T_4 + T_3. These results demonstrate that HRP-II is an obligatory intermediate in the reaction, as would be expected of a radical-mediated reaction. Free DIT reacted with both HRP-I and HRP-II in one-electron transfer reactions, and the time courses for these reductions resembled those observed with DIT + Tg. Moreover, free DIT was stimulatory at substoichiometric concentrations. These observations suggest that in DIT-stimulated coupling, free DIT radicals act to shuttle oxidizing equivalents from the peroxidase intermediates to DIT and MIT residues in Tg. This could account for the remarkable efficiency of HRP-I-mediated coupling. We have also demonstrated in steady state experiments with catalytic amounts of TPO and LPO that coupling is markedly inhibited by nitrosobenzene, a known radical scavenger. Supported by NIDDK-03612 (A.T.).

197. COMPARISON OF THE UPTAKE OF TRIIODOTHYROACETIC ACID (TRIAC) AND T3 IN CULTURED ANTERIOR PITUITARY CELLS. M.E. Everts, T.J. Visser, R. Docter, M. de Jong, E.P. Krenning and G. Hennemann, Departments of Internal and Nuclear Medicine, Erasmus University Medical School, Rotterdam, The Netherlands.

When Triac is used to suppress TSH secretion in patients, it has to be applied in large doses, because of its short half-life. Although the normal serum level of Triac is rather low (50 pM), recent studies suggest that this may increase considerably during non-thyroidal illness (NTI). Therefore, Triac may be of significance for the suppression of TSH secretion in NTI, which occurs in spite of low serum T_3 and T_4 (1,2). We compared the uptake of [^{125}I]Triac and [^{125}I]T_3 and the effects of the unlabeled hormones on TSH release in anterior pituitary cells. Cells were isolated from adult male euthyroid rats, and cultured (500,000 cells/well) for 3 d in MEM with 10% fetal calf serum (3). Incubations were performed at 37 C in MEM/0.5% BSA without or with TRH (100 nM) and/or Triac, T_3 or T_4 (0.001-1 μM). Uptake was measured with [^{125}I]Triac (100,000 cpm; 120 pM) or [^{125}I]T_3 (50,000 cpm; 50 pM). The ratio of the free fractions of Triac, T_3 and T_4 in medium with 0.5% BSA was 1:8:1, respectively. Exposure of the cells to 100 nM TRH for 2 h, stimulated TSH release by 80-110%. Triac and T_3 (1 nM-1 μM, total hormone) were equally effective in reducing this response significantly, but T_4 was 10 times less effective. The uptake of [^{125}I]Triac per pM free hormone after 15 min, 1 and 4 h of incubation, was twice as large as that of [^{125}I]T_3. The presence of 1 μM Triac decreased the uptake of both isotopes at all time intervals. Neither [^{125}I]Triac nor [^{125}I]T_3 were metabolized within 4 h. After 1 h of incubation, 31% of cellular [^{125}I]Triac and 30% of cellular [^{125}I]T_3 was found in the nuclear fraction. The 15-min uptake of [^{125}I]Triac was reduced by simultaneous incubation with 10 nM Triac (35%, $P<0.001$). The maximum effect was seen with 10 μM Triac (56%, $P<0.001$). A similar effect was found with 10 μM T_3, T_4 or Tetrac. Preincubation (30 min) and incubation (15 min) with oligomycin (10 μM) reduced the cellular ATP content by 55% ($P<0.001$), the 15-min [^{125}I]Triac uptake by 25% ($P<0.001$), and the 15-min [^{125}I]T_3 uptake by 77% ($P<0.001$). The temperature dependence of [^{125}I]Triac and [^{125}I]T_3 uptake was the same, i.e. the uptake increased 5-fold when the temperature was increased from 0 to 22 C, and two-fold with a further increase to 37 C. The effect of 10 μM monensin on [^{125}I]T_3 uptake (37%, $P<0.001$) was twice as large as that on [^{125}I]Triac uptake (15%, $P<0.025$). Taken together, our data suggest that [^{125}I]Triac is rapidly taken up by the anterior pituitary by a carrier-mediated mechanism, that is only to a minor extent dependent on ATP (oligomycin) or the Na^+ gradient (monensin). Because Triac, on basis of the free hormone concentration, was more potent than T_3 or T_4 in suppression of TSH release, and the production of Triac (1) and its free fraction (2) substantially increase during NTI, it is possible that Triac is important for the control of TSH secretion during NTI.

1) Thyroid 1992, 2:S-39; 2) Medical Hypotheses 1993, 40:38-43; 3) Endocrinology 1993, 132:1278-1285

201. INHIBITORY ROLE OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS IN THE REGULATION OF THYROID FUNCTION.

Insulin-like growth factors (IGFs) are essential for TSH-mediated induction of differentiated function in human and sheep thyroid cells in culture. Medium conditioned by cells from both cultures contains insulin-like growth factor binding proteins (IGFBPs) whose synthesis and secretion are controlled sensitively by factors regulating the growth and function of the cells. The role of the individual IGFBPs in regulating the response of thyroid cells to IGFs is unknown. However following TSH addition in both cultures, IGFBP secretion is rapidly reduced. Although the species share this in common, the spectrum of IGFBPs secreted by human and sheep functional thyroid cell cultures differs markedly. The characteristic doublet of IGFBP-3 was present in functional cultures of human cells but in sheep thyroid cells, IGFBP-3 was only synthesized following challenge with inhibitors of differentiated function such as epidermal growth factor and stimulators of protein kinase C. In human cells IGFBP-3 mRNA and protein were increased following addition of transforming growth factor β. When human cells were treated with TSH, forskolin or cAMP analogs, there was an 80% reduction in IGFBP-5 mRNA levels and significant but lesser decreases in IGFBP-3 mRNA. IGFBP-4 mRNA was not regulated by TSH or stimulators of cAMP. IGFBP-2 and IGFBP-5 mRNAs were inhibited within 6h by TSH in sheep cultures with a parallel decrease in protein levels. IGF analogs (Des-IGF-I and Long R3 IGF-I (GroPep)) which bind IGFBPs with much lower affinity than IGF-I, were used to examine the effects of the IGFBPs on thyroid function. For these experiments, 7 day old cultures were incubated with TSH (300μU per ml) and IGF-I or the analogs for 72h and iodide uptake and organification were measured following a 3h pulse with ^{125}I. The analogs were more potent stimulators of the uptake and organification of iodide when used at the same concentration as IGF-I (30ng per ml). At high concentrations (100ng per ml) IGF-I was able to stimulate these parameters as well as the analogs. This disparity in the potency of IGF-I compared to the analogs, was most marked when the cells were plated at high density. The same IGF-I preparation, when used in assays of FRTL5 cell growth and colon cell growth, was equipotent to the analogs, confirming its documented activity. The data indicate that IGFBPs secreted by thyroid cells in culture are inhibitory to the actions of IGFs. The rapid reduction in IGFBP synthesis and secretion by TSH may be a mechanism to reduce these inhibitory effects thus permitting the cells to respond with increases in thyroid growth and function to exogenous or autocrine IGF-I.

203. INCREASED METASTATIC POTENTIAL OF POORLY DIFFERENTIATED HUMAN HEPATOCELLULAR CARCINOMA CELLS IS ASSOCIATED WITH AN OVEREXPRESSION OF THYROID HOROMONE β1 NUCLEAR RECEPTOR. Kwang-huei Lin[1] and Sheue-yann Cheng[2]. [1]Chang-Gung College of Medicine and Technology, Taoyuan, Taiwan; [2]Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Tumor metastasis is the result of very complex processes. Specific genes which directly or indirectly mediate these processes are largely unknown. However, reduced expression of the anti-metastatic gene *nm23* has recently been reported to be associated with several metastatic tumors. To understand the role of thyroid hormone in tumor metastasis, we studied the expression of the *nm23* gene in nine human hepatocellular carcinoma cell lines. Previously we have shown that these cell lines differentially express human β1 thyroid hormone receptor (h-TRβ1). SK-Hep-1, Mahlavu and HA22T express h-TRβ1 protein at a high level, J5, J7 and J3-28 at a moderate level, and HepG2, Hep3B and PLC/PRF/5 at a very low level. The expression of h-TRβ1 is inversely correlated with the degree of differentiation in these cells. In addition, the proliferation of cells in which h-TRβ1 is overexpressed is stimulated by 3,3',5-triiodi-L-thyronine (T_3). By Northern blot analysis, we detected a 0.8 kb *nm23* RNA in all cell lines, but with different degrees of abundance. The *nm23* RNA levels for Mahlavu, J5, HA22T, SK-Hep-1, J7, J3-28, PLC/PRF/5 and Hep3B cells were between 20-80% of that in HepG2 cells, which showed a level as high as that detected in normal RHEK-1 (a human epithelial cell line) and MRC-5 (a human lung fibroblast cell line). Using immunoprecipitation, two *nm23* proteins with molecular weights of 17.5 and 18 kd were specifically detected in all cell lines. Consistent with the mRNA levels, expression of *nm23* proteins was lowest in HA22T, Mahlavu, SK-Hep-1 and J5 cells, intermediate in J7, J3-28 and PLC/PRF/5 cells and highest in HepG2 and Hep3B cells. These results indicate an inverse correlation in the expression of *nm23* gene and h-TRβ1. Using an *in vitro* metastatic assay, we found that metastatic potential is highest in SK-Hep-1 and Mahlavu cells which express low levels of *nm23* proteins and lowest in HepG2, Hep3B and PLC/PRF/5 which have high levels of *nm23* proteins. Treatment of these cell lines with T_3 led to a reduction of h-TRβ1 protein and a decrease in metastatic potential. These results indicate that the increased metastatic potential is associated with a high level of h-TRβ1 and that T_3 modulates metastatic potential by regulating the expression of h-TRβ1.

207. NEUROTHYRONINES: MORPHOLOGIC EVIDENCE DEMONSTRATING A NON-GENOMIC ROLE FOR T3 IN LOCUS CERULEUS (LC) AND ALL OTHER BRAIN STEM NORADRENERGIC (NA) NUCLEI.

An association between thyroid hormones and adrenergic neurotransmitters has been established on the basis of considerable clinical, physiologic, functional, and molecular evidence. We now report several findings derived from T3 immunohistochemistry (T3-IHC) which greatly strengthen the case for a direct association between T3 and NA functions in rat brain. Surprisingly, and previously unreported despite the use of a variety of techniques to study thyroid hormone distribution in brain, highest concentrations of T3 were in LC and all other brain stem NA nuclei. Moreover, label (specific for triiododiphenylether compounds) within the cells of these nuclei was uniquely distributed. In almost all other T3-IHC-labeled brain cells, major concentration was over the nucleoplasm, with lesser concentrations in cytosol and nerve cell processes. However, the highly conspicuous label in NA cells was distributed in clumps within their perikarya and in beads along the course of their thick cell processes (axons and dendrites) while their nuclei were conspicuously free of reaction product. Moreover, the wide distribution of T3 and T3 nuclear receptors can now be understood as reflecting the widespread distribution of NA projections; there is newly-revealed concentration of the hormone in neuropil, neuronal cell bodies and interconnecting nerve cell processes in terminal fields of NA projection sites. Taken together, the evidence suggests that NA systems may be the sites of maximal T3 investment in rat brain.

208. HETEROGENEITY IN mRNA ENCODING THYROGLOBULIN'S C TERMINUS. ME Mason, SJ Hoback, JT Dunn, University of Virginia, Charlottesville, VA.

Thyroglobulin (Tg) contains specific sites for thyroid hormone formation and abnormalities in Tg may cause human disease. We examined the mRNA encoding regions of 4 important hormonogenic sites in 7 human thyroids (normal, goiters, Graves disease) by the following methods. RNA was extracted from thyroid tissue and reverse transcribed, and selected regions of Tg cDNA were amplified by the polymerase chain reaction. Amplified sequences corresponded to Tg cDNA bases 11-317 (N terminus, including hormonogenic site A) and 7626-8360 (C terminus, including hormonogenic sites B, C and G). Amplified products were purified, digested with restriction endonucleases and subcloned into plasmid vectors. These clones were sequenced and compared to the Tg sequence published by Malthiery (Eur. J Biochem, 165, 1987). We found that Tg's N terminus had a G instead of T at nucleotide 78 in all clones examined (7 clones from 5 subjects), suggesting an error in the published sequence. This alteration in the nucleotide sequence does not change the translated amino acid at residue 26 (valine). Thus, the N terminus appeared truly invariant. In contrast, clones of Tg's C terminus exhibited considerably more variability in nucleotide sequence. The most predominant alteration was deletion of CT at bases 7870-71, a frame shift mutation changing the deduced amino acid sequence beginning at residue 2624, that would also introduce a stop codon at nucleotide 8013. This deletion was present in a variety of diseased and normal thyroids. Several other changes in the C terminus were noted, including a deletion of 134 bases in two siblings with Pendred's syndrome, and several single base changes. These observations indicate heterogeneity in the mRNA encoding Tg's C terminus, in contrast to that in the N terminus. These alterations are in the region of important hormonogenic sites and thus may affect thyroid hormone synthesis and contribute to the development of goiter.

214. **CLINICAL RELEVANCE OF AUTOANTIBODIES WITH DUAL SPECIFICITY FOR THYROGLOBULIN AND THYROPEROXIDASE.** U. Feldt-Rasmussen, L. Hegedüs, M. Ferrand, J. Ruf and P. Carayon. Dept of Med P, Div of Endocrinology, Rigshospitalet, and Med. Dept of Endocrinology, Herlev Hospital, Copenhagen University, Copenhagen, Denmark; U38 INSERM, Faculté de Médecine, Marseille, France.

Recent evidence has indicated that thyroid autoimmune disorders may be associated with the presence of circulating autoantibodies (aAb) with dual specificity for thyroglobulin (TG) and thyroperoxidase (TPO). Whether the determination of these TGPO aAb has any clinical relevance either alone or in combination with measurement of TG and TPO aAb has not been investigated. The availability of purified preparations of both human TG and TPO allowed the development of a specific and sensitive RIA for TGPO aAb in serum. In the present study, we compared bispecific TGPO aAb levels and total TG and TPO aAb levels, respectively, in serum of 84 normal controls and 226 patients with various thyroid and autoimmune diseases, including non-toxic goiter ($n=50$), toxic nodular goiter ($n=13$), thyroid carcinoma ($n=20$), primary idiopathic myxedema ($n=15$), postpartum thyroiditis ($n=11$), Hashimoto's thyroiditis ($n=38$), pernicious anemia ($n=27$), rheumatoid arthritis ($n=19$) and insulin dependent diabetes mellitus ($n=33$). In addition, 16 patients with Hashimoto's thyroiditis were studied before therapy and after more than 3 months of treatment with L-T_4. It was shown that TGPO aAb were generally but not always present in serum of patients with Hashimoto's thyroiditis, which also contained TG and TPO aAb. In contrast, TGPO aAb were undetectable in normal controls (excepting a few cases reaching borderline levels) as well as in sera from the majority of the other patients tested. Selecting sera positive for TGPO and either TG or TPO aAb showed a statistically significant correlation between TGPO and TG ($n=26$, $P<0.005$) but not TPO aAb. Interestingly, TGPO aAb levels decreased significantly in patients with Hashimoto's thyroiditis after L-T_4 therapy ($P<0.05$), some of them shifting from TGPO aAb positive before treatment to negative after treatment. TGPO aAb determination thus distinguishes patients with Hashimoto's thyroiditis from patients with other thyroid and/or autoimmune disease. The specific presence of TGPO aAb in a subset of patients with Hashimoto's thyroiditis and their variation during L-T_4 therapy remain to be understood. This could contribute to understanding the mechanisms of autoimmune thyroid disease.

220. EXPRESSION AND TSH BINDING OF MUTANT TSH-RECEPTOR (PRO52 → THR) OCCURRING IN AUTOIMMUNE HYPERTHYROIDISM

Mutations in the TSH-receptor (TSHR) are suspected to participate in the multifactorial etiology of autoimmune hyperthyroidism. Recently, we described the first known heritable mutation in the TSHR gene leading to the replacement of a proline by threonine at amino acid position 52 in the extracellular domain of the receptor protein. Secondary structure prediction indicated a major change of protein structure as a result of the mutation. In order to examine the ability of the mutant receptor to insert into plasma membrane and to bind TSH following studies were performed. A new eukaryotic expression vector for wildtype (wt) and mutant (mut) TSHR was constructed including the entire coding region of the human TSHR cDNA from restriction sites AvaI to HpaI. Cos-7 cells were transfected using the calcium phosphate transfection method. Competition studies with [^{125}I] labeled and unlabeled bovine TSH were performed 72 h after transfection and resulted in similar dissociation constants of both receptors (K_d-wt = 0.95 nM and K_d-mut = 0.85 nM). In conclusion these data indicate, that the mutant receptor protein is functionally expressed and therefore present on the surface of the cells. This fact is the precondition for possible direct interactive relations with the immune system.

221. HETEROZYGOUS SEGREGATION OF A CYTOSINE TO THYMINE TRANSITION IN THE THYROGLOBULIN GENE IN A FAMILY WITH CONGENTIAL GOITER.

H. M. Targovnik, A.E.C. Billerbeck, G.D. Frechtel, G.E.Cerrone, J.Vono, F.Mendive, F. Pedrinola, G.A.Medeiros-Neto. Cát. Génetica y Biologia Molecular, Hosp. de Clinicas José de San Martin, Fac.Farm.Bioq.Univ.Buenos Aires, Argentina and Lab. Tireoide, Hosp.Clínicas, Fac.Med.Univ.São Paulo, Brazil.

Two siblings with congenital goiter and impairement of Tg synthesis were previously reported and the responsible mutation is attributable to a cytosine (C) to thymine (T) transition creating a stop codon at position 1510. The point mutation is removed by the preferential accumulation of a 171 nt deleted Tg mRNA (deletion mapped between positions 4567 and 4737) The C to T transition at nucleotide position 4626 converts the TCGA sequence into TTGA. The mutation removes a Taq I site at this position in the mutant Tg gene. We made use of this property to determine whether in this pedigree the nonsense mutation is linked to the observed inheritance of the Tg defect. For this purpose we amplified genomic DNA covering the Tg mRNA sequence between nucleotide 4567 and 4737 from 13 members of the family. The clinical studies on this family have indicated that two siblings, JNA and MA and one nephew, RRS, were goitrous and had congenital hypothyroidism. Other relatives and spouses had normal thyroid function. Taq I digested **all** of the amplified DNA in seven subjects whereas cleavage of amplified DNA of the father and five siblings was only 50%. It is clear that the father and three of these siblings are carriers of the C to T mutation (normal thyroid function). However JNA and MA had goiter and hypothyroidism and assuming that the two alleles are codomimmantly expressed it is tempting to speculate that an additional mutation may be found in these patients. The parents may have different Tg defective genes, the father being a carrier of the nonsense mutation and, theoretically, the mother would be genotyped as heterozygous for an undetermined mutation. MA and JNA inherited one copy of the nonsense mutation from their father and one copy of the undetermined mutation from their mother. The nephew (RRS) is homozygous for the undetermined mutation and both parents are heterozygous for the same mutation. Southern blot results excluded the possibility of PCR artefact. Our results strongly suggest that an additional mutation is present in affected individuals

2:00 P.M. 48. RADIATION-INDUCED THYROID CANCER: A POOLED ANALYSIS OF SEVEN STUDIES. E. Ron, J.H. Lubin, R.E. Shore, K. Mabuchi, B. Modan, L.M. Pottern, A.B. Schneider, M..A. Tucker, and J.D. Boice, Jr., Epidemiology and Biostatistics Program, NCI, NIH, Bethesda, MD, Department of Environmental Medicine, New York University Medical Center, NY, NY, Departments of Epidemiology and Epidemiologic Pathology, Radiation Effects Research Foundation, Hiroshima, Japan, Department of Clinical Epidemiology, Chaim Sheba Medical Center, Tel Hashomer, Israel, and Department of Endocrinology and Metabolism, Michael Reese Hospital, University of Illinois, Chicago, Illinois.

The thyroid gland of children is especially vulnerable to the carcinogenic action of ionizing radiation. To provide insights into various modifying influences on risk, a pooled analysis was conducted of seven major studies for which organ doses to individual subjects were available. Nearly 500 thyroid cancers were evaluated. Linearity best described the relationship between dose and risk of thyroid cancer, even down to levels on the order of 0.10 Gy. At the highest doses, associated with cancer therapy (>10 Gy), there appeared to be a down turn or levelling of risk. The pooled excess relative risk per Gy (ERR/Gy) and excess absolute risk per 10^4PY-Gy (EAR/10^4PY-Gy) for persons exposed during childhood was 7.7 (95% CI = 2.1, 28.7) and 4.4 (95% CI = 1.9, 10.1), respectively. The attributable risk percent was 47% for the group of exposed subjects. However, these summary estimates were strongly affected by age at exposure even within this limited age range. The ERR was higher (p=.07) for females than males, as was the EAR due to the higher rate of naturally occurring thyroid cancer among women. The excess risk followed neither a simple multiplicative nor additive pattern in relation to background occurrence. There was no excess risk until five years after exposure. Although the ERR was still elevated 40 years or more after exposure, the risk began to decline about 30 years after exposure. Risk also decreased significantly with increasing age at exposure, with little risk apparent after age 20 years. Based on very limited data, there was some suggestion that spreading dose over time (from a few days to >1 year) may lower risk, possibly due to the opportunity for cellular repair mechanisms to operate. The thyroid gland in children appears to have the highest risk coefficient of any organ, and is the only tissue with convincing evidence for risk under 0.25 Gy.

2:15 P.M. 158. RET MUTATIONS AND PENTAGASTRIN TESTING IN FAMILIAL MEDULLARY THYROID CARCINOMA AND MULTIPLE ENDOCRINE NEOPLASIA 2A. B.G. Robinson, D. Marsh, V. Hyland, S. Andrew, D. McDowell and P. Clifton-Bligh. Royal North Shore Hospital, Sydney, Australia.

Mutations in the RET oncogene have now been identified in families with medullary thyroid cancer (MTC) and multiple endocrine neoplasia type 2A (MEN2A). We have developed a method for rapidly diagnosing mutations using polymerase chain reaction (PCR) amplification of mutation containing exons followed by restriction enzyme digestion of PCR products. Seven separate mutations have been identified in fifteen Australasian families. Three of these mutations are present both in families who manifest only the MTC phenotype and are also present in families who have MEN2A. All of these mutations result in substitution of a cysteine residue in the extracellular domain of the ret protein by an alternate amino acid. In families in whom mutations have been identified it has been possible to evaluate pentagastrin stimulation test results in individuals found to be gene carriers and those found to be normal. Pentagastrin (0.5 ug/kg) was administered and plasma samples collected at 0,1,2,5 and 10 minutes for measurement of calcitonin by radioimmunoassay. Twenty non gene carriers were studied: the mean basal calcitonin was 66.9±22.4 pg/ml and the peak calcitonin was 86.8±34.8 pg/ml. The highest peak values in these normal controls was 189 pg/ml (male) and 91 pg/ml (female). One suspected gene carrier based on linkage analysis had a thyroidectomy performed when his calcitonin level peaked at 182 pg/ml. He had histologically confirmed C-cell hyperplasia as well as parathyroid hyperplasia. He was subsequently shown to be a gene carrier by direct mutation detection. Three "at risk" individuals in MEN2A/FMTC families with calcitonin levels peaking at 194 pg/ml, 189 pg/ml and 166 pg/ml respectively were shown to be non-gene carriers and thyroidectomy was not performed. A fourth individual whose calcitonin level peaked at 98 pg/ml using a 2 site immunoenzymetric assay (Medgenix Diagnostics SA, Belgium) underwent thyroidectomy. C-cell hyperplasia failed to be diagnosed and he was subsequently shown not to be a carrier of the mutation segregating with FMTC in his family. These studies have indicated that utility of direct mutation detection and pentagastrin stimulation in the management of the MEN2 syndromes.

2:30 P.M. 182. SCREENING FOR RET GENE MUTATIONS IN MULTIPLE ENDOCRINE NEOPLASIA (MEN) TYPE 2 AND IN SPORADIC MEDULLARY THYROID CARCINOMA (MTC): CLINICAL APPLICATIONS. F. Pacini, I. Ceccherini*, E. Martino, C. Romei, R. Elisei, E. Molinaro, F. Mancusi, G. Romeo*, and A. Pinchera. Istituto di Endocrinologia, University of Pisa, and *Istituto G. Gaslini, Genova, Italy.

Germline missense mutations of the RET proto-oncogene have been recently demonstrated as causative in MEN 2 (A and B) and in familial MTC. In this study we looked for germline mutations of the RET gene in 1 pedigree with MEN 2B, in 4 with MEN 2A, one of which was affected with the particular variant of MEN 2A and Cutaneous Lichen Amyloidosis (CLA), and in 16 cases of sporadic MTC. RET proto-oncogene mutations in exon 10, 11 and 16 were searched for by SSCP and sequence analysis or by restriction analysis of PCR amplification products, when specific restriction site were altered in the mutated sequence.

A Met918-Thr mutation in exon 16 was found in the MEN 2B pedigree (in which only one member was affected). A Cys634- Arg missense mutation in exon 11 was found in 2 pedigrees with MEN 2A and a Cys634-Tyr mutation in one MEN 2A and in the MEN 2A+CLA. In this pedigree the mutation was found in all 5 affected members and in 1 out of 7 apparently unaffected members. Upon recall, this last subject, a 25-year old men, had no clinical thyroid abnormality, basal serum calcitonin (CT) was undetectable (<12 pg/ml) and peak response to pentagastrin was 41 pg/ml (at the upper limit of normal in our laboratory). With his informed consent, total thyroidectomy was performed. At histology 2 small foci of MTC (2 mm in the right and 3 mm in the left lobe) were found. Immunohistochemistry with anti-CT antibody confirmed the C cells origin of the tumors and showed C cells hyperplasia in the normal thyroid tissue surrounding both nodules.

We then examined the tumoral and the constitutional DNAs from 16 patients with the apparently sporadic form of MTC. Point mutations of the RET proto-oncogene were found in 9 cases (Met918-Thr in 4, Cys634-Arg in 4, Cys634-Tyr in 1). In 2 patients the corresponding germline DNA also carried the mutation, allowing the classification of these cases as hereditary rather than sporadic cases.

In conclusion, our study confirms the presence of specific germline mutation in the different forms of MEN 2 and indicates that the MEN 2A variant with CLA carries the same mutation of MEN 2A. Furthermore, somatic point mutations were found in 50% of sporadic MTCs. Two important clinical applications derive from our data: a) screening for RET mutation allows to classify as hereditary some MTC apparently presenting as sporadic; b) the attribution of gene carrier status by screening of family members for RET gene mutations allows the very early detection and treatment of pre-clinical MTC. On this basis, we suggest that total thyroidectomy in gene carrier subjects be carried out as early as possible.

2:45 P.M. 200. **OVEREXPRESSION OF STIMULATORY GUANINE NUCLEOTIDE-BINDING PROTEIN ($G_{s\alpha}$ PROTEIN) IN PAPILLARY THYROID CARCINOMAS IS NOT RESTRICTED TO TUMORS WITH $G_{s\alpha}$ ONCOGENES.** M.Derwahl*. C.Hamacher*, G.Papageorgiou+, N.Speidel*, K.M.Müller#, H.Schatz*, Medizinische Universitätsklinik* and Institut für Pathologie#, 44789 Bochum, Germany and Evangelismos Hospital, Athens, Greece+

In a subset of thyroid adenomas and differentiated thyroid carcinomas somatic point mutations of the gene coding for the $G_{s\alpha}$ protein (GSP oncogenes) have been found. These mutations cause constitutive activation of the adenylate cyclase cascade. In the present study we investigated the interrelationship between the expression of $G_{s\alpha}$ protein and the presence of GSP oncogenes in papillary thyroid carcinomas (n = 44). $G_{s\alpha}$ expression was analyzed by immunostaining and Western blot analysis using an antibody raised against the recombinant human $G_{s\alpha}$ protein. Surrounding normal thyroid tissue was used as control. In addition, Northern blot analysis of $G_{s\alpha}$ mRNA expression was performed. GSP oncogenes were detected by oligonucleotide hybridization technique and subsequent nucleotide sequencing of PCR-amplified DNA (codon 201 and 227 of $G_{s\alpha}$ gene). In addition, growth rate (Ki-67 antigen expression) of the carcinomas and thyroglobulin synthesis was analyzed by immunstaining.

We found that in comparison to the surrounding tissue nearly 70% of papillary thyroid carcinomas showed a very intense cytoplasmic $G_{s\alpha}$ immunostaining. This $G_{s\alpha}$ overexpression was confirmed on the protein and mRNA level by Western blot and Northern blot analysis, respectively. However, only in 15% of these tumors GSP oncogenes (codon 201 or 227) were detectable which is in accordance with the results of others. No difference between $G_{s\alpha}$-overexpressing carcinomas with or without GSP oncogenes was seen with respect to thyroglobulin synthesis or proliferation rate of these tumors.

In conclusion, we here demonstrate that overexpression of $G_{s\alpha}$ protein in papillary thyroid carcinomas is detectable in both carcinomas with or without GSP oncogenes. Furthermore, both groups do not differ in proliferation rate or thyroglobulin expression.

2:00 P.M. 50. HUMAN TRANSTHYRETIN (TTR) LIGAND COMPLEXES: CRYSTAL STRUCTURE OF TTR-3',5'-DICHLORO-2-CARBOXY-DIPHENYLAMINE COMPLEX. Vivian Cody, Joseph R. Luft and Walter Pangborn, Medical Foundation of Buffalo, 73 High St., Buffalo, NY 14203; Sharon Munro, David Chalmer and David Criak, Monash University, Parkville, Australia.

Structure activity data show that thyroid hormone analogues have different binding affinities for transthyretin (TTR), a tetrameric serum protein with two identical hormone binding domains, depending on their substituent patterns. For example, the relative binding affinity of thyroxine (T_4), 3',5',3-triiodothyronine, and tetraiodothyroacetic acid are 100, 33 and 680%, respectively. In addition, many pharmacological agents and natural products such as plant flavonoids, non-steroidal analgesics and inotropic bipyridines are strong competitors for T_4 binding to TTR. Recent structure-activity data show that analgesic phenylanthranilic acids such as flufenamic acid, mefanamic or fenclofenac are more potent competitors than T_4 for TTR binding. Conformational analysis of these analgesics (Weeks, et al, 1986: Dhanaraj & Vijayan, 1988) reveal that these diphenylamines adopt a skewed conformation similar to T_4 with the 2-carboxy phenyl ring coplanar with the bridging imino group which forms an intramolecular hydrogen bond, and the chlorophenyl ring perpendicular to it. Analysis of the structure activity relationships among the strongest competitors for binding to TTR indicates that flufenamic acid (3-CF_3 anthranilic acid) has 175% the affinity of T_4 while mefenamic (2',3'-dimethyl) acid has only 27% the binding affinity. Analogues with no 2-carboxylate are inactive. Based on these findings, a series of anthranilic acid derivatives were synthesized which complete for T_4 binding to TTR and show the most potent analogue was a 3',5'-dichloro derivative (VCP-6) which has 523% of T_4 binding to TTR. To better understand the structural basis for selectivity and specificity for binding competition, we have co-crystallized human TTR with VCP-6 and report its three dimensional structure. These crystals are isomorphous to other $P2_12_12$ TTR complexes which have two independent monomers in the asymmetric unit cell. Data were collected to 2.1Å resolution on an RaxisIIC Imaging Plate area detector for the VPC-6 complex. Interpretation of the electron density calculated at 2.3A showed that the position of the halogen atoms, contoured at $>3\sigma$ in an F_O-F_C difference map, indicate a forward mode of binding, similar to T_4; that is the halogen ring is bound toward the center of the channel in both domains. Computer modeling of the binding of VCP-6 in the hormone binding sites suggest that the enhanced binding observed in these analogues is a result of the added attractive interactions of the 2-carboxylate which forms a strong hydrogen bond to Lys-15 (NZ...O3 2.88Å) and the disposition of the chloride atoms in the hydrophobic pockets observed for thyroid hormone binding. Supported in part by DK-41009.

2:15 P.M. 75. THE SAME MISSENSE MUTATION IN THE ALBUMIN GENE IS ASSOCIATED WITH FAMILIAL DYSALBUMINEMIC HYPERTHYROXINEMIA IN 8 UNRELATED FAMILIES. T. Sunthornthepvarakul, P. Angkeow, R.E. Weiss, S. Refetoff, Departments of Medicine & Pediatrics, The J.P. Kennedy Jr. Mental Retardation Research Center, The University of Chicago, Chicago, IL.

Familial dysalbuminemic hyperthyroxinemia (FDH) is the most common cause of inherited increase of serum T4 in Caucasians. It is the result of increased T4-binding to serum proteins and is inherited as a dominant trait. Prior to the current study, the presumed nature of the abnormal serum protein with high affinity for T4 was based on electrophoretic and immunologic similarities to normal albumin. Using polymorphic markers in the close proximity as well as within the albumin gene, we recently established a tight linkage (lod score, 5.25) between FDH and the albumin gene locus in a large Amish family. This finding prompted the cloning and sequencing of the entire coding region of the albumin gene of a subject with FDH from this family. A single nucleotide substitution, not present in previously reported albumin variants, was found in one of the two alleles. It consists of a G to A transition in codon 218 resulting in the replacement of the normal arginine with histidine (R218H). This mutation was found in 9 affected members of the family that were tested but not in 8 unaffected relatives or 18 unrelated individuals with normal tests of thyroid function. Unexpectedly, the same missense mutation was found in the albumin gene of 12 subjects with FDH belonging to 7 unrelated families but not in their normal relatives. In every individual with FDH, the novel mutation was associated with the Sac I albumin gene polymorphism (frequency 48%). This finding strongly suggests a founder effect since the likelihood of co-association by chance is less than 0.4%. It is in agreement with the finding of much higher prevalence of FDH in people of Hispanic origin.

2:30 P.M. 149. ROLE OF HETERO- VERSUS HOMODIMERS IN THE DOMINANT NEGATIVE ACTION OF HUMAN MUTANT THYROID HORMONE β1 RECEPTORS IN PATIENTS WITH RESISTANCE TO THYROID HORMONE (RTH). R. Wong, X. Zhu*, M.A. Pineda, S-y Cheng* & B.D. Weintraub. NIDDK & *NCI, National Institutes of Health, Bethesda, MD 20892.

RTH is a syndrome characterized by refractoriness of the pituitary and peripheral tissues to the action of thyroid hormone, is usually transmitted in an autosomal dominant fashion and manifests marked heterogeneity in tissue resistance. hTRβ1 mutants have reduced affinity for T3 and exhibit a dominant negative effect whereby the mutant hTRβ1 allele antagonizes the function of normal h-TRα- and β-alleles. Previous studies have yielded conflicting data about the relative roles of hetero- versus homodimers in mediating this dominant negative effect but have primarily employed transfection using a single cell-type as well as idealized thyroid response elements (TREs) and exogenous retinoid X-receptors with which to characterize protein-protein complexes. In the current study, we examined the effects of three naturally-occurring hTRβ1 mutants on three natural TREs - an inverted repeat, chicken lysozyme silencer (Lys); a direct repeat, malic enzyme (ME) and a combination of a direct repeat and palindrome, growth hormone (GH) (gift of Dr. G. Brent, Boston) in two different cell-types. Transfection studies with wild-type (WT) hTRβ1 in HeLa and NIH3T3 cells showed that the T3-induced transactivation on these TREs varied markedly between cell-types suggesting that different thyroid hormone auxiliary proteins or adaptor proteins contribute to these differences. The hTRβ1 mutants displayed the expected T3-induced dose-responsiveness, with the greatest difference in transactivation occurring on the ME-TRE between the two cell-types. Moreover, using the identical TRE, the magnitude of the dominant negative effect of these hTRβ1 mutants also varied between the cell-types. In gel-mobility analyses, prominent heterodimers formed with both WT and mutant hTRβ1 on Lys-TRE using HeLa nuclear extract, and much weaker heterodimers with 3T3 nuclear extract, corresponding to a 10-fold T3-induced transactivation observed with WT hTRβ1 in HeLa cells compared to a 5-fold T3-stimulation in 3T3 cells. On the ME-TRE, heterodimers formed with WT and mutant hTRβ1 with HeLa nuclear extract, but using 3T3 nuclear extract, both homodimers and heterodimers formed. Furthermore, the dominant negative effect of these hTRβ1 mutants in 3T3 cells correlated well with the intensities of the homodimer bands. Previous studies have correlated the dominant negative effects of mutant receptors with the type of mutation as well as the TRE-motif. However, our data suggest that the cell type in which a mutant receptor operates crucially determines the relative amounts of hetero- or homodimers and that this, combined with the nature of the mutation and the TRE-motif, modulates the dominant negative action of mutant receptors and contributes to the heterogeneity of organ resistance.

2:45 P.M. 161. GENE AMPLIFICATION AS A CAUSE FOR INHERITED THYROXINE-BINDING GLOBULIN (TBG) EXCESS. Y. Mori, A. Inagaki, H. Takeuchi[1], Y. Igarashi[1], J. Sugiura[2]. First Dept. of Internal Medicine, Nagoya U. School of Medicine, Nagoya, [1]Dept. of Pediatrics, Hamamatsu U. School of Medicine, Hamamatsu and [2]Toyota Memorial Hospital, Toyota, Japan.

Inherited TBG-Excess remains unknown as to its mechanism, although mutations responsible for complete or partial deficiencies have been identified by gene sequencing. In this study, the dosage of TBG gene, locates on X-chromosome (q21-22), was determined in two Excess families. Genomic DNA was applied to duplex PCR in which 2 target genes, TBG and βGlobin (chromosome11) or TBG and Duchenne muscular dystrophy(DMD:Xp), were amplified simultaneously and amplification was terminated in the exponential phase. The PCR products were separated by high performance liquid chromatography using DEAE-NPR column and monitored with UV at 260 nm. The TBG gene dosage relative to βGlobin is shown in the table. Compared to normal males, the TBG gene dosage in affected males, I-1 and II-2, was triple and double respectively, corresponding to their serum TBG levels. Affected females had one more gene dosage than affected males. Nevertheless, TBG gene amplification couldn't be detected in fluorescence in situ hybridization. In regard to the DMD gene, no amplification was observed by duplex PCR. The 1.2 kbp promoter region of TBG gene from affected males showed a same activity with normal controls. The amplified region was considered to contain the entire TBG gene but to be small for the detection in the chromosome. In conclusion: gene amplification and not structural gene alteration might be responsible for TBG-Excess.

Subject	Sex	Serum TBG (μg/ml)	HPLC (mean of triplicate)	
			TBG/βGlobin	ratio to Normal M.
Family I 1.	M	60	1.03	3.12
2.	F	32	1.37	4.15
Family II 1.	M	44	0.64	1.94
2.	F	33	0.96	2.91
Normal control	M (n=10)	21	0.33	1.00
	F (n=10)	23	0.65	1.97

3:30 P.M. 13. **EFFECTS OF ACUTE HYPOTHYROIDISM ON HUMAN BRAIN PHOSPHORUS METABOLISM.** K. B. Ain and C. D. Smith, Depts. of Internal Medicine and Neurology, University of Kentucky Med. Center, Lexington, Kentucky; and Dept. of Medicine, Veterans Administration Med. Center, Lexington, Kentucky.

Acute hypothyroidism produces definite neuropsychological effects in adult humans. Despite this, previous studies have failed to demonstrate metabolic changes from hypothyroidism in adult human brain. We used P-31 nuclear magnetic resonance (NMR) spectroscopy to evaluate the effects of acute hypothyroidism on cerebral phosphate metabolism. Phosphorus-31 NMR spectroscopy was performed with a surface coil designed to sample signals from the anterior-superior frontal lobe. Nine adult patients with thyroid carcinoma were studied when prepared for radioiodine scanning after discontinuing levothyroxine (LT_4) therapy for six weeks. Repeat spectroscopy scans were performed on the same patients after seven to eight weeks of LT_4 therapy and compared to the earlier scans. Thyrotropin values were elevated at 135 (mean, range 39 - 310 mU/L) during the initial spectroscopy scan and ranged from <0.1 to 7.9 mU/L at the time of the second scan. Paired analysis of P-31 NMR spectra revealed the phosphocreatine/inorganic phosphate (PCr/Pi) ratio to increase from 2.270 ± 0.295 (mean ± SD) to 2.606 ± 0.219 after treatment with LT_4 (P=0.015). The Pi/ß-adenosine triphosphate (ATP) ratio demonstrated antiparallel changes from 0.591 ± 0.044 to 0.492 ± 0.092 (P=0.011). There were no significant differences seen in relative concentrations of Pi, phosphodiester, PCr, γ-ATP, α-ATP, ß-ATP or pH; nor in the ratio of PCr/ß-ATP. The change in the PCr/Pi ratio can be used to calculate the change of velocity of oxidative metabolism between hypothyroid and euthyroid states. The magnitude of this change observed in human brains is similar to the change of metabolism velocity observed by others studying human skeletal muscle under similar conditions. In conclusion, we observe reversible alterations in adult cerebral phosphate metabolism during acute hypothyroidism that parallel many of the changes described in skeletal muscle. This provides the first direct evidence of metabolic effects of acute hypothyroidism on adult human brain.

3:45 P.M. 51. **THE DIAGNOSTIC VALUE OF ^{99m}TC-SESTAMIBI IN THE LOCALIZATION OF RECURRENT DIFFERENTIATED THYROID CANCER.** H.Elser, I.Mattern-Alvarez, P.Georgi. Department of Nuclear Medicine, University of Heidelberg, Germany

Differentiated thyroid carcinoma (DTC) are potentially curable tumors. 131-I-whole-body-scintigraphy (WBS) and thyreoglobulin level have been shown to be both sensitive and highly specific for the follow-up of DTC. However false negative results are known. ^{99m}Tc-Methoxy-iso-Butyl-Isonitrile (MIBI) is a newly developed radiotracer for myocardial imaging that concentrates dependent on perfusion in mitochondrial membranes. The purposes of this investigation were to characterize the uptake of MIBI in recurrencies of DTC and to determine the feasibility and advantages of utilizing this compound as a tumor imaging agent. 46 patients with previous total thyroidectomy, radioiodine-therapy and sometimes previous external radiation with known or suspected recurrencies of DTC were investigated. Each patient underwent MIBI scanning, WBS, ultrasound, and computed tomography. Furthermore serum thyreoglobulin levels were determined. All together 64 MIBI scans were performed. In all cases of suspected lymph nodules involvement, cytological and/or histological confirmation was recommended. Results: False positive pulmonary uptake was demonstrated only in one patient. For MIBI results no correlation to thyroid hormonal status was observed. Image quality was excellent. The uptake of 99mTc-MIBI into recurrencies of DTC was rapid, recurrencies were evident within 10 minutes following intravenous injection. Conclusions: MIBI is of diagnostic value in recurrent DTC unrelated to thyroid hormonal status.

Sensitivity	MIBI	131-I-WBS
Lqcal rec.	94.3%	62.8%
Pulmon. rec.	77.4%	38.7%
Sceletal rec.	83%	44.4%

4:00 P.M. 86. TREATMENTS WITH 131-I IMPART LESS ABSORBED RADIATION TO THYROID CANCER THAN PREDICTED BY DOSIMETRY. J. C. Sisson, R. Ackermann, S. Zempel and S. Spaulding, University of Michigan, Ann Arbor, MI.

We, as others, have observed lower tumor/background ratios in thyroid cancers on images made after therapeutic doses and compared with those made after 2 mCi of 131-I in a diagnostic study. To explain the phenomenon, we quantified the radioactivity in the tumors. Regions of interest on conjugate scintigraphic views were quantified to give, after subtracting an appropriate background activity, counts per minute (cpm) and μCi (determined from a correlation with cpm in an external mock source of known activity) in selected tumors. Values obtained from images made after a 2 mCi diagnostic dose were compared with those obtained after a therapeutic dose, each at 2 (or 3) days after the respective carrier-free 131-I dose. In 10 patients receiving 30-325 mCi in therapy, the Rx/Dx ratios for tumors were 0.03-0.79; only two patients had a Rx/Dx ratio of >0.5. Comparable differences were not seen in dosimetry of the whole body nor of the blood.

To determine if the 2 mCi diagnostic dose impaired the the tumor uptake of the therapeutic dose, we evaluated two patients by measuring concentrations of 123-I in selected tumors at 1 and 2 days after 20 mCi were given orally on two occasions. One 123-I dose was given 2 days before and the second 123-I dose was given 5 days after the 2 mCi 131-I dose. Two days after the second 123-I dose, the therapeutic dose of 131-I was given. From data obtained at 22 or 46 h (radioactivity extrapolated to 0 time), fractions of doses in the tumors were calculated in each study and then compared as ratios:

	123-I (#2)/123-I (#1)	123-I (#1)/131-I (Dx)	131-I (Rx)/131-I (Dx)
Pt 1 (Rx 147 mCi)			
tumor A	0.58	0.75	0.063
tumor B	0.73	0.92	0.056
Pt 2 (Rx 346 mCi)			
tumor A	1.14	1.06	0.31
tumor B	1.35	1.00	0.42

In two patients the effective half lives of diagnostic (data over 1-7 days) and therapeutic (data from 2 and 7 days) doses were compared. T 1/2 values: in two tumors in one patient, Dx 37 and 38 h and Rx 50 and 48 h; tumor in other patient, Dx 102 h and Rx 82 h.

Conclusions. Therapeutic doses give fractional uptakes of 131-I in thyroid cancers that are generally <0.5 of those predicted by diagnostic dosimetry. The discrepency appears to be primarily in uptake and less, if at all, in retention of 131-I. The mechanism for the discrepency is not clear but could result from a sensitive Wolff-Chaikoff effect in the tumor triggered by the larger iodide dose from the treatment, or a selective early radiation effect on the uptake/organification activities of cancers.

4:15 P.M. 173. HIGH PREVALENCE OF THYROID NODULES IN OCCUPATIONALLY RADIATION EXPOSED SUBJECTS.

The possible risk for thyroid nodules and cancer due to occupational exposure to ionizing radiation has not been extensively explored yet. We have studied the prevalence of thyroid nodules, cross-sectionally, by ultrasonography in males medical X-ray workers registered in the list of the Radioprotection Service of the USL-12 (Pisa; mean urinary iodine=88 mcg/day), with a cumulative occupational exposure to radiation higher than 100 mSv or a duration of occupational exposure longer than 10 years in the Units of Hortopaedia and Hemodinamic (total number 59; mean age was 48.7±7.1 years; mean duration of the occupational exposure was 23.7±7.0 years). A control group was chosen from the employers (130 males; mean age 44.8±6.2 years) of a shipyard factory in a iodine deficient area (Lunigiana; mean urinary iodine=49.8 mcg/day), without radiation occupational exposure. Neck ultrasonography was always performed by the same two operators, using a probe (Toshiba, Tosbee) with a settorial 7.5 MHz transducer. Only thyroid nodules with a diameter higher than 5 mm, and with agreement of both operators were considered. Among the occupationally exposed subjects thyroid nodules were detected in 24 (40.6%). Among the subjects of the non-exposed group thyroid nodules were detected in 25 (19.2%). Fine needle aspiration resulted negative for thyroid malignancy. FT3 and FT4 were in the normal range in all subjects with thyroid nodules. A higher prevalence of history of residence in iodine deficient areas was present in the control group (chi-square; p<.01). The prevalence of thyroid nodules resulted higher (chi-square=8.63; p=.0033) in exposed male subjects. Comparing exposed and non-exposed groups, stratified by 30-39, 40-49 and 50-59-year age subgroups, we observe a higher significant Relative Risk (RR) for thyroid nodules in the exposed subjects (Mantel-Haenszel weighted RR = 1.80; 95% Confidence Limits = 1.15-2.83), due overall to a higher risk in the younger subjects. Considering three levels of exposure (non-exposed; exposed with ≤ 19 years of work; exposed with ≥ 20 years of work) as a trend factor and using the linear trend chi-square test, stratified for age, a significant result was obtained (p<0.01). In conclusion the preliminary results of our study suggest, in agreement with previous retrospective epidemiological studies, that occupational radiation exposure may be a risk factor for thyroid nodules.

4:30 P.M. 209. BONE MASS IS NORMAL IN MEN CHRONICALLY TREATED WITH SUPPRESSIVE DOSES OF LEVOTHYROXINE.

C. Marcocci, F. Golia, G. Bruno-Bossio, E. Vignali, A. Pinchera- Istituto di Endocrinologia, Università di Pisa, Pisa, Italy.

Several studies have evaluated the effects of thyroid hormone therapy on bone mass in women, whereas only limited informations are available in man. We have previously shown that such treatment was not associated with a decrease of bone density in premenopausal women. In the present study we have measured bone mineral density (BMD) in 20 men treated with suppressive doses of levothyroxine (L-T4). Indications for L-T4 therapy were nontoxic goiter (n=2) or total thyroidectomy for differentiated thyroid cancer (n=18). The age ranged between 27 and 54 yr (mean 44.8 yr) and the duration of L-T4 therapy between 4.5 and 21.5 yr (mean 10.1 yr). The daily dose of L-T4 taken at the time of the study (113-218 μg, mean 176 μg) was not significantly different from that used during the entire period of treatment. The cumulative dose of L-T4 ranged between 190 and 1,698 mg (mean 668 mg). Thyroid status was evaluated by yearly measurement of free T4 (FT4), free T3 (FT3) and TSH. At the time of the study FT4 was increased in 6 patients (30%) and FT3 in 1 (5%); mean values were 15.3±0.9 pg/ml and 3.8±0.2 pg/ml, respectively. Serum concentrations of calcium, PTH, osteocalcin and SHBG did not differ between patients and controls. BMD was measured by dual-energy X-ray absorptiometry (Lunar DPX) at the lumbar pine (L2-L4) and femur (neck, Ward's triangle and trochanter); the results obtained were compared with those of normal subjects matched for age and body habitus. We found no significant difference between patients and controls at any site of measurement: L2-L4= 1.197±0.04 vs 1.222±0.01; femoral neck= 0.981±0.03 vs 0.967±0.01; Ward's traingle= 0.857±0.03 vs 0.872±0.02; trochanter= 0.871±0.03 vs 0.818±0.01). BMD at any site was not correlated with duration of L-T4 therapy, L-T4 dose, cumulative L-T4 intake, and serum FT4 or FT3.

In conclusion the results of the present study indicate that chronic treatment with suppressive doses of L-T4 in man, as well as in women, has no significant effect on bone mass.

4:45 P.M. 217. Follow up of Graves' Disease patients treated with antithyroid drugs alone combined with TSH suppression therapy by Triiodothyroacetic (TRIAC) or Triiodothyronine (T3).

C.Jaffiol, N.Khalifah, J.C.Manderscheid, L.Baldet, J.Bringer, R.Martinel.

Some therapeutic trials have pointed out the advantages of TSH suppressive therapy when combined with anti-thyroid drugs (ATD) : T4 or T3 are commonly used assuch. TRIAC, a potent TSH supressant may offer some advantages due to its good clinical tolerance.

A random sample of 51 patients (47 F - 4 M)with Graves'Disease without prior treatment was selected. One of three treatments was applied : G1 (n=24)Carbimazole + TRIAC ; G2 (n=15) Carbimazole + T3, G3 (n=12) Carbimazole alone.

The following indices were evaluated before and at regular intervals (3-6 months) after therapy : weight, heart rate,thyroid size, grade of ophtalmopathy,early radio iodine uptake (ERU), FT4,FT3,TSH, anti-thyroid Ab. Therapy was discontinued when ERU was within normal limits (< 10 %). Some patients needed surgery or radio-iodine ablation. We compare the followings between the 3 groups : duration of therapy, thyroid size and ERU during follow up, pronostic value ofgoitre size and ERU early post-therapy (0-6 months) and clinical tolerance of TRIAC and T3.

Remission rate was lower in G3 (66.67 %) than in G1 (87.50 %) or G2 (80%). Therapy was shorter with TRIAC (13,1 ± 9,2 m) than either G2 (17,85 ± 5m) or G3 (17.3 ± 5 m). Goitre incidence increased during therapy in G3 to 40 % at recovery ; it decreased in groups I and 2 to 18 % and 11,7 % respectively ($p < 0.007$). Reduction of thyroid size and of ERU early post-therapy has pronostic value. A significant relationship was observed between reduction of thyroid mass and duration of therapy (p=0.01). Drug intolerance occured in 4 % of TRIAC and 13.3 % of T3 treated patients.

In conclusion, TSH suppression combined with anti-thyroid drugs leads to a significant reduction in thyroid size and a significantly increased rate of remission. Reduction of goitre size and ERU at 6 months is a valuable index of good prognosis. TRIAC may be prefered for combination with anti thyroid drugs on account of its good clinical tolerance.

8:00 A.M. 24. **PREDICTION OF POSTPARTUM ONSET OF GRAVES' THYROTOXICOSIS BY MEASUREMENT OF THYROID STIMULATING ANTIBODY IN EARLY PREGNANCY**

N. Amino, Y. Hidaka, H. Tada, H. Tamaki, T. Kashiwai, Y. Iwatani and N. Mitsuda
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Autoimmune thyroid diseases often occur after delivery and at least 40% of Graves' patients with child-bearing age develop their disease during the postpartum period. However, it had been difficult to know who will develop Graves' thyrotoxicosis after delivery. We tried to establish a systematic method in predicting postpartum onset of Graves' thyrotoxicosis. First, we measured anti-thyroid microsomal antibody (MCAb) by agglutination method in early pregnancy in 3405 consecutive pregnant women who attended our maternity clinic. Two hundred and sixty-two subjects (7.7%) were newly found to have positive MCAb and 71 of these women could be followed monthly during pregnancy and after delivery until 6 months postpartum. For these 71 subjects, TSH-binding inhibitory immunoglobulin(TBII) and thyroid stimulating antibody (TSAb) were measured in their early pregnancy, and were measured serially until 6 months after delivery for the subjects with either positive TBII or TSAb. TSAb was measured by highly sensitive bioassay using FRTL-5 cells. Thyroid function tests(FT4, FT3 and TSH) and goiter size were recorded at every observation. Among 71 subjects, 1 had positive TBII and 7 showed positive TSAb in their early pregnancy without any thyroid dysfunction. All 7 developed thyroid dysfunction in postpartum period. Five of them (70% of TSAb positive subjects) developed Graves' disease, two showing persistent and three transient. None of 64 TSAb-negative subjects developed Graves' thyrotoxicosis, though 44 developed various types of thyroid dysfunction due to postpartum autoimmune thyroiditis.

In conclusion, the high risk individuals of postpartum onset of Graves' thyrotoxicosis can be found in their early pregnancy by the detection of TSAb. Over all occurrence of postpartum Graves' disease in the general population is estimated above 0.54%, that is, one of 200 postpartum women may develop Graves' thyrotoxicosis, although thyrotoxicosis may be transient in half of the patients.

8:15 A.M. 49. EXTREMELY LOW DOSES OF HEPARIN CAN CAUSE ARTIFACTUAL ELEVATIONS IN THE SERUM FREE THYROXINE CONCENTRATION AS MEASURED BY EQUILIBRIUM DIALYSIS. C.M. Mendel, J.C. Jaume, P.H. Frost, F. Greenspan and C. Laughton. Cardiovascular Research Institute and Thyroid Molecular Biology Unit, VAMC, Department of Medicine, University of California, San Francisco CA.

Heparin can cause an artifactual elevation in the concentration of unbound (free) thyroxine (T4) in the plasma, particularly when measured by equilibrium dialysis. The lipase released into the plasma by heparin acts on substrate (triglycerides=TG) in the plasma in vitro to release unesterified (free) fatty acids (FFA) which, in high concentrations, inhibit the binding of T4 to its plasma binding proteins. This artifact occurs only in the presence of sufficient substrate (serum TG > 180 mg/dl), and is more pronounced in methods requiring long incubation times. We observed this artifact in three patients receiving Intralipid and subcutaneous (sc) heparin. Free T4 (FT4), when measured by standard equilibrium dialysis, was elevated in all patients, but was normalized when the in vitro generation of FFA, during equilibrium dialysis, was prevented by prior treatment of the samples with protamine to inhibit lipoprotein lipase and with an antibody to hepatic triglyceride lipase. This observation caused us to investigate whether intravenous (iv) doses of heparin that are commonly used to flush iv lines (typically 100 U) could cause this artifact. We gave increasing doses of heparin at weekly intervals to each of three normal volunteers and measured FFA generation in their plasma (supplemented with 250 mg/dl triglycerides) under conditions simulating equilibrium dialysis. We found that, indeed, iv doses of heparin as low as 0.08 U/kg (5.6 U in a 70-kg subject) as well as a standard dose of sc heparin (5000 U) could cause enough in vitro generation of FFA to artifactually increase the serum FT4 concentration when measured by equilibrium dialysis. These results indicate that equilibrium dialysis may not be the best method for assessing serum free T4 concentrations in hospitalized patients, and should be taken into account when interpreting previous studies demonstrating inhibitors of T4-serum protein binding in sera from hospitalized patients.

8:30 A.M. 77. FOLLICULAR LESIONS OF THE THYROID: THE FUTILITY OF FROZEN SECTION EVALUATION. H Chen, M.D.*, T Nicol, M.D.+, A Busseniers, M.D.+, and R Udelsman, M.D.* The Division of Endocrine and Oncologic Surgery, Department of Surgery* and The Department of Pathology+, The Johns Hopkins Hospital, Baltimore, Maryland 21287.

Follicular lesions of the thyroid present a diagnostic and therapeutic challenge. Although Fine Needle Aspiration (FNA) is very accurate in differentiating other thyroid neoplasms, it rarely distinguishes follicular adenomas from carcinomas, and frequently results in the diagnosis of "follicular lesion" or "follicular neoplasm". Therefore, many advocate the routine use of frozen section (FS) to guide intraoperative management. To determine the utility of frozen section evaluation, we reviewed 125 consecutive patients from May 1984 to January 1994 with follicular thyroid lesions by FNA or FS. All patients underwent surgery at the Johns Hopkins Hospital. All FNAs, FS, and permanent sections were re-reviewed. FNAs were categorized as inadequate, indeterminate, benign, follicular neoplasm, or suspicious/diagnostic of malignancy. FS were categorized as benign, indeterminate, follicular lesion (cannot distinguish benign from malignant), or suspicious/diagnostic of malignancy. The median age at the time of surgery was 43 years (range: 18 to 82 years). There were 90 females (76%) and 35 males (24%). Of 125 patients, 118 underwent FNA and 120 FS. Sensitivity, specificity, and accuracy are shown below.

STUDY	SENSITIVITY	SPECIFICITY	ACCURACY
FNA	70%	79%	75%
FS	55%	71%	52%

FS were categorized in 104/120 as "follicular lesion", rendering no useful clinical information. In 16/120 cases FS yielded "diagnostic" information. In only 4 of the 16, however, did FS correctly modify the operative procedure at a cost of $12,510/useful FS. Notably, in 6 cases, an incorrect FS actually misled the surgeon and resulted in 4 misguided operative procedures. Therefore, we recommend that the operative management of follicular thyroid lesions be based on FNA and clinical parameters. The routine use of FS is of minimal diagnostic value, prolongs the operation, and may lead to an incorrect intervention. Avoidance of futile frozen section evaluation will result in a savings of $41,700 per 100 patients. Furthermore, it will not compromise patient care and may actually improve the clinical decision process.

8:45 A.M. 195. **CARDIAC VALVE INVOLVEMENT IN AUTOIMMUNE THYROID DISEASE.** S. Mohr-Kahaly*, G. Kahaly. Departments of Cardiology* and Endocrinology/Metabolism, Johannes Gutenberg-University Hospital, Mainz, Germany.

In patients (pts) with Graves' disease (GD), an increased glycosaminoglycan production is observed in the retrobulbar space, in the pretibial region and in the cardiac valves. The augmented accumulation of glycosaminoglycans in the mitral valve leads to thickening of the leaflets. A prolapse of this thickened (>5 mm) mitral valve, also called myxomatous, into the left atrium is then registered. In a previous study, the prevalence of mitral valve prolapse (MVP) was investigated in 60 pts with GD and ophthalmopathy and in 20 pts with toxic nodular goitre (TNG). 20/60 (33%) pts with GD but none in the TNG group showed a thickened myxomatous valve ($p=0.001$). Thyroid function did not influence the incidence and intensity of the prolapse. In this prospective study, the prevalence of MVP was determined in 50 pts (40 fem, 19-67 yrs, median 39 yrs) with chronic lymphocytic thyroiditis using pulsed waved - Doppler and conventional (M-mode and two-dimensional) echocardiography. MVP was defined as a systolic buckling >3 mm. 2410 pts (1511 f, 31-77 yrs, 44 yrs) from the echocardiography laboratory served as controls. A myxoid degeneration of the mitral leaflets was found in 18 of 50 (36%) pts with Hashimoto's thyroiditis and in 201 of 2410 (8%) controls ($p=0.002$, Wilcoxon 2-sample test). No correlation between the prevalence of MVP and the presence of hypothyroidism, serum antithyroid antibodies, or the duration of lymphocytic thyroiditis was found. In 24/50 (48%) pts, a prolapse of both anterior and posterior mitral leaflets was present, whereas in 20/50 (40%) pts, a prolapse of the anterior leaflet only was found. Using transoesophageal echocardiography, thickness of the mitral valve was precisely measured (median 6.7 mm, 5-9 mm). Median systolic buckling was 4.5 mm, 3-6 mm. Color-coded Doppler echocardiography showed in 5/50 (10%) a mild mitral regurgitation grade I. In 4/50 (8%) and 1/50 (2%) pts with thyroiditis, a prolapse of the aortic and tricuspid valves, respectively was also present. Thus, the prevalence of myxomatous valves is significantly increased in pts with autoimmune thyroid disease and valvar prolapse is not attributable to myocardial dysfunction caused by a direct effect of thyroid hormones. A proof of a common autoimmune mechanism would probably require immunohistological studies of mitral valve and thyroid tissue. The present hypothesis is a defect of genetic coding for control of mitral valve tissue formation, turnover or subsequent modification at a locus adjacent to one exerting some influence over the control of thyroid function or all three.

10:30 A.M. 16. SYNERGISTIC INTERACTION BETWEEN MUSCLE ENHANCER FACTOR 2 (MEF2) AND T_3 RECEPTORS (TR) IN STIMULATING RAT CARDIAC SARCOPLASMIC ENDOPLASMIC RETICULUM Ca^{2+} ATPase (SERCA2) GENE TRANSCRIPTION. A. Moriscot, M.R. Sayen, R. Hartong, and W.H. Dillmann, Univ of California, San Diego, CA.

T_3-stimulates the transcription of the SERCA2 gene in cardiac myocyte and this effect is mediated through three separate thyroid response elements (TRE1, TRE2, TRE3) in the regulatory region (regreg) of the gene upstream from the transcription start site. It is currently unclear if T_3 action is modified in cardiac myocytes by tissue-specific transcription factors. In sequencing 3.5 kb of SERCA2 regreg upstream from the transcription start site, we noted six putative MEF2 sites. To investigate a potential interaction between T_3 and MEF2, heart derived H9c2 cells were co-transfected with plasmids containing 3.5 kb of SERCA2 regreg linked to the CAT reporter. Different expression plasmids coding for TRα1 and MEF2 were co-transfected. Cells were maintained in the presence or absence of 10^{-7} M T_3 for 24 hrs. Transfection of TRα1 or MEF2 by themselves had no effect on SERCA2 driven reporter activity. In contrast, co-transfection of TRα1 plus MEF2 induces a 2.7 ± 0.15 fold increase in CAT reporter activity. Addition of T_3 leads to a 3.2 ± 0.2 fold increase in reporter activity thus providing for a small further increment in reporter activity. To evaluate the mechanism and specificity of the interaction, a plasmid containing TRE1 linked to a TK promoter and a CAT reporter was co-transfected with TRα1 itself and/or MEF2 expression plasmids. MEF2 had no effect on CAT expression in the presence of TRα1 with or without T_3 indicating the requirement for MEF2 to attach to its DNA binding site for exerting a synergistic interaction with TRα1. A CREB binding site was identified in the SERCA2 regreg which is functional since transfection of expression plasmids for CREB and constitutively active protein kinase A with a 3.5 kb SERCA2 regreg driven CAT reporter leads to a 2 fold increase in transactivation. MEF2 co-transfection plus CREB and PKA has no further effect on reporter activity indicating that MEF2 does not interact with other transcription factors. CONCLUSION: 1) TRα1 and MEF2 interact synergistically in enhancing SERCA2 transgene transcription; 2) TRα1 and MEF2 interact in a specific manner most likely depending on MEF2 attaching to its specific DNA binding site; 3) TR effects on transcription can be enhanced by muscle-specific factors in cardiac myocyte derived cells.

10:45 A.M. 26. **IDENTIFICATION OF A *cis*-ACTING DESTABILIZING REGION WITHIN RAT TSHβ mRNA THAT MEDIATES T_3-INDUCED DEGRADATION OF TSHβ mRNA.**

P. J. Leedman, R. A. Spanjaard, A. R. Stein and W. W. Chin, Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Howard Hughes Medical Institute and Harvard Medical School, Boston, Massachusetts, 02115.

Thyroid hormone (T_3) suppresses TSHβ gene expression by transcriptional and post-transcriptional mechanisms. T_3-induced reduction in TSHβ mRNA stability is associated with a shortened poly(A) tail and is dependent upon ongoing protein synthesis. However, the molecular mechanism(s) mediating this phenomenon is unknown. We reasoned that a T_3-responsive *cis*-acting destabilizing element(s) should reside within TSHβ mRNA, and aimed to identify this sequence by utilizing transient transfection studies in cultured murine TtT97 thyrotrope tumor cells. Various portions of the rat TSHβ cDNA were subcloned into the RSV-CAT vector, downstream of the CAT protein coding sequence. These constructs were electroporated into primary cultures of TtT97 cells, which were maintained in T_3-deficient medium. After the addition of T_3, the cells were harvested and CAT activity measured. RSV-LUC was co-transfected, and the results were normalized against luciferase activity. The results were expressed as a ratio of the CAT activity derived from cells transfected with constructs containing TSHβ cDNA divided by the CAT activity from cells transfected in parallel with RSV-CAT alone. Addition of 100 nM T_3 to cells transfected with the full-length TSHβ cDNA resulted in a decrease in the CAT activity ratio at 4 and 8 hr to ~15 and 20% of control values, respectively. Next, a construct (TSHβ-7) containing only the 3' 250 bp of the coding region and the entire 3'-UTR (75 bp) was tested. After incubation with 100 nM T_3, the CAT activity ratio of TSHβ-7 at 4 and 8 hr decreased to ~10 and ~15% of control, respectively. This decrease suggested that a putative *cis*-acting element was present within this region. Rat pituitary GH_3 cells were used to investigate the tissue specificity of this response. A T_3-induced decrease in the CAT activity ratio was not demonstrated in GH_3 cells, consistent with the notion that a thyrotrope-specific *trans*-acting factor may be responsible for conferring this effect. In conclusion, the 3' ~320 bases of the rat TSHβ mRNA contain a T_3-responsive *cis*-acting destabilizing element. Interestingly, this region encompasses the 3'-UTR, the first 40 bp of which we have previously characterized as containing a binding motif for a T_3-regulated rat pituitary cytoplasmic *trans*-acting RNA-binding protein. It is possible, therefore, that this *trans*-acting factor may play a role in the destabilization of TSHβ mRNA. Further characterization of this *cis*-acting element should provide critical insight into the molecular mechanisms governing the T_3-induced modulation of TSHβ mRNA stability.

11:00 A.M. 84. **THYROID HORMONE RESPONSE ELEMENTS AND T_3 RECEPTOR CROSS TALK MODULATE 9-CIS RETINOIC ACID INDUCED ACTIVATION OF RETINOID X RECEPTORS: A NEW PARADIGM OF DUAL HORMONAL REGULATION?** P.G. Walfish*, Y-F. Yang*, X. Zhu*, L. Xia* and T.R. Butt+. Samuel Lunenfeld Research Institute of Mount Sinai Hospital & University of Toronto Medical School*, Toronto, Ontario, Canada M5G 1X5 and SmithKline Beecham+, King of Prussia, PA 19403.

Yeast (S.cerevisiae) is devoid of endogenous T3 receptors (TRs) and retinoid X receptors (RXRs). Yeast systems were used to determine whether different thyroid hormone response elements (TREs) and rat (r) T3 receptors ($TR\beta_1$) could regulate 9-cis retinoic acid (9-c-RA) activation of different RXR isotypes. The expression plasmids contained the following TR and RXR receptors: chicken (c) RXRγ or mouse (m) RXRα, wild-type rTRβ1 or one of two D300A and Δ286-305 rTRβ1 E1 subdomain mutants which have well characterized $\approx$ 40% and > 95% respective reductions in heterodimerization with RXRs. Various combinations of these receptors were co-expressed with a reporter plasmid containing the ß-galactosidase gene under the control of one of the following TREs: rGH palindrome x3 (PAL), the chicken lysozyme silencer F2 inverted repeat x1 (F2) or a 4 bp spaced AGGTCA repeat x1 (DR4). When wild-type $TR\beta_1$, mRXRα and cRXRγ were expressed as homodimers, the order of potency for both constitutive and cognate hormone (1μM) induced responses on these different TREs was PAL > F2 > DR4. mRXRα was two to three fold more potent than cRXRγ and rTRβ1 on PAL and F2 TREs, but both TRβ1 and RXR homodimers were relatively inactive on TRE-DR4. When wild-type $rTR\beta_1$ was co-expressed with RXRs and compared to RXR homodimer effects, enhanced constitutive and 9-c-RA (1μM) induced activation was observed for cRXRγ but not mRXRα on TRE-PAL, whereas cRXRγ was 1.5 to 3.0 fold more active than mRXRα on TRE-F2, while the largest relative increase of 6 and 19 fold respectively was observed on TRE-DR4. When substituted D300A or Δ286-305 TRβ1 E1 subdomain mutants were co-expressed with RXRs and compared to wild-type $TR\beta_1$ activation effects on these RXRs, the mutants did not significantly reduce 9-c-RA (1μM) induced activation of RXRα and had only a weak 12 and 34% respective reduction of RXRγ on the TRE-PAL; whereas, a similar 39-46% and 71-80% respective decrease was noted for mRXRα and cRXRγ on TRE-F2; and the largest similar relative respective reductions in 9-c-RA responses of 40-48% and 86-98% was documented for mRXRα and cRXRγ on TRE-DR4. **Conclusions: 1)** TREs PAL and F2 regulated constitutive and 9-c-RA induced effects of RXR homodimers. **2)** TREs DR4 and F2 had enhanced 9-c-RA induced activation of RXR heterodimers resulting from T3 receptor cross talk. **3)** Such nuclear signalling mechanisms could represent a new molecular paradigm of dual (T3 and 9c-RA) hormonal regulation wherein TREs with varying responsivity to homodimers and heterodimers interact with TRs and different RXR isotypes to modulate transactivation induced by cognate ligands.

11:15 A.M. 85. THE IMPORTANCE OF SEQUENCE VERSUS SPACING FOR DIRECT REPEAT THYROID HORMONE RESPONSE ELEMENTS. R.W. Katz and R.J. Koenig, Endocrinology Division, University of Michigan Medical Center, Ann Arbor, Michigan

Direct repeats of the hexamer AGGTCA can function as response elements for T3, all-trans retinoic acid (RA) and 1α, 25 dihydroxyvitamin D (vitD). The specificity of the response derives from the spacing between the hexamer half-sites, with a 3 bp spacer conferring a vitD response, a 4 bp spacer a T3 response, and a 5 bp spacer an RA response (3-4-5 rule). Recently, we have shown that the highest affinity binding site for T3 receptor (TR) α1 is the octamer TAAGGTCA, not the hexamer AGGTCA. Thus, in retrospect, the 3-4-5 rule was derived from studies using an imperfect TR binding site. The studies described below were performed to test whether the 3-4-5 rule holds for direct repeats of the octamer sequence. Methods: JEG-3 cells were transfected by calcium phosphate precipitation. The cells received 3 µg of the empty vector pCDM or pCDM expressing TRα1, RA receptor α (RARα), or vitD receptor (VDR). The cells also received 4 µg of a chloramphenicol acetyltransferase (CAT) plasmid derived from pUTKAT3, in which a basal thymidine kinase (tk) promoter drives CAT expression. pUTKAT3 derivatives contained potential response elements 5' to the tk promoter. These sequences included a single copy of the octamer TAAGGTCA or direct repeats with spacings of 3, 4, 5, or 9 bp between the core hexamers (denoted 8DR3, 8DR4, 8DR5, and 8DR9). Similar constructs were made with the sequence GCAGGTCA, which represent traditional hexamer direct repeats (6DR3, etc). Cotransfections included pRSVGH, which expresses human GH, and all CAT activities were normalized to hGH to control for transfection efficiency. Ligand inductions utilized 10 nM T3, 1 µM RA, or 100 nM vitD. Gel shift assays also were performed with radiolabeled probes derived from the above response elements plus E. coli generated TRα1 and RXRα. Results: 8DR3, 8DR4, and 8DR5 were equally T3 responsive, yielding 12-15 fold T3 inductions of CAT (activities without T3 were similar). 8DR9 was substantially weaker. In contrast, 6DR4 was more T3 responsive than 6DR3 and 6DR5 (6 fold vs 2-3 fold), as predicted by the 3-4-5 rule. VitD response was 6.4 fold for 8DR3 but only 2-3 fold for 8DR3 and 8DR5. RA response was 8.4 fold for 8DR5 but 4-5 fold for 8DR3 and 8DR4. Gel shift assays showed than TR-RXR heterodimers formed equally well on 8DR3, 8DR4 and 8DR5. TR homodimers formed only slightly better on 8DR4 than 8DR3 and 8DR5. Conclusions: 1) 8DR3, 8DR4 and 8DR5 can impart equally strong T3 responses, indicating the 3-4-5 rule is less important for T3 inductions from octamer DRs; 2) 8DR3 gives greater T3 induction than vitD induction, and 8DR5 gives greater T3 induction than RA induction; 3) as shown by others, the 3-4-5 rule does hold for T3 responses from hexamer DRs; 4) the T3 response on 8DR3-5 could potentially be due to TR homodimers and/or TR-RXR heterodimers.

11:30 A.M.
107. **AMINO-TERMINAL PHOSPHORYLATION OF THYROID HORMONE RECEPTOR BETA MODULATES ITS BINDING TO AND FUNCTION ON THYROID HORMONE RESPONSE ELEMENTS.** O. Cohen, A.N. Hollenberg, T.R. Flynn, M.K. Hegarty, J.S. Flier, and F.E. Wondisford. Thyroid Unit and Endocrine Division, Beth Israel Hospital and Harvard Medical School, Boston MA.

Phosphorylation of both thyroid hormone receptor (TR) isoforms has recently been demonstrated. However, the location of phosphorylation sites on the TR-beta isoform (TR-β) and their functional consequence are unclear. We investigated the role of TR-β phosphorylation, as a possible mechanism for cross talk between thyroid hormone and cAMP signaling pathways. To localize phosphorylation sites in TR-β, N- and C-terminal truncations of a human TR-β cDNA were constructed. *In vitro* transcribed and translated wild type (WT) and truncated TRs were phosphorylated by protein kinase A (PKA) and ^{32}P ATP, immunoprecipitated, and analyzed by SDS-PAGE. A PKA phosphorylation site was localized to between amino acids 45 and 65 of human TR-β. This region contains a PKA phosphorylation consensus site. The DNA-binding characteristics of phosphorylated WT and N-terminal mutations of TR-β were then studied using a gel-mobility shift assay. TR-β bound as homodimers and heterodimers with retinoid X receptors (RXR) to three different positive TREs [Direct repeat +4 (DR+4), palindrome (Pal), Inverted Pal]. Phosphorylation of TR-β significantly reduced homodimer binding, while enhancing heterodimer binding to all these elements. These changes were PKA and ATP concentration-dependent and were abolished by a PKA inhibitor peptide or by heating the PKA. TR-β mutants lacking the 45-65 region did not show phosphorylation related changes in complex binding. Functional studies of the TR-β in heterologous cells suggest that cAMP analogs enhance T_3-mediated stimulation of a Pal containing reporter construct. These data indicate that cAMP enhances thyroid hormone stimulation by phosphorylation-induced changes in TR DNA-binding.

11:45 A.M.
216. FUNCTIONAL ANALYSIS OF A TRANSACTIVATION DOMAIN IN THE THYROID HORMONE β RECEPTOR V.K.K.Chatterjee, Y.Tone, T.N.Collingwood and M.Adams Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Hills Rd, Cambridge CB2 2QQ, U.K.

Nuclear receptors have been shown to exhibit variable constitutive transcriptional activity which localises to the amino-terminal (A/B) region, together with a ligand-dependent transactivation function mapping to the carboxy-terminal (D/E/F) domain. The latter also contains a series of hydrophobic heptad repeats which have been shown to be important for dimerisation. We have analysed the transcriptional properties of the human thyroid hormone β receptor (TRβ) in transfection assays by co-expressing chimaeric proteins, consisting of TRβ domains coupled to the DNA binding domain of the transcription factor GAL4, with a reporter gene containing GAL4 binding sites. Repression of basal transcription in the absence of ligand mapped to the D/E/F domain and was not enhanced by the presence of the A/B region. However, mutations (L423R, L428R) of hydrophobic residues in the ninth heptad repeat markedly impaired basal repression. Ligand-dependent transactivation also mapped to the D/E/F domain, but was completely abolished by deletion of nine carboxyterminal residues which may form an amphipathic alpha helix. To analyse this motif in detail, both hydrophobic and negatively charged residues were mutated to alanine, and reporter gene activation by mutant proteins assayed in a T3 dose-dependent manner. A number of mutants (P453A, F455A, L456A, F459A) exhibited right-shifted activation profiles consistent with their impaired hormone-binding affinities (WT $K_a = 2.2x10^{10}M^{-1}$, P453A = $0.7x10^{10}M^{-1}$, F455A = $0.6x10^{10}M^{-1}$, L456A = $0.4x10^{10}M^{-1}$, F459 = $0.03x10^{10}M^{-1}$). However, three mutants showed negligible or severely reduced ligand-dependent activation although their T3 binding affinities were comparable to wild type receptor (E457A= $3.4x10^{10}M^{-1}$, E457D= $2.1x10^{10}M^{-1}$, L454A=$0.9x10^{10}M^{-1}$). Furthermore, these mutations were equally deleterious when introduced into the native full length receptor and tested with a reporter gene containing a natural thyroid response element. To test mutant receptor interactions with RXR in cultured cells, the chimaeric proteins were co-expressed with a second fusion protein (VP16-RXR) containing the activation domain of VP16 coupled to RXR. All the carboxyterminal mutants interacted with VP16-RXR with equal efficacy comparable to wild type receptor, whereas the interaction of ninth heptad mutants (L423R, L428R) with VP16-RXR was markedly impaired. Finally, in vitro receptor interactions with the basal transcription factor TF-IIB were examined and shown to be preserved for all mutants. Our data indicates that both hydrophobic and charged residues in the carboxyterminus of TRβ are necessary for hormone-dependent transactivation. Basal repression by unliganded TRβ may in part be mediated by ninth heptad interactions with factors other than TF-IIB.

7. HYPOFUNCTION OF THE HYPOTHALAMUS-PITUITARY-THYROID AXIS IN CADMIUM-TREATED RATS. M.Pavia,Jr., B.Paier, M.I.Noli, M.Gonzalez Pondal, K. Hagmüller and A.A.Zaninovich, Univ.of Buenos Aires, Hospital de Clínicas, Argentina and Univ.of Graz, Dept.of Zoology, Graz, Austria.

Heavy metals such as cadmium (Cd) and zinc (Zn) are toxic environmental contaminants. Cd inhibits rat liver 5'-deiodinase (5'-D) activity, an effect also induced by Zn. However, it was shown than Cd- but not Zn-treated rats had inappropriately low serum T4 concentration. To clarify this point groups of 200 g BW male Wistar rats were injected with Cd chloride 2.5 mg/kg BW or Zn sulphate, the same dose, ip 24 h before the following experiments were performed: 1) TRH test: 1 ug TRH/100 g BW iv and blood drawn 10 and 20 min later. 2) Pituitary 5'-D activity: glands were homogenized, added 25 mM dithiothreitol, 1 mM Cd or Zn and 1 uCi ^{125}I-T4, incubated for 90 min at 37°C and chromatographed. 3) Thyroid/plasma ratio: 2 uCi ^{131}I were given iv and thyroidal and plasma radioactivity determined 4 h later. 4) T4 kinetics: 2 uCi ^{125}I-T4 were injected iv and blood samples, urine and feces were collected during 48 h. Serum T4, T3 and TSH were measured by RIA. RESULTS: In normal rats serum TSH averaged 3.2±1.3 ng/ml, after Cd it was 3.1±1.5 ng/ml and after Zn 2.4±0.9 ng/ml (NS). Cd- and Zn-treated rats were slightly hyperresponsive to TRH (NS). Pituitary 5'-D was blocked by Cd and by Zn ($P<0.01$). Cd decreased serum T4 levels to 21.1±7.6 nmol/l ($P<0.01$) (normal rats had 38.2±14.4 nmol/l) whereas Zn induced an increase (54.4±15.7 nmol/l,$P<0.05$). T4 MCR ($P<0.05$), degradation rate ($P<0.05$), ^{131}I-thyroidal uptake ($P<0.01$) and thyroid /plasma radioactivity ratio ($P<0.01$) were all decreased by Cd.

In summary, the lack of pituitary response to low serum T4 and T3 in Cd-treated rats appears to be unrelated to 5'-D inhibition, as this effect should have led to an increased TSH release. Instead, the changes in pituitary-thyroidal physiology are compatible with a Cd-induced nonthyroidal illness syndrome.

9. GALACTOSYLTRANSFERASE AND MANNOSIDASE II mRNA LEVELS INCREASE WITH DIFFERENT KINETICS IN THYROTROPHS OF HYPOTHYROID MICE. T.E. Helton and J.A. Magner, Section of Endocrinology, East Carolina University (ECU) School of Medicine, Greenville, NC 27858.

Hypothyroid animals secrete not only more TSH, but also qualitatively different TSH having altered oligosaccharides. To explore the cellular mechanism of oligosaccharide modulation by thyroid status, 40 mice were treated ± PTU, and pituitaries were removed after 1, 2, 3, 4, and 6 weeks. Serum T4 levels confirmed that mice receiving PTU were hypothyroid even after 1 week. Thyrotrophs and corticotrophs were identified in 5 um-thick pituitary slices using immunocytochemistry; *in situ* hybridization was performed using ^{35}S-labeled 48-mer DNA probes to galactosyltransferase (GTase) and mannosidase II (Manase). Control probes also were used. Autoradiography was performed for 4 weeks, cells and silver grains were scored, and the differences reported below were significant by ANOVA. Compared to euthyroid thyrotrophs, the GTase mRNA level in thyrotrophs increased 305% after mice received PTU for 1 week, and remained elevated for 6 weeks; the mean increase within thyrotrophs over the 6 week period was 151%, whereas there was little change in corticotrophs. These GTase data are based on scoring of 3036 cells and 7211 silver grains. Compared to euthyroid thyrotrophs, the Manase mRNA level in thyrotrophs remained unchanged while mice received PTU for 1, 2, 3, and 4 weeks, but then increased 100% after week 6 of PTU, whereas there was no change in corticotrophs. These Manase data are based on scoring of 2193 cells and 4147 silver grains. Thus, thyroid status modulates mRNA levels of two glycosyltransferases in thyrotrophs, perhaps by affecting gene transcription or mRNA stability. Moreover, the kinetics of the modulation of two glycosyltransferase mRNAs differ. This is the first report of the modulation of these glycosyltransferase mRNA levels in thyrotrophs, and may explain, in part, the mechanism by which different isoforms of TSH are secreted in different physiologic states. Supported by Boots Pharmaceuticals, the ECU Department of Medicine, and a starter grant from ECU.

22. INTERACTION OF THYROID HORMONE ON α- AND β- ADRENERGIC STIMULATION ON SARCOPLASMIC RETICULUM (SR) Ca^{2+} ATPase GENE EXPRESSION IN CARDIAC MYOCYTES. P. S. Wu and W. H. Dillmann, University of California, San Diego, CA

The similarities in the cardiac manifestations of thyrotoxicosis and catecholamine excess, and the therapeutic benefits of sympathetic blocking agents in hyperthyroidism are well established. But the molecular basis of the interactions between thyroid hormone and the adrenergic system in the heart is complex and remains poorly understood. We previously showed the expression of the SR Ca^{2+} ATPase gene is highly influenced by thyroid hormone (T_3). The present study was designed to determine whether T_3 modulates the effect of α- and β-adrenergic stimulation on the SR Ca^{2+}ATPase gene expression in cardiac myocytes. Neonatal rat cardiac myocytes were isolated and cultured for 48 hours in serum containing media before being maintained in serum-free media for an additional 24 hours. The cells were then treated with the following agents, either alone or in combination with 10^{-7}M T_3; phenylephrine (10^{-4}M) and propanolol ($2x10^{-6}$M) producing a pure α- adrenergic stimulation, the β-adrenergic agonist isoproterenol (10^{-6}M), and forskolin (10^{-5}M) which directly stimulates cardiac adenylate cyclase. SR Ca^{2+}ATPase gene expression was determined by Northern analysis of messenger RNA isolated from the cells. We confirmed our previous report that T_3 markedly increases the Ca^{2+}ATPase mRNA by 250%. Phenylephrine in the presence of propanolol was found to reduce the expression of Ca^{2+}ATPase gene by 50% and this downregulation is not overcome by the addition of T_3. Isoproterenol and forskolin did not affect the expression of Ca^{2+}ATPase mRNA but in combination with T_3, both these agents increased the gene expression by 150%. These results suggest that α- and β-adrenergic stimulation produce differing effects on the SR Ca^{2+}ATPase gene expression in cardiac myocytes which are modified by T_3. In the presence of α-adrenergic stimulation, the upregulation effect of T_3 on Ca^{2+}ATPase is completely suppressed, whereas, under β-adrenergic stimulation, this response is blunted. Our study showed complex interactions exist between T_3 and the adrenergic system on the expression of SR Ca^{2+}ATPase gene in the heart.

31. THYROID HORMONE INCREASES THE PARTITIONING OF GLUCOSE TRANSPORTERS TO THE PLASMA MEMBRANE IN ARL 15 CELLS: SURFACE GLUT1 PHOTOLABELING WITH [^{3}H]ATB-BMPA. R.S. Haber*, S.P. Weinstein*, A. Pritsker*, C. Wilson†, and S.W.Cushman†, *Mount Sinai School of Medicine, New York, New York, and †National Institutes of Health, Bethesda, Maryland.

The stimulation of glucose transport by L-triiodothyronine (T_3) in the rat liver-derived ARL 15 cell line has been shown to be partly attributable to increased GLUT1 glucose transporter gene expression. However, an early stimulation of 2-deoxyglucose (2-DG) uptake at 4-6 hours precedes the observed increase in GLUT1 protein levels (evident at 24-48 hours), and the greater stimulation of uptake after 48 hours of T_3 treatment is not fully accounted for by the increase in GLUT1 protein. We therefore hypothesized that GLUT1 glucose transporters in ARL 15 cells are distributed between inactive intracellular and active plasma membrane pools, and that T_3 treatment increases the partitioning of GLUT1 to the cell surface. A direct test of this hypothesis was made possible by the recent availability of the impermeant bis-mannose photolabel ATB-BMPA (2-N-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis(D-mannos-4-yloxy)-2-propylamine)for accurate quantitation of surface glucose transporters. Confluent monolayers of ARL 15 cells grown in medium with thyroid hormone-depleted serum were treated with 100 nM T_3 or diluent alone. To quantitate cell surface GLUT1, cell monolayers were incubated with [2-^{3}H]ATB-BMPA and exposed to UV irradiation (300 nm) for 2 minutes. GLUT1 protein was immunoprecipitated and subjected to SDS-PAGE, and the radioactivity in the GLUT1 peak (45-50 kD) was counted. To determine the relative amounts of surface vs. total cellular GLUT1, parallel cell monolayers were photolabeled in the presence of 0.025% digitonin, permitting access of the photolabel to intracellular GLUT1. In control cells, only about 20% of total cellular GLUT1 was present at the cell surface. T_3 treatment for 6 hours increased the rate of [^{3}H]2-DG uptake by 30%, 92%, and 95% in three independent experiments ($p<0.001$ for T_3 effect). Concomitantly, surface GLUT1 photolabeling increased by 17%, 81%, and 72%, respectively ($p<0.001$). We confirmed that total cellular GLUT1 measured by immunoblotting was not increased by T_3 treatment at 6 hours, indicating that T_3 caused a re-distribution of intracellular GLUT1 to the plasma membrane. T_3 treatment for 48 hours increased the rate of [^{3}H]2-DG uptake by 143%, 172%, and 216% in three experiments, and increased surface GLUT1 photolabeling by 88%, 161%, and 184%, respectively ($p<0.001$). Increases in total cellular GLUT1 photolabeling (15%, 91%, and 47%) were smaller. T_3 treatment for 48 hours thus increased the fraction of total cellular GLUT1 at the plasma membrane, from 21 ± 1% to 34 ± 3% (mean ± SE, n=3 experiments, $p=0.02$). We conclude: 1) that GLUT1 glucose transporters in ARL 15 cells are partitioned between surface and intracellular pools, as in other cell lines; 2) that most of the early stimulation of glucose transport by T_3 (at 6 hours) is mediated by an increase in the fractional partitioning of GLUT1 to the plasma membrane; and 3) that after 48 hours of T_3 treatment, the enhanced partitioning of GLUT1 to the plasma membrane superimposed on an increase in total cellular GLUT1 causes a further increase in surface GLUT1, and in glucose transport.

37. EFFECT OF THYROID HORMONE ON SYNAPTOSOMES: COUPLING OF SYNAPTOSOMAL T3 RECEPTOR TO G-PROTEINS IN CHICK EMBRYOS. A. Giguère, C. Beaudry, S. Fortier, N. Gallo-Payet, D. Bellabarba, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec, Canada

We have recently described a T3 receptor in synaptosomes of chick embryo brain. In order to understand how the hormone may act on its synaptosomal receptor we have investigated if this receptor could be linked to G-protein. We first examined the influence of GTP-γS on T3 binding to the synaptosomal receptor. 100 μM GTP-γS lowered the binding capacity of the high affinity site by 2.5 fold (Bmax from 29.7 to 11.7 pmole T3/μg prot.), a finding consistent with the coupling of receptor to G-proteins. Then we analyzed the effect of T3 on the ADP-ribosylation of G-proteins by pertussis toxin. This procedure has been used to determine the conformation of G-proteins, since their Gi and Go types are ADP-ribosylated if they are in an inactive form. The results indicated that preincubation with T3 induces a dose-dependent increase of the ADP-ribosylation of the α subunit of Go, with a maximal effect (30%) observed with 10 nM T3. This finding indicates that the hormone enhanced the level of inactive Go protein. Finally we investigated the influence of T3 on the intrinsic GTPase activity of G-proteins, measured by the hydrolysis of [γ^{32}P]-GTP and we observed a 30% diminution of enzyme activity in presence of 100 nM of T3. Kinetic studies suggest that T3 lowered the binding of GTP to the α subunit, but had no effect on the rate of its hydrolysis. This modulation of GTP binding to G-protein confirms that the T3 synaptosomal receptor is coupled to G-proteins and that the hormone acts at this level by decreasing the activity of these proteins and possibly formation of second messengers. Modulation of second messenger production is known to be an important phenomenon in the regulation of neuronal proliferation and differentiation. Therefore these results suggest that the action of T3 on brain maturation could be, at least in part, perform by this pathway via its synaptosomal receptor.

39. SEQUENCE OF NUCLEAR AND MITOCHONDRIAL ACTIONS IN THYROID HORMONE ACTIVATION OF METABOLIC EFFECTS. Kenneth Sterling and Milton A. Brenner, Bronx VA Medical Center, Bronx, NY and the Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY

The existence of extranuclear pathways of thyroid hormone action has been supported by findings from several laboratories which include studies of actions observed after administration of a bolus injection of triiodothyronine (T_3) into hypothyroid rats. Binding of the hormone by organelles such as mitochondrial membrane has been observed as well as early (30 min) increases in oxygen consumption and ATP formation by mitochondria. In the present work the activation of a component of mitochondrial inner membrane, adenine nucleotide translocase (AdNT), has been measured, and shown to have risen by 15% or more, compared to hypothyroid controls 30 min after iv injection of a minute dose of T_3 (40 ng). In vitro studies were also undertaken with preincubation of isolated hypothyroid mitochondria with 30 nM T_3 for 5 min. This brief preincubation resulted in a consistent rise in AdNT activity of 33% compared with control mitochondria preincubated with vehicle lacking hormone.

These studies were considered in relation to earlier experiments of ours in which normal rats which are injected intraperitoneally with a large dose (20 ug) of T_3 which revealed a gradual rise of AdNT activity from 20 hrs to 4 days, when activity had more than tripled. In the later time periods studied, the increases were viewed as reflective of enhanced protein formation of the carrier AdNT. This interpretation is supported by the findings of Luciakova and Nelson (Eur J Biochem 207:247-251, 1992) in which Northern blots revealed progressive rise of the specific mRNA directing the synthesis of AdNT over the course of 2 days. Thus we believe that T_3 has an early direct effect in activating the membrane carrier, AdNT, followed by nuclear directed enhanced formation of this protein.

44. ANNEXIN IV IS INDUCED BY THYROID HORMONE IN α1-RECEPTOR-DEPENDENT HUMAN FIBROBLASTS: APPLICATION OF DIFFERENTIAL DISPLAY ANALYSIS. J. Menke, B. Torres, R. Klann, J. Wiley, B. Bercu, and S. Usala, East Carolina Univ., Greenville, NC, Univ. of South Florida, Tampa, FL.

We have found primary fibroblasts from a patient with severe thyroid hormone resistance require thyroid hormone for normal growth in culture. This patient from Kindred S was homozygous for a dominant negative β-receptor mutation. In contrast, fibroblasts from normal and heterozygous patients of Kindred S were not dependent on T_3 for growth. Likewise, fibroblasts from a patient without β-receptor from Kindred F1 demonstrated a T_3-independent replication rate. We hypothesized that the "double dose" of mutant receptor in the homozygous S fibroblasts passively and/or actively repressed critical growth genes and that this repression could be partially reversed by T_3-activated α1-receptor. In order to identify T_3-regulated genes associated with cellular growth we have used differential display (DIFFDIS) analysis of mRNA from normal and homozygous S fibroblasts. Total RNA was prepared from normal and homozygous S fibroblasts 48 hours after culture in stripped media with or without 10^{-7}M T_3. Approximately 1000 genes were preliminarily screened and the great majority did not appear to be regulated by thyroid hormone. However, a 311 bp cDNA, visualized with the 5'-GCAATCGATG-3' and T_{12}AG primer set, was up-regulated by T_3 in both the homozygous S and normal fibroblasts. This cDNA was isolated, cloned, and sequenced, and perfectly matched the 3'-sequence of annexin IV. Significantly, annexin IV is a calcium-dependent lipid-binding protein that has been previously reported to be regulated by thyroxine in rat muscle and liver (Rainteau et al., J.B.C. 263:12844,1988). We conclude that since the homozygous S fibroblasts are dependent on α1-receptor for thyroid hormone action that the annexin IV gene is a candidate for transcriptional regulation by the α1-receptor. We also conclude that differential display analysis with the homozygous S fibroblasts is a feasible approach for characterizing the cascade of T_3-inducible genes involved in cellular replication.

59. **CHARACTERIZATION OF A NEW FAMILY WITH INHERITED PITUITARY RESISTANCE TO THYROID HORMONE.** O.E. Janssen and A.E. Heufelder, Medizinische Klinik, Klinikum Innenstadt, Ludwig-Maximilians-Universität, Munich, Germany.

The syndromes of resistance to thyroid hormone are characterized by a reduced effect of thyroid hormones on peripheral tissues, the pituitary, or both. The majority of reported cases belong to the latter category, also known as generalized resistance to thyroid hormone (GRTH), a disorder with autosomal dominant inheritance coinciding with mutations of the thyroid hormone receptor-ß (TRß) in most families studied to date. Due to the global tissue hyposensitivity, untreated subjects usually achieve a normal metabolic state with elevated levels of circulating thyroid hormones. In contrast, pituitary resistance to thyroid hormone (PRTH) manifests as thyroid hormone excess at peripheral tissues and presumably occurs sporadic. However, PRTH may encompass a number of unrelated conditions including non-neoplastic pituitary hyperplasia, defective pituitary 5'-deiodinase, and subjects with GRTH with a more pronounced pituitary hyposensitivity to thyroid hormone. We now present data concerning clinical and biochemical characterization of a new subject with familial occurence of PRTH. The patient presented with tachycardia, palpitations, nervousness, weight loss, excessive sweating and a small diffuse goiter. Laboratory findings were as follows:

test	value	normal range	test	value	normal range
TSH	2.5 µU/ml	0.4 - 4.0	TRH stim. TSH	5.0 µU/ml	3.0 - 18.0
TT3	403 ng/dl	80 - 180	TBG	2.2 mg/dl	1.5 - 2.8
TT4	20.3 µg/dl	4.5 - 10	Tg-Ab	171 U/ml	< 350
FT3	7.4 ng/l	1.9 - 5.0	Mic-Ab	< 100 U/ml	< 350
FT4	6.7 ng/dl	0.8 - 1.8	TSH-R-Ab	92 % bind.	85 - 100

Except for iron-deficiency, all other values were within the normal range. Isoelectric focusing of serum revealed a normal pattern of T_4-binding proteins. Elevated FT_4 levels were confirmed by equilibrium dialysis. Other affected members of the family include the patients mother, sister and brother, as well as her 6-year old daughter, who shows symptoms of the attention-deficit-hyperactivity-disorder, known to be associated with GRTH. CONCLUSION: The symptoms and laboratory findings of the members of the family examined are consistent with inherited PRTH, of which only three other cases have yet been reported.

62. CIRCADIAN VARIATION IN CIRCULATING TSH OLIGOSACCHARIDES: OBSERVATIONS FROM FREQUENT BLOOD SAMPLING IN FOUR HUMAN SUBJECTS. J.A. Magner, J. Kane and N. Scherberg, East Carolina University School of Medicine, Greenville, NC, Michael Reese Hospital, Chicago, IL, and University of Chicago, Chicago, IL.

Multiple isoforms of TSH exist with different metabolic clearance rates due to variations in oligosaccharide structures. The serum level of TSH varies in a circadian fashion with a peak at about 11 pm. In theory, the serum level might rise because more TSH is being secreted, or because TSH isoforms having a longer circulation time and a different oligosaccharide structure are being released at certain times; we examined the latter possibility by drawing blood around the clock from four subjects. Each TSH specimen was treated ± neuraminidase, and was analyzed in duplicate by affinity chromatography using ricin lectin. The percentages of sialylated TSH isoforms (mean ± range) in the subjects during each time period were:

SUBJECT	2 PM	11 PM	4 AM	11 AM	2 PM	11 PM
1	3.2	2.0±0.7	5.1±0.6	43.4±4.9*	10.9	
2		47.0±2.3	48.9±1.5	56.7±2.3		
3		14.1±1.9*	4.2±0.5	3.5±0.1	3.3±0.4	18.2±0.3*
4		43.3±2.8	50.5±1.1	55.3±5.1	51.0±1.7	

Asterisks indicate significantly high values, by ANOVA, for a given subject. Thus, subjects 1 and 3 exhibited circadian variation in TSH sialylation, but the changes were not in phase. Subjects 2 and 4 showed no variation in sialylation with time, suggesting that there may be substantial individual differences in such changes. This is the first report of diurnal variation of TSH oligosaccharide structure. One may speculate that diurnal cycling of hypothalamic factors influences thyrotroph posttranslational processing and/or selective TSH isoform secretion in some persons.

64. PULSATILE THYROTROPIN (TSH) AND PROLACTIN (PRL) SECRETION DURING 10-DAY CONTINUOUS THYROTROPIN-RELEASING HORMONE (TRH) INFUSIONS. M.H. Samuels and P. Kramer, Oregon Health Sciences University, Portland, Oregon and University of Texas Health Sciences Center, San Antonio, Texas.

TSH and PRL are secreted in a pulsatile fashion in normal and hypothyroid subjects. The origin of these pulses is unknown; since both hormones are stimulated by TRH, pulsatile TRH input from the hypothalamus may control pulsatile TSH and PRL secretion. In this case, continuous TRH infusions should abolish TSH and PRL pulses. To test this hypothesis, constant TRH infusions (1 μg/min) were administered over 9 days to 6 subjects with treated primary hypothyroidism and normal basal TSH levels. Hypothyroid subjects were chosen to avoid TRH-induced thyroid hormone elevations that could potentially affect TSH pulses. Blood samples were drawn every 15 min for 24h prior to (BASELINE) and on the final day (TREATED) of TRH infusions. TSH and PRL levels were measured by immunoradiometric assays. Hormone pulses were located by Cluster analysis. Results were as follows (means ± SEM):

	BASELINE	TREATED
24h mean TSH (mU/L)	3.06 ± 1.25	26.01 ± 7.44*
24h TSH pulse freq	13.5 ± 1.1	16.5 ± 1.8
24h TSH pulse amp (mU/L)	3.53 ± 1.41	30.72 ± 8.76*
24h mean PRL (ng/mL)	15.48 ± 1.49	23.10 ± 3.50
24h PRL pulse freq	8.7 ± 0.7	9.7 ± 0.9
24h PRL pulse amp (ng/mL)	21.35 ± 2.05	32.71 ± 4.97

* = $p < 0.02$ by paired t-test

In these treated hypothyroid subjects, constant TRH infusions for 9 days markedly increased mean serum TSH levels and TSH pulse amplitude without altering TSH pulse frequency. Mean PRL levels and PRL pulse amplitude rose 50%, but these changes were not significant, and PRL pulse frequency was not affected by TRH infusions. These results suggest that TRH controls TSH (and perhaps PRL) pulse amplitude. However, TRH does not appear to participate in the generation of TSH or PRL pulses, and the origin of these pulses remains unclear.

67. EPIDERMAL GROWTH FACTOR (EGF) TRANSCRIPTIONALLY DOWN-REGULATES THYROTROPIN-RELEASING HORMONE RECEPTOR MESSENGER RIBONUCLEIC ACID IN RAT PITUITARY CELLS. S. Konaka, M. Yamada, T. Monden, T. Satoh, T. Iwasaki M. Murakami, T. Iriuchijima and M. Mori, First Department of Internal Medicine, Gunma University School of Medicine, Maebashi, Gunma, Japan

Epidermal growth Factor (EGF) has been reported to regulate the thyrotropin-releasing hormone (TRH) responses in rat pituitary GH4C1 cells by changing the number of TRH receptors (TRH-Rs). In this report, we investigated the mechanism by which EGF regulates TRH-R numbers in GH4C1 cells using Northern blot analysis, a protein and RNA synthesis inhibitor, and nuclear run-on assay. Northern blot analysis and binding studies revealed that treatment of cells with EGF reduced both TRH-R bindings and TRH-R mRNA levels in a dose- and time- dependent manner, while no significant changes were observed in β-actin mRNA levels. EGF (1nM) caused a 40 % decrease in the TRH-bindings and a 80 % decrease in its mRNA level after 48 hr. Treatment of the cells with actinomycin D to inhibit new RNA synthesis increased TRH-R mRNA level to 170 % of control at 2 hr incubation, and then caused a time-dependent decline. Following EGF treatment, actinomycin D caused a 30 % increase in TRH-R mRNA level, and then caused the same decline. The data suggest that EGF can inhibit an increase in TRH-R mRNA level induced by actinomycin D, and the stability of TRH-R mRNA was not significantly affected in EGF treated cells. Nuclear run-on assay revealed that the rate of transcription of the TRH-R gene was significantly inhibited in cells treated for 48 hr with 1nM EGF (Approx. 60 % of control). Furthermore, incubation in the presence of cyclohexamide resulted in a 60 % increase under basal conditions and completely blocked EGF-mediated decrease in TRH-R receptor mRNA levels. We conclude that 1) EGF decreases expression of TRH-R mRNA in large part by reducing its transcriptional rate, and this action requires synthesis of new protein, and 2) inhibitors for protein and RNA synthesis cause a significant increase in the basal TRH-R mRNA levels, suggesting that there may be a protein suppressing TRH-R mRNA level in pituitary.

72. COMPARISON AMONG THREE TRUNCATED T3 RECEPTORS IDENTIFIED IN PATIENTS WITH GENERALIZED RESISTANCE TO THYROID HORMONE (GRTH). H.Nakamura, Y.Miyoshi*, S.Sasaki*, T.Tagami*, K.Nakao*, M.Taniyama+, T.Yoshimi. Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu; Kyoto University School of Medicine*, Kyoto; Showa University School of Medicine+, Tokyo, Japan.

We compared here the functions of three different truncated T3 receptor(TR)β1s identified in unrelated patients with GRTH under the same experimental conditions. Two consecutive base substitutions in TRβ1 gene in a 6-yo girl changed the 451st Phe codon(TTC) to stop codon(TAA), creating TRβ1 truncated 11 amino acids from the C-terminal(F451<) (Thyroid 3(supple)T-3,1993). She had mental retardation, delayed speech development, attention deficit hyperactivity disorder(ADHD) and SITSH (T3 826ng/dl, fT4 12.9ng/dl, TSH 0.6μU/ml). Another truncated TRβ1 with 13-amino acid deletion (F449<) was found in a 16-yo boy who showed SITSH (fT3>20pg/ml, fT4 >8ng/dl, TSH 2.2μU/ml) and low TBG (3.4μg/ml), but not any clinical signs and symptoms except for goiter. In addition to these mTRs we identified in Japanese patients, the third truncated TRβ1, C446< with 16-amino acid deletion, identified in a 31-yo American male was provided by Dr. R.I. Dorin (Mol Cell Endocrinol 99:81, 1994). The clinical features of this patient included ADHD, mental retardation, deafness, short stature and goiter.

Each mutant TR (mTR) was expressed in Cos-7 or CV1 cells. T3 binding activity in nuclear extracts analyzed by Scatchard plots was remarkably low in every mTR with no significant difference. None of mTRs showed any transcriptional activity, assayed using TRE pal_2- and DR4-CAT reporter genes, even with 1μM T3. When dominant negative activity of mTRs was studied by co-transfecting with wild TRβ1 (molar ratio 1:1) and a reporter gene, each mTR showed very strong activity. Interestingly, the severity of dominant negative activity was equal in F449< and C446<, and that of F451< was significantly stronger than the others. A gel shift study revealed no apparent difference among mutant and wild TRs. The mTRs suppressed RAR functions and F451< again exhibited the strongest inhibition.

In summary, (1) F451< with 11-amino acid truncation showed stronger dominant negative activity than mTR with 13- or 16-amino acid truncation. (2) Clinical features such as mental retardation, deafness and ADHD did not necessarily correlate with severity of mTR's dominant negative activity.

73. IS THE CLINICAL PRESENTATION OF RESISTANCE TO THYROID HORMONE (RTH) PREDICTABLE THROUGH THE FUNCTIONAL ANALYSIS OF MUTANT THYROID HORMONE RECEPTOR (TR) ßS?

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RTH is characterized by variable tissue hyposensitivity to thyroid hormone. Mutations of the TRß gene have been identified in nearly 100 families with RTH. In most cases, free thyroid hormone levels in affected members are nearly double the upper limit of normal and T3-binding of mutant TRs is less than one third that of wild type (WT) TRß. In the present study, we analyzed the properties of mutant TRs from families with RTH who have a relatively mild elevation in circulating free T4 (Family Mb[G316H], Ma[R320H] and Mj[I431T]). Transcriptional activity as well as dominant negative effect (DNE) on WTTRß were tested on positive (palindrome (pal) or inverted palindrome (F2)) and negative (human α-subunit (α-sub)) thyroid hormone response element (TRE) regulating expression of the luciferase gene. Binding of mutant TRßs to DNA were tested by electromobility shift assay on F2. A mutant TRß with severe impairment of T3 binding and strong DNE, Mf (G345R) was used for comparison.

		WT	R316H	R320H	I431T	G345R
FT4I(%upper normal limit)		—	120	145	129	226
T3 binding affinity (% of WT)		100	5	42	5	<3
Transactivation*	pal	1	250	10	100	>1000
	F2	10	1000	100	250	>1000
	α-sub	<1	10	<1	10	>100
DNE (1nM/10nM)†	pal	0 / 0	70 / 70	80 / 60	60 / 50	85 / 95
	F2	0 / 0	60 / 90	80 / 80	50 / 80	80 / 95
	α-sub	0 / 0	20 / 0	15/0	10 / 0	70 / 40
Binding to F2 TRE	homodimer(HD)	+++	-	++	-	+++
dissociation of HD by 10^{-7}M T3		yes	NA	yes	NA	no
	heterodimer	+++	+++	+++	++	+++

* *nM of T3 required for 1/2 max transactivation (pal or F2) or repression (α-sub) of luciferase gene expression.*

† *(T3 concentration) %interference with WT TRß transactivation (pal or F2) or repression (α-sub) in the presence of mutant TRß and indiciated T3 concentraion.*

Though R316H and I431T had severely impaired T3-binding and transactivation, their DNE were much weaker than G345R, but comparable to R320H. The level of FT4I required to maintain a normal serum TSH level as well as the dose of exogenous T3 required to produce a similar suppression of TSH appear to depend predominantly on DNE of these TRß mutants rather than T3-binding. Differences in interaction of mutant TRs with TREs may partially account for these findings. The peripheral tissue responses to T3 in affected individuals were highly variable and correlated poorly with in vitro results suggesting other genetic factor(s) also play a role in the phenotype of RTH.

81. EVIDENCE THAT 125I-THYROXINE (T4*) UPTAKE IN RAT BRAIN REFLECTS BINDING TO TYPE II 5'-DEIODINASE. J.T.Gordon,L.V. Outterbridge, E.E.Tomlinson and M.B.Dratman, Medical College of Penna., U.of Penna.,and V.A.Medical Center,Philadelphia,PA.

Unlike T_3, which is taken up into synaptosomes by a sodium- and energy-dependent mechanism, T_4 appears to enter synaptosomes by diffusion. There is at best ambiguous evidence that T_4 enters any CNS structure via a carrier-assisted mechanism. To the contrary, autoradiography of frozen brain sections after iv T_4^* reveals that T_4^* fails to differentially label brain networks when T_4 to T_3 conversion is blocked by prior Na ipodate administration. Under those circumstances, T_4^* labelling is diffuse and nonspecific, never revealing the evidence for specific localization and axonal transport seen after iv T_4^* when brain type II 5'-deiodinase (5'-D-II) is not blocked. We have, therefore, suggested that apparent examples of mediated uptake of T_4 in brain may represent binding of T_4 to plasma membrane sites such as 5'-D-II assemblies. Recently we reported that CNS 5'-D-II, like brown adipose 5'-D-II, is maintained by synergism of a_1- and B-adrenergic effects. If brain 5'-D-II is the site for both T_4 binding and deiodination, both phenomena should be inhibited similarly by noradrenergic receptor-blocking agents. We have now observed the effect of prazosin (4mg/kg) and propranolol (20mg/kg) on in vivo ^{125}I-T_4^* uptake in brain of hypothyroid male rats killed 3 h after iv T_4^*. Apparent uptake of T_4^* i.e. T_4^* binding plus T_3^* (T_4^*) uptake [T_4^*+2T_3^* (T_4^*)] in brain decreased 3% (N.S.) after prazosin, an a_1- blocking agent, and 13% (N.S.) after propranolol, a B-blocking agent, but 41% (P<.0005) after both blocking agents together. Exactly the same pattern of effects on T_4^* deiodination by 5'-D-II was produced by these two agents. Conclusion: 5'-D-II in rat brain may be the site for both binding and deiodination of T_4.

92. CHARACTERIZATION OF EXPRESSION AND FUNCTION OF T3 RECEPTOR α AND β VARIANTS IN NORMAL AND CHRONICALLY DISEASED HUMAN LIVER. A. Chamba, J. Hopkins, A. Strain,* J. Neuberger*, R. Bland, M.C. Sheppard and J.A. Franklyn, Department of Medicine and *Liver Unit, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, UK.

It is well recognised that the liver represents an important target tissue for thyroid hormone action but little is known regarding the relative expression and function of α and β T3 receptor (T3R) variants, especially in man. We have therefore characterized expression of T3R α1, α2 and β1 receptors in normal and diseased human liver and have determined expression of target genes regulated by T3 as a marker of T3R function. Western blot analysis using specific polyclonal antibodies to human T3R α1, α2 and β proteins (Falcone et al, Endocrinol 131, 2419) revealed abundant expression of all receptor variants in nuclei of normal and diseased liver (T3R α1 - single 48 kDa band, T3R β - 52, 55 and 65 kDa bands; predominant α1 60 kDa and β 65 kDa bands in the human hepatoma Hep G2 cell line). Quantification of Western blots by scanning laser densitometry revealed no significant differences in T3R expression between normal and diseased (primary biliary cirrhosis) liver (T3R α1: normal 0.80±0.18 OD units, PBC 0.83±0.29; T3R β 52 kDa band: 1.08±0.3 vs 1.33±0.3; 55 kDa band: 0.65±0.1 vs 0.9±0.3; 65 kDa band: 1.32±0.4 vs 1.31±0.3, mean±SE, n=6) and there were similarly no differences between normal liver samples and those affected by cryptogenic cirrhosis, alcoholic cirrhosis or sclerosing cholangitis. Immunocytochemistry using the same antibodies revealed similar levels of intranuclear staining for T3R α1, α2 and β variants when normal and diseased liver were compared, staining being confined to hepatocytes. Despite abundant T3R expression determined by Western analysis and immunocytochemistry, Northern blot hybridization of specific T3R α1, α2 and β cDNAs to total RNA (50 μg per lane) from normal and diseased liver failed to reveal specific T3R mRNA bands and similar analysis of poly A+ RNA (10μg per lane) revealed only a feint α2 mRNA band of 3.2kb (in contrast to readily detectable α1, α2 and β mRNAs in total RNA prepared from rat cerebral cortex). Despite marked reductions in circulating concentrations of free T3 in patients with chronic liver disease compared with controls, Northern blot analysis revealed similar levels of expression of the T3 regulated genes encoding TBG and TTR in normal and diseased human liver suggesting maintenance of tissue euthyroidism (TBG/18S ratio: normal 0.23±0.04 OD units, diseased (PBC) 0.30±0.07; TTR/18S ratio 1.01±0.4 vs 0.98±0.2; mean±SE, n=4). These findings suggest that T3R α and β proteins play an important role in human liver allowing maintenance of a euthyroid state in chronic disease despite a reduction in serum T3.

97. STEROID HORMONE RECEPTORS BLOCK THYROID HORMONE-MEDIATED TRANSCRIPTIONAL ACTIVATION. P.M. Yen, E.C. Wilcox, and W.W. Chin, Division of Genetics, Brigham and Women's Hospital; Howard Hughes Medical Institute and Harvard Medical School, Boston, MA.

Thyroid hormone receptors (TRs) and steroid hormone receptors belong to a large superfamily of nuclear hormone receptors. The interactions between these receptor subfamilies are poorly understood. In this study, we used co-transfection assays to examine the effects of estrogen and glucocorticoid receptors (ERs, GRs) on TR-mediated repression of basal transcription by unliganded TR, and transcriptional activation by liganded TR on two different thyroid hormone response elements (TREs). Our results demonstrate, for the first time, that steroid hormone receptors can block T_3-mediated transcriptional activation with little or no effect on basal repression. This blockade was enhanced by both cognate steroid hormones and several antagonists. Furthermore, studies using various ER and GR truncation mutants suggest that, while DNA-binding is not required, the DNA binding domain may be the locus for the blockade of transcriptional activation. In electrophoretic mobility shift assays, ER was unable to bind to the TREs in the presence or absence of estrogen. These results suggest that the mechanism for blocking TR-mediated transcriptional activation does not require steroid hormone receptor binding to TREs. Rather, it may involve titration of a critical co-activator(s) required for T_3-mediated transcriptional activation. It is possible that this novel cross-talk pathway may modulate TR-mediated transcriptional activation in steroid hormone-responsive tissues.

98. 9-*CIS* RETINOIC ACID REGULATION OF RAT GROWTH HORMONE GENE EXPRESSION: POTENTIAL ROLE OF MULTIPLE NUCLEAR HORMONE RECEPTORS. A. Sugawara, Division of Genetics, Department of Medicine, Brigham and Women's Hospital; Howard Hughes Medical Institute and Harvard Medical School; Boston, MA.

Rat growth hormone (rGH) gene expression is increased by both thyroid hormone (T_3) and all-*trans* retinoic acid (RA) via a composite hormone response element containing three putative half-sites (rGH-HRE). However, it is not known whether 9-*cis* retinoic acid (9cRA) also can regulate rGH gene expression. In this study, we performed a Northern blot analysis which demonstrated that 9cRA as well as T_3 and RA increased rGH mRNA expression in GH_3 cells. However, no further stimulation was observed when cells were treated with both T_3 and 9cRA. Transient transfection studies in rat pituitary GH_3 cells, using reporter plasmids containing the rGH-HRE and mutated half-sites, revealed that 9cRA-stimulation of rGH transcription was mediated by the rGH-HRE, and all three half-sites were necessary. In order to elucidate the particular receptor complexes involved in this 9cRA effect, we next co-transfected a reporter plasmid containing the rGH-HRE, and expression plasmids encoding several different receptors into a cell line which contains little or no endogenous retinoic acid receptor (RAR) or thyroid hormone receptor (TR). Interestingly, in the presence of either retinoid X receptor (RXR) alone, RAR alone, or both receptors, 9cRA caused similar induction of transcriptional activity. However, co-transfection of TR with these receptors suppressed basal and blocked 9cRA-induced transcriptional activity in the absence of T_3. Our data suggest that 9cRA-stimulation of rGH transcription is likely mediated by 9cRA bound RXR- and/or RAR-, but not by TR-, containing complexes. We also show that unliganded TR can block 9cRA stimulation of rGH transcription. Our studies provide evidence that several different members of the nuclear hormone receptor family can interact on this composite DNA element, with transcription stimulated or blocked depending on the presence or absence of cognate ligands.

100. **EFFECT OF 3,5-DIIODO-L-THYRONINE ON PITUITARY GENE EXPRESSION IN VITRO.**
S. Ball, C. Horst*, H. Rokos*, W.W. Chin. Division of Genetics, Department of Medicine, Brigham and Women's Hospital; Howard Hughes Medical Institute and Harvard Medical School, Boston, MA; and Marion Merrell Dow Research Institute, Henning Berlin R&D, Berlin*.

The major iodothyronine secreted by the thyroid gland, thyroxine (T4), is metabolized through outer ring monodeiodination to the active hormone, triiodothyronine (T3). Further outer ring deiodination of T3 produces 3,5 diiodothyronine (T2). This compound is generally considered to be inactive. However, it has recently been reported that T2 has significant thyromimetic activity on the pituitary, at doses that do not produce systemic effects (Horst, C., Harneit, A., Seitz, H.J., Rokos, H. (1993). J. Endocrinol. Invest. 16. (Suppl. 2): 91). Thus, T2 may be a preferential agonist at the pituitary level. In this study, we examined whether T2 can regulate pituitary gene expression in cultured pituitary cells, and whether T2 can bind to T3 receptors (TRs). GH3 cells were treated with various doses of T2 (Henning Berlin) and T3 (Sigma), for 24 hours. Growth hormone (GH) and TRβ2 mRNA levels were determined by Northern blot analysis, and mRNA levels corrected for cyclophilin mRNA. The ability of T2 to dissociate TR homodimers from DNA was tested by EMSA, using *in vitro* translated TRβ1, and an inverted palindrome probe (F2). The method used to synthesize T2 precludes contamination with T3; formal assessment by HPLC revealed T3 contamination of less than 0.01%.

		1/2 Max. response	Max. response
GH mRNA	T2	40 nM	1.00 μM
	T3	0.4 nM	0.01 μM
TRβ2 mRNA	T2	0.8 nM	0.10 μM
	T3	0.9 nM	0.05 μM

Table 1. Dose-response of GH and TRβ 2 mRNA to T2 and T3 in GH3 cells.

Maximum responses to the iodothyronines were quantitatively similar. T3 was approximately 100 times as potent as T2 in stimulating GH mRNA levels. T3 was only two-fold as potent as T2 in maximally decreasing TRβ2 mRNA levels. Indeed, concentrations of the iodothyronines giving half-maximal reduction in TRβ2 levels were equivalent. EMSA revealed significant dissociation of TRβ1 homodimers at 1 nM T2, with maximal dissociation at 1 μM, similar to T3. In summary, we demonstrate that T2 is capable of regulating T3-dependent gene expression in cultured pituitary cells, and can bind to *in vitro* translated TRs. These data provide both functional and physical evidence that T2 has significant thyromimetic activity.

104. DNA BINDING AFFINITY OF hTRβ1 MUTANTS AS HETERODIMERS WITH TRAPS FROM DIFFERENT TISSUES.

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Patients with generalized resistance to thyroid hormone (RTH) show various organ specific features, for example mental retardation, growth abnormalities, liver damage or delayed bone age. This may reflect aberrant mutant TRβ1 heterodimerization with specific thyroid hormone receptor auxiliary proteins (TRAPs) from different tissues, altering the mutant's ability to transactivate tissue-specific genes. We examined the heterodimerization of TRβ1 mutants and TRAPs of several rat tissues (cerebrum, cerebellum, liver, heart, lung, spleen, kidney and testis) by gel mobility shift assay(GMSA). Mutant TRβ1 proteins, synthesized in reticulocyte lysate, were incubated with ^{32}P rat malic enzyme (rME) thyroid hormone response element (TRE) and nuclear extracts of rat tissues. The TRβ1 mutants used were CL(R320H), Mf(G345A), Mh(P453H), Mc(P453S) and GH(R316H). Their ratios of T_3 affinity to Ka of TRβ1 wild type(TRβ1wt) were 0.42, <0.03, 0.19, 0.30 and 0.06, respectively. Two major bands were observed in this GMSA. Cerebellum and lung extracts formed a slowly migrating band (possibly RXRβ heterodimers), while liver and kidney extracts formed a faster migrating band (possibly RXRα heterodimers), and cerebrum, heart, spleen, and testis had both bands. There were no obvious differences in heterodimerization between TRβ1wt, and all TRβ1 mutants, when using tissue extracts and DNA in presumably excess ratio to TR. Scatchard analysis of DNA binding was performed by competing with 5, 10, 50, and 300 fold excess non-radiolabeled rME-TRE. After GMSA, the amount of heterodimer band was measured by densitometry. Mutant GH TR, which causes "pituitary RTH", formed heterodimers with lower DNA affinity than TRβ1wt with all extracts, whereas other mutant heterodimers had DNA affinity similar to that of TRβ1wt. The mutant GH, Mc and TRβ1wt receptor expression vectors were cotransfected into COS1 cells, together with a double-palindromic TRE fused to a Tk-luciferase gene as a reporter. The mutant GH had weaker dominant negative effect than Mc. Conclusions: 1) As anticipated, TRβ1wt and mutants dimerize with different TRAPs in different tissues. 2) The affinity of the tested mutant TRs did not show any tissue-specific defects in binding to TRAPs. 3) Deficiency in heterodimerization by mutant GH correlates with a reduced dominant negative function.

108. THE C-TERMINUS OF THE BETA-SUBUNIT IS IMPORTANT FOR THYROTROPIN SECRETION. D. T. Herodotou, and F.E. Wondisford. Thyroid Unit, Beth Israel Hospital, Harvard Medical School, Boston, MA.

The striking conservation of 12 cysteine (C) residues at identical positions in all pituitary glycoprotein hormone β-subunits suggests that disulfide bonds between cysteine pairs are similar or identical among β-subunits and are important for folding of the subunits. There is agreement for TSH-β subunit bonds between cysteine residues: C16-C67, C19-C105, and C88-C95. We have recently identified a novel mutation in a Brazilian kindred with congenital central hypothyroidism. This mutation resulted in replacement of C105 with a valine (V) residue and an additional 9 amino acid non-homologous extension. This β-subunit mutation resulted in impaired production of TSH in this kindred and in heterologous cell culture systems and led us to investigate the role of C-terminal residues in TSH assembly, secretion, and bioactivity. Human TSH-β minigenes, containing either a wild type (WT) sequence, a codon 105 frameshift mutation (C105V), or a C-terminal truncation, were constructed, and confirmed by DNA sequencing. A mammalian expression vector containing either a WT or mutant TSH-β minigene was cotransfected with a human common α-subunit expression vector into 293 cells; and stable transfectants, expressing WT or mutant recombinant human TSH, were selected. The C105V mutation would be predicted to affect the folding of the β-subunit and thus hinder its assembly with the α-subunit. Our preliminary data confirm this prediction. TSH concentrations from cell cultures transfected with the C105V mutant were at least 10-fold lower than those from cell cultures transfected with the WT construct in three different immunoassays. A second construct truncating the TSH-β subunit just after C105 impaired TSH secretion to the same extent, indicating that inclusion of C105 was not sufficient to restore normal TSH secretion. Truncation of the TSH-β subunit just after tyrosine 112, however, resulted in normal secretion. These data suggest that C-terminal residues between 106 and 111 are critical for TSH secretion. They may facilitate the folding of the β subunit or its assembly with the α-subunit. Analysis of truncations between 106 and 110 are planned to localize further the critical TSH-β subunit residues.

111. AN ARTIFICIAL THYROID HORMONE RECEPTOR MUTANT WITHOUT DNA BINDING CAN HAVE DOMINANT NEGATIVE EFFECT. R-T Liu, S. Suzuki and T. Takeda, Thyroid Study Unit, The University of Chicago, Chicago, IL 60637

The syndrome of generalized resistance to thyroid hormone encompasses a heterogenous group of conditions which are caused by mutation of thyroid hormone receptor β1 (TRβ1), usually in the ligand-binding domain. When co-expressed in transient transfection assays, mutant receptors inhibit the activity of normal receptors, demonstrating dominant negative effect (DNE). However, the mechanism of DNE is not well understood. One study (Nagaya et al, J Biol Chem 1992) emphasized the essential role of intact DNA-binding activity of mutants in order to exert the DNE, since introduction of an additional mutation in the DNA-binding domain (DBD) into a mutant receptor eliminated its DNE. During our study of the DNE of TRvα2 (Endocrine Society Annual Meeting 1994), a DBD mutant of TRα1, which has substitution of cysteine at position 73 in the "P box" of the first zinc finger by serine, was also obtained. This mutant does not activate transcription from TREs although it binds T3. In transient co-transfection studies, TRα1 DBD mutant inhibited the transactivation by wild-type TR either in the presence or absence of T3 on three different TRE-linked TK-luciferase reporter genes; double palindrome, rat malic enzyme (rME), and inverted palindrome. Co-transfection study of the previously reported TRβ1 DBD mutant also showed a DNE. Co-expression of TRvα2 can enhance the DNE of these two DBD mutants. TRα1 DBD mutant alone can also inhibit the transactivation from a TK-luciferase reporter gene either linked with rME or not. These are the same findings as we observed using TRvα2. Our results indicate that a DBD mutant can have DNE, possibly through a mechanism similar to TRvα2 which may interfere with basal trancription factors. The clinical significance of these DBD mutants is currently unclear, but it is logical to expect that they do occur in nature.

113. IMPORTANCE OF THE GC BOX FOR CONSTITUTIVE ACTIVITY OF THE PROMOTER OF HUMAN THYROID HORMONE RECEPTOR β1

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Two major isoforms of thyroid hormone receptors (TRs), TRα1 and TRβ1, are present in mammals. TRα1 mRNA is expressed at an early stage in development, while TRβ1 mRNA appears at a late stage of maturation and is widely distributed in adult tissues. It is known that Sp1 transcriptional factor is ubiquitously expressed in tissues and increases transcriptional activity through binding to a specific sequence (5'-GGGCGG-3') called the GC box. Sequencing analysis revealed that there are five GC boxes in the hTRβ1 promoter region between -1325 and the transcription start site. To evaluate importance of the GC boxes in basal expression of hTRβ1, I assessed transcriptional activity of the TRβ promoter attached to a luciferase reporter following transient transfection into HepG2 cells, in which TRβ1 is normally expressed. 5'-deletional analysis showed that the transcriptional activity of the promoter between -130 and +44, which has the most proximal GC box, conserved 80 percent of the basal activity of the entire promoter, and that further deletion to -96, which removes the last GC box, sharply suppressed expression. Two nucleotides substitution in the most proximal GC box of the whole promoter suppressed transcriptional activity to 40 percent of the normal promoter. These findings indicate that Sp1 transcriptional factors contribute to the basal expression level of TRβ1 through binding to the most proximal GC box. The other GC boxes in the promoter may be involved in different physiologic aspects of receptor expression.

115. THYROID HORMONE POTENTIATES rHuIFN-γ-INDUCED HLA-DR EXPRESSION IN HeLa CELLS BY A PROTEIN KINASE-DEPENDENT MECHANISM. H.-Y. Lin, F.B. Davis, L.J. Martino, M.G. Hogg, H.R. Thacore and P.J. Davis, Albany Medical College, Albany, and SUNY at Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY.

HLA-DR antigen expression is induced by recombinant human interferon-γ (rHuIFN-γ) in a variety of cells, including HeLa cells. We have recently shown that thyroid hormone potentiates by more than 100-fold the antiviral action of rHuIFN-γ in human fibroblasts and HeLa cells (J Clin Endocrinol Metab, in press) and does so by a mechanism that does not require protein synthesis and is blocked by staurosporine, a protein kinase inhibitor. In the present studies we examined the effect of rHuIFN-γ and thyroid hormone on class II HLA-DR expression in HeLa cells. Western blotting of cell lysate proteins after PAGE was based on mouse monoclonal HLA-DR II α-chain-specific antibody (Dako Corporation, 1:100) and visualization by chemiluminescence (ECL detection system, Amersham). HeLa cells grown to confluence were incubated with different concentrations of rHuIFN-γ (10-1000 IU/ml). L-Thyroxine (T_4)(10^{-9} M free T_4 concentration) was added simultaneously with rHuIFN-γ and up to 48 hr after start of incubation of cells with the IFN. When added simultaneously with rHuIFN-γ, T_4 caused potentiation of HLA-DR IIα antigen expression at 48 hr, compared to rHuIFN-γ alone. Potentiation of the rHuIFN-γ effect on HLA-DR II expression has also been shown with 3,5,3'-L-triiodothyronine (T_3). When added 48 hr after initiation of incubation of cells with rHuIFN-γ, T_4 expedited the decline of HLA-DR signal in the ensuing 48 hr. Staurosporine (1.0 nM) blocked potentiation of rHuIFN-γ-induced class II HLA expression by T_4. In the absence of rHuIFN-γ T_4 had no effect on HLA antigen expression. Thus, in HeLa cells thyroid hormone has two actions on rHuIFN-γ-induced HLA-DR IIα expression, enhancing the appearance of antigen by a protein kinase-dependent pathway and affecting the turnover of antigen.

118. THE POTENTIATION BY THYROID HORMONE OF rHuIFN-γ-INDUCED ANTIVIRAL ACTIVITY IS PROTEIN KINASE AND PHOSPHOLIPASE C-DEPENDENT. H.-Y. Lin, F.B. Davis, H.R. Thacore and P.J. Davis. Dept. Medicine, Albany Medical College, Albany and Dept. Microbiology, SUNY at Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY.

We have recently shown that L-thyroxine (T_4) and 3,5,3'-L-triiodothyronine (T_3) potentiate the antiviral state induced by recombinant human interferon-γ (rHuIFN-γ), but not IFN-α, in human dermal fibroblast and HeLa cell cultures. Thyroid hormone, alone, has no antiviral effect. The present studies delineate the signal transduction pathways involved in this action of T_4 and T_3 in HeLa cells. Cells were cultured in DMEM with 10% fetal bovine serum depleted of thyroid hormone, and grown to 80% confluence. Cells were treated for 24h with rHuIFN-γ, 1.0 IU/ml. During the last 4h, T_4, T_3 or hormone analogue was added to the medium. Inhibitors of phospholipase C (U73122 [U]), protein kinase C (staurosporine [S]), calmodulin (W-7) and protein synthesis (cycloheximide [CX]) were also added to the medium during the 4h T_4 exposure. After incubation, antiviral activity was determined using vesicular stomatitis virus (VSV) as the challenge virus, and virus replication measured by determination of viral plaque-forming units (pfu). Virus yield in control cells ranged from 1.3 to 2.6 x 10^8 pfu/ml. There was a consistent 10-fold reduction in virus yield with rHuIFN-γ, 1.0 IU/ml. T_4, (10^{-7} M [total hormone]), further reduced virus yield 87- to 96-fold compared to yield with rHuIFN-γ. L-T_4 and L-T_3 were equipotent, while D-T_4, D-T_3, tetrac and triac were inactive. Addition of 10 nM S during the 4h exposure completely blocked the T_4 effect, but only if the inhibitor was present during the first 2h of T_4 exposure. The inhibitor U (100 nM) also blocked the T_4 effect, but only if present during the first 1h of T_4 treatment. In contrast, the inactive analogue U73343 had no effect on T_4 potentiation. W-7 (50 μM) did not alter T_4 potentiation of rHuIFN-γ's antiviral effect, nor did CX, 25 μg/ml, although the latter inhibited 96% of [^{35}S]-methionine uptake during this period. These studies show that 1) in physiologic concentrations, thyroid hormone potentiates the antiviral effect of rHuIFN-γ, 2) this potentiation is not dependent on either new protein synthesis or a calmodulin-mediated process, and 3) the hormone effect is supported by phosphoinositide pathway-related signal transduction.

120. PROTRH GENE EXPRESSION IN THE HYPOTHALAMUS IS STIMULATED BY GLUCOCORTICOIDS *IN VITRO* WITHIN ONE HOUR.IMD Jackson and L-G Luo,
Division of Endocrinology, Brown University, Rhode Island Hospital, Providence RI 02903

Glucocorticoids have been reported to inhibit the hypothalamic-pituitary-thyroid axis in the human as well as the rat. However, the direct effect on TRH biosynthesis and secretion has not been determined. We have recently established fetal rat (day 17) diencephalic neuronal cultures which demonstrate proTRH gene expression in the presence of 5'bromo-2-deoxyuridine(BrdU), a cell differentiating agent. The hypothalamic region was dispersed enzymatically and plated at a density of 3000 cells/mm^2 in DMEM/L 15 culture medium supplemented with 10% fetal calf serum and 50 uM BrdU. Utilizing this model, we have recently reported that diencephalic neurons exposed to dexamethasone(Dex) at 10-^{8}M for two weeks showed increased proTRH mRNA (Jackson et al, Abstract, 76th Ann Meeting Endoc Soc,1994). Additionally, proTRH mRNA was shown to be co-localized with the protooncogenes *cfos* and *cjun* (Luo and Jackson, Soc for Neuroscience Abst. 1994). In this study, we have examined the time frame for stimulation of proTRH gene expression by glucocorticoids and whether the response is entrained to that of *cfos* and *cjun*. The mRNAs were assayed by Northern blot analysis and the results were normalized for *B*-actin a "house-keeping" gene. After the diencephalic neurons were cultured for 3 days, Dex (10-^{8}M) was added to the medium. Total RNA was isolated at 10 separate time points over the subsequent 96 hr.. No effects were evident at 15 min but a significant increase in proTRH and *cfos/cjun* mRNAs were demonstrated at 60 min (1.9, 2.4 and 2.9 fold; $p<0.01$). By 3 hours, proTRH mRNA had increased by 5.9 fold and at 6 hr to 6.6 fold. The levels of *cfos* and *cjun* at 6 hours were also increased 6.4 and 5.7 fold respectively ($p<0.01$). **Summary and conclusion**: these studies demonstrate the early time course of proTRH and *cfos/cjun* mRNA responses to glucocorticoid stimulation in fetal rat diencephalic neurons. The rapid increase of proTRH mRNA to glucocorticoid stimulation is consistent with an effect at the transcriptional level, while the time course of the *cfos/cjun* response is in keeping with their mediation of the proTRH mRNA rise

123. RESPONSE OF TRIIODOTHYRONINE-DEPENDENT ENZYME ACTIVITIES TO INSULIN LIKE GROWTH FACTOR-1(IGF-I) AND GROWTH HORMONE (GH) IN PRIMARY CULTURE OF RAT LIVER CELLS. C.G.Pellizas, A.Coleoni, A.M. Cabanillas, A.Masini-Repiso & M.E.Costamagna. Clinical Biochemistry Department., School of Chemical Sciences. National University of Cordoba. Argentine .

Triiodothyronine(T_3) stimulates the rat GH gene transcription. This effect of T_3 is antagonized by IGF-I, a factor that is under GH control. We examined the possibility that a GH or IGF-I excess could in turn modulate the metabolic effect of T_3 in other target tissues such as liver. To verify this hypothesis, the effect of IGF-I and GH on the activity of two T_3-dependent enzymes: cytosolic malic enzyme (ME) and mitochondrial α-glycerophosphate dehydrogenase (α-GPD) was studied in rat liver cells in primary culture. GH (800 ng/ml) incorporated for 48h to the cell culture, reduced α-GPD activity (ΔA/min/mg DNA, mean ± SEM: 0.0551 ± 0.0007; control: 0.1347 ± 0.0143, $p< 0.01$) and ME activity (ΔA/min/mg DNA, mean ± SEM: 0.0447 ± 0.0025; control 0.0706 ± 0.0141, $p< 0.01$). In similar conditions IGF-I (5 U/ml) induced a reduction of α-GPD (0.0915 ± 0.0105; control 0.1345 ± 0.0153, $p< 0.01$) and ME (0.0513 ± 0.0068; control 0.1214 ± 0.0202, $p< 0.01$) activities. Timecourse studies indicated that IGF-I (15 U/ml) reduced α-GPD and ME ($p< 0.01$) 24h after its addition to the culture medium whereas the GH (800 ng/ml) effect was recorded only after 36h ($p< 0.01$). This delayed effect of GH suggested that its effect could be mediated by IGF-I synthesis. To test this hypothesis, the effect of GH (800 ng/ml) on the two enzymes activities was studied in the presence of IGF antibodies (1:240 or 1:480 dilution)(anti-IGF-I/Somatomedine C rabbit antiserum, UB3-189, NIDDK). A gradual increase of α-GPD ($p< 0.01$) and ME ($p< 0.01$) activities was observed in the presence of GH plus IGF-I antiserum.The T_3-induced α-GPD and ME activities with a saturating T_3 concentration (1 μM) for 24h were significantly lower in the presence of IGF-I (10 U/ml) (α-GPD, mean ± SEM: control 0.1103 ± 0.0151; T_3 1μM 0.1683 ± 0.0190; T_3 + IGF-I 0.1255 ±0127*; ME, mean ± SEM: control 0.0979 ± 0.0206; T_3 1 uM 0.2909 ± 0.0362; T3 + IGF-I 0.1777 ± 0.0275*,(*$p< 0.01$ respect control group and T_3 treated group) . **In conclusion:** in a rat liver cell culture GH excess reduced the metabolic effect of T_3 evaluated by two liver T_3-dependent enzyme activities. This effect is at least in part mediated by IGF-I. Since in the presence of IGF-I the maximal enzyme activities attained after a high T_3 concentration did not reach the control value, the effect of IGF-I might be independent of saturation of nuclear T_3 receptors. A reduction of T_3 receptor number by IGF-I is a possible explanation.

127. PROTEIN KINASE INHIBITOR (H7) BLOCKS THE EFFECT OF TRIIODOTHYRONINE (T3) ON ACETYLCHOLINESTERASE ACTIVITY (AChE) AND ITS mRNAs, IN NEUROBLASTOMA CELLS THAT OVEREXPRESS THE THYROID RECEPTOR β1. J. Puymirat, and J.H. Dussault, CHU Laval Research Center, Quebec, Canada.

We have previously shown that T3 treatment of neuroblastoma cells that overexpress the β1 thyroid receptor (TRβ1) induces functional differentiation of these cells as indicated by the marked increase in AChE activity (*Lebel et al., Proc. Natl. Acad. Sci., 1994*). The effect on AChE activity was dose-dependent and the time-course analysis reveals that this effect occurs after 24 hr of T3 treatment, with maximal effect occurring after 48 hr of treatment. The increase of AChE activity is paralleled by an increase of AChE mRNAs. The induction of AChE mRNAs which occurs after 24 hr of treatment, suggests that this effect is likely to be an indirect one. There are several data showing that phosphorylation may play a role in thyroid hormone-regulated gene expression. Using both a protein phosphatase inhibitor, okadaic acid (OA), and a protein kinase inhibitor, H7, we examined the effects of these agents on T3-induced accumulations of AChE activity and its mRNAs.

OA treatment of cells grown in the absence of T3 increases AChE activity in a dose-dependent manner with a maximal increase (5-fold) observed at 20 nM OA. The simultaneous addition of T3 (30 nM) and OA (5 to 20 nM) in culture, caused a synergistic effect in AChE activity. H-7 protein kinase inhibitor treatment of cells decreased the T3-induced of AChE activity in a dose-dependent manner, with a 50 % of maximal inhibition occurring at 10-15 μM H-7. H7 had no effect on the activity of AChE in cells not treated with T3. Furthermore, we examined the effect of H7 on AChE-mRNAs. Treatment of cells with H7 (25μM) for 24 hours completely inhibited the T3-induced accumulations of AChE-mRNAs. Finally, we showed that T3 treatment of cells increases by 1.4-fold the activity of serine / threonine protein kinases, as determined in an *in vitro* kinase assay, using Myelin Basic Protein (MBP) as substrate. This inhibitory effect of the protein kinase inhibitor was not caused by decreased binding of T3 to its receptor since H7 (25 μM) had no effect on T3 binding capacity (2.12 ± 0.9 and 2.16 ± 0.5 pmole / mg DNA, for untreated and H7 treated cells, respectively).

These results indicate that on-going protein phosphorylation is required specifically for stimulation of AChE gene expression by T3.

128. **A THYROTROPE-DERIVED CELL LINE WHICH HAS LOST THYROID HORMONE REGULATION LACKS TRβ2 AND RXRγ mRNA.** B.R. Haugen, W.M. Wood, D.F. Gordon, V.D. Sarapura, and E.C. Ridgway, University of Colorado Health Sciences Center, Denver, Colorado.

The biological effects of thyroid hormone which influence gene expression, cell growth and differentiation are mediated through various receptors. We have been investigating the unique expression and thyroid hormone regulation of the thyrotropin (TSH) β and α subunits in two thyrotrope-derived cell types. TtT-97, a thyrotrope-derived tumor line, expresses the TSHß subunit and has TSHß mRNA expression and growth which is regulated by thyroid hormone. α-TSH, a unique thyrotrope-derived cell line, retains α-subunit expression, but does not express TSHβ subunit mRNA and is not regulated by thyroid hormone. In order to better understand the molecular mechanisms governing TSHβ gene expression and thyroid hormone regulation in thyrotropes we have identified differential mRNA expression in these cell types of two genes which are known to mediate thyroid hormone action. Thyroid hormone receptors (TR) and retinoid X receptors (RXR) are known to mediate the effects of thyroid hormones by forming heterodimers which influence gene transcription. Using Northern blot analysis, we have identifed differences in the levels of mRNAs encoding these transcription factors between the TtT-97 and α-TSH cells. TR α1 mRNA is equally expressed in both cell types, while TRß1 mRNA is diminished and TRß2, a pituitary-specific receptor, mRNA is absent from the α-TSH cells. Retinoid X receptors are known to form heterodimers with TRs to mediate thyroid hormone action. Northern blot analysis of TtT-97 and α-TSH mRNA shows that RXRα and RXRß mRNA is equally abundant in both cell types, while RXRγ mRNA is detectable only in TtT-97 cells. In order to understand the lack of TRβ2 and RXRγ mRNA in α-TSH cells, we hypothesized that the piutitary-specific transcription factor, Pit-1, or the thyrotrope-specific splice variant, Pit-1T may play a role in the expression of these receptors. Pit-1 and Pit-1T proteins are expressed in TtT-97 cells but not α-TSH cells. Reintroduction of either Pit-1 isoform into α-TSH cells by transient transfection stimulates the TRβ2 promoter to a level seen in TtT-97 cells. In conclusion, the thyrotrope-derived α-TSH cells, which lack TSHß gene expression and thyroid hormone regulation, are lacking TRß2 and RXRγ mRNA as well as Pit-1 isoform proteins. The complex interaction and regulation of these factors may be responsible for the aberrant α-TSH cell phenotype.

129. REGULATION OF THE HUMAN TRH GENE (hTRH) BY HUMAN THYROID HORMONE β_1 RECEPTOR (hTRβ_1) MUTANTS. P. Feng, Q.L. Li, T. Sato, R. Wong*, and J.F. Wilber. Div. of Endocrinology, Univ. of Maryland School of Medicine, Baltimore, MD 21201, and NIDDK, NIH, Bethesda, MD 20892*

TRH gene expression in the hypothalamic paraventricular nucleus is suppressed by thyroid hormone (T_3) excess in vivo. Human TRH-promoter activity can also be inhibited significantly by T_3 in human neuroblastoma cells (HTB-11) co-transfected with TRs. It is known that mutations in the human TRβ gene are associated with the syndrome of generalized resistance to thyroid hormone. To examine this further, we investigated the effect of mutant hTRβ_1 on the dominant negative regulation of human TRH gene by T_3. Transient gene expression assays were performed in hHTB-11 cells transfected with a hTRH promoter-luciferase construct (-242 to +54 bp) (10μg/plate), and co-transfected with wild-type (WT) hTRβ_1 and/or mutant hTRβs (0.5μg/plate). Mutant TR-ED has a T_3-binding affinity only 20% that of WT-hTRβ_1 and mutant TR-PV is unable to bind T_3. Transfection efficiency was monitored by β galactosidase activity. Results: Liganded WT-hTRβ_1 was able to inhibit transcriptional activity of hTRH by 41% at 10^{-9}M T_3. However, mutant TR-ED exerted only one third inhibition (-14%) on the TRH promoter activity compared to WT TRβ_1. By increasing T_3 concentration to 10^{-7}M, the inhibitory effect of TR-ED on the TRH promoter activity could be achieved to the same level induced by WT TRβ_1 with 10^{-9}M of T_3. Of great interest was that mutant TR-ED was able to block the inhibition effect of WT TRβ_1 on the TRH promoter activity at lower T_3 concentration (10^{-9}M), with co-transfection of TR-ED and TRβ_1 at ratio 10:1. These effects corresponded quantitatively to the reduced T_3 binding. However, unliganded TR-ED stimulated the TRH promoter activity by 153±26%, similar to the activation induced by WT TR-β_1 (151±22%). In contrast, the mutant TR-PV did not exert any inhibitory effect on the TRH promoter activity in the presence of T_3 ranging from 10^{-9} to 10^{-6}M. Unliganded TR-PV, moreover, exhibited significant stimulatory effect on TRH promoter activity (134±10%). Conclusions: 1) The TR ligand binding domain is essential for their dominant negative potencies on the hTRH promoter, but does not appear to be necessary for the stimulatory effect of unliganded receptors. 2) TRβ_1 mutants can inhibit the negative transcriptional regulation of WT-TRβ_1 in proportion to their reduced affinities for T_3, leading to the abnormalities of the T_3 negative feedback on the hypothalamic TRH gene expression.

142. ASPARAGINE-LINKED OLIGOSACCHARIDE STRUCTURES DETERMINE CLEARANCE AND ORGAN DISTRIBUTION OF PITUITARY AND RECOMBINANT THYROTROPINS (TSH). M. W. Szkudlinski, J. E. Tropea, M. Grossmann and N. R. Thotakura, MCEB, NIDDK, National Institutes of Health, Bethesda, MD 20892

We have recently shown that recombinant human TSH (rhTSH) with highly sialylated oligosaccharide chains had a higher *in vivo* bioactivity and lower metabolic clearance rate than the predominantly sulfated pituitary human TSH (phTSH) (Szkudlinski et al., Endocrinology, 140: 1490, 1993). In the present study we have determined the relative importance of various organs and specific clearance mechanisms in the plasma disappearance of differentially sialylated and sulfated TSH preparations. Oligosaccharide structures were determined by High pH Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) as well as the Reagent Array Analysis Method (RAAM). Various radioiodine-labeled or unlabeled TSH preparations were injected via the femoral vein of male Spraque-Dawley rats. At various time points (5-180 min) after injection, blood, liver, kidney, spleen, lung, heart and thyroid samples were collected. Uptake of TSH was determined by TCA-precipitation of tracer and/or by TSH chemiluminescence assay in the organ homogenates. It was found that rhTSH is distributed predominantly to the kidneys at 5, 15, 30 and 45 min after injection. In contrast, asialo-rhTSH, phTSH, and solely sulfated bovine TSH (bTSH) were cleared predominantly by the liver (at 5 and 15 min), with a later renal phase (at 30 and 45 min). The serum half-lives of the examined preparations were, in descending order: rhTSH > phTSH-rhTSH (subunit hybrids) > phTSH = rat TSH (rTSH) > bTSH > asialo-rhTSH-rhTSH (subunit hybrids) = asialo-rhTSH, and were inversely proportional to the liver uptakes. Only minute amounts of TSH (less than 1%) enter the thyroid and other organs (heart, spleen and lungs). Blockade of the GalNAc-sulfate receptors (by injection of bLH, oLH or dextran sulfate) resulted in a significant decrease of liver uptake of partially sulfated phTSH and rTSH. Similarly, asialo-rhTSH clearance by the liver was significantly inhibited by co-injection of asialo-fetuin, but not fetuin; neither fetuin nor asialo-fetuin affected rhTSH clearance. Thus, phTSH, rTSH and bTSH preparations containing sulfated oligosaccharide side chains are cleared by the GalNAc-sulfate specific receptors in the liver. In contrast, rhTSH analogs desialylated in one or both subunits are cleared by the liver asialo-glycoprotein receptors, whereas rhTSH with highly sialylated oligosaccharides in both subunits accumulates predominantly in the kidneys, even at the early phase of clearance, indicating that sialylated glycoprotein hormones escape from specific receptor-mediated clearance mechanisms in the liver. Moreover, phTSH-rhTSH subunit hybrids are largely protected from liver-specific recognition. These data indicate that sialylated and sulfated oligosaccharide structures, each to a different extent, determine glycoprotein hormone distribution as well as plasma levels, and thereby *in vivo* potency.

145. MUTANT ALLELE PCR & SEQUENCING (MAPS): A NOVEL METHOD FOR IDENTIFICATION OF MUTATIONS OF THE HUMAN THYROID RECEPTOR-β (hTR-β) GENE IN PATIENTS WITH RESISTANCE TO THYROID HORMONE (RTH). M.B. Grace and G.S. Buzard*, MCEB/NIDDK and *BCDP, PRI/DynCorp NCI-FCRDC, National Institutes of Health, Bethesda, MD 20892

Rational design of the molecular techniques that characterize genetic polymorphisms in heterozygous alleles is a major precondition for large scale clinical applications of mutation analysis. Although there are many methods by which the more than 60 mutations in the hTR-β gene have been characterized, the sequencing of PCR products has been especially problematic. The standard sequencing methods are not always adequate for maximal efficiency, reliability or simplicity. Nevertheless, nucleotide sequencing is essential to the final characterization of point mutations. To meet this need, we have exploited the potential of the allele-specific associated polymorphism (ASAP) technique. ASAP is a polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP)-based screening method in a mini-gel format with ethidium bromide staining of unlabeled single stranded DNA (ssDNA). Mutant alleles have demonstrated reproducible conformation dependent electrophoretic migration shifts which we have termed ASAP-fingerprints. We have used ASAP routinely to screen and predict new or previously known mutations in exons 9 and 10 of the hTR-β gene in related and unrelated kindreds with RTH. Besides detecting mutations, ASAP gels were used preparatively to provide purified enriched mutant alleles separated from wild type ssDNA by eluting mutant versus normal allele bands with micro-capillary tubes. Micro-plugs containing gel-embedded ssDNA template were isolated for mutant allele PCR & sequencing (MAPS). To assess this template for sequencing, we regenerated ASAP fingerprints from the genomic DNA of kindreds with 7 different exon-9 mutations of the hTR-β gene. After MAP enrichment and ASAP confirmation, the MAP products were sequenced by dideoxynucleotide sequencing using either T7 polymerase, or cycle sequencing, with either radionucleotides or dye-labeled dideoxynucleotide terminators, and either autoradiography or an automated sequencer. Biotinylated primers and affinity capture were used in conjunction with MAPS analysis to facilitate sequencing. The combination of technologies (SSCP/ASAP/MAPS) has eliminated many of the interpretative and technical problems associated with the sequencing of heterozygous alleles, and has the potential of providing a blueprint for automated detection and characterization of genetic mutations.

154. SELECTIVE AFFINITY LABELING OF THYROID HORMONE RECEPTOR (TR) MONOMERS WITH A CARBOXY-ESTERIFIED DERIVATIVE OF BROMOACETYL T_3 ($BrAcT_3$-OMe). M. Safran, P.M. Yen, H. Rokos and J.L. Leonard, Molecular Endocrinology Laboratory, Univ. Mass. Med. Ctr., Worcester, MA, Div. of Genetics, BWH and Harvard Medical School, Boston, MA and Marion Merrell Dow Res. Inst., Henning Berlin GMBH, GFR.

TR-dependent transactivation of gene expression is a dynamic process requiring interactions between T_3, TR and TREs. To determine the consequences of covalent attachment of T_3 to the ligand binding site of TR on transactivation, alkylating derivatives of T_3 were screened for their ability to bind to TR. NH_2-modified T_3 analogs, $BrAcT_3$ and N-AcT_3, failed to compete with T_3 for the TR. However, COO-esterified analogs were good competitors for TR and esterification of the COO- group of $BrAcT_3$ ($BrAcT_3$-OMe) increased its ability to compete for TR ~ 10-100 fold. Thus, Br$Ac[^{125}I]T_3$-OMe was evaluated as an affinity label for TR. 400 pM Br$Ac[^{125}I]T_3$-OMe readily labeled the ~50 kDa baculovirus expressed TR. Labeling was rapid, blocked by excess T_3 and the affinity labeled TR was specifically immune precipitated with anti-TR antisera. Paradoxically, ~10μM $BrAcT_3$-OMe was required to decrease TR homodimer binding on gel shift assays and to transactivate in transient expression assays. Further, $BrAcT_3$-OMe labeled TR retained its ability to bind T_3, suggesting that at least two binding sites were present. To resolve this dilemma, TR was separated by sucrose gradient centrifugation and the distribution of the TR determined by T_3 binding analysis. Two peaks of TR were present at 6.1S and 3.7S corresponding to TR dimers and TR monomers respectively. Br$Ac[^{125}I]T_3$-OMe labeled TR migrated exclusively as a monomer and covalent modification of TR by the affinity ligand blocked the ability of T_3 to bind to the 3.7S monomer. These data indicate that i) $BrAcT_3$-OMe is a good affinity label for TR and selectively interacts with TR monomers; ii) T_3 and $BrAcT_3$-OMe compete for the ligand binding site on TR monomers; and iii) alanine side chain modified T_3 cannot interact with TR dimers in solution. This novel reagent will provide insight into ligand-TR interactions and the functional roles of TR monomers and dimers.

155. **T_4-DEPENDENT REGULATION OF THE SECRETION AND EXTRACELLULAR ORGANIZATION OF LAMININ AND FIBRONECTIN IN ASTROCYTES.** A.P. Farwell. Molecular Endocrinology Lab, U. Mass. Medical School, Worcester, MA.

Astrocytes secrete the extracellular matrix proteins laminin (LM) and fibronectin (FN), which then bind to receptors on the surface of astrocytes known as integrins. The distribution LM and FN on the astrocyte surface imparts directional cues to the elongating neurite in the developing brain. Integrins also bind to the microfilament network within the astrocyte and T_4 regulates the pattern of integrin distribution in astrocytes by modulating the organization of the microfilaments. In this study, the secretion and distribution of LM and FN in astrocytes was examined. Confluent cultures of rat astrocytes were grown for 24 h in defined media ± 10 nM T_4, collected by trypsinization and a monocellular suspension made by filtration through a 20 μ mesh. Cells were seeded onto glass coverslips coated with poly-*d*-lysine. Cells were fixed to coverslips with 4% paraformaldehyde at 3, 6 and 24 h after seeding. To differentiate between an intra- or extracellular location of FN and LM, immunocytochemistry was performed on permeabilized and non-permeabilized cells. LM was identified within 3 h after attachment in both the T_4-treated and the T_4-deficient cells and was restricted to the perinuclear space. A more diffuse intracellular distribution was observed by 6 h. Secreted LM was observed on the T_4-treated astrocytes after 24 h while intracellular staining became much less pronounced. In contrast, little or no LM was identified on non-permeabilized T_4-deficient cells at 24 while intracellular staining was detected at the same level as the T_4-treated cells. Extracellular FN was detected at 24 h in all cells. However, FN was organized in linear arrays on the T_4-treated astrocytes while diffuse punctate staining was observed on the T_4-deficient astrocytes. These data suggest that T_4 regulates the secretion and the surface distribution of LM and, to a lesser extent, FN, in astrocytes. The T_4-dependent regulation of LM and FN distribution on the surface of astrocytes provides a mechanism by which this morphogenic hormone can influence neuronal migration and development.

162. **A NOVEL MUTATION AT OUTSIDE OF THE HOT SPOT REGIONS OF THE THYROID HORMONE β RECEPTOR IN A FAMILY WITH THYROID HORMONE RESISTANCE.**

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Resistance to thyroid hormone (RTH) is an inherited disorder characterized by the reduced tissue sensitivity to thyroid hormone. Over 30 different mutations in the thyroid hormone receptor (TR) β gene have been identified in subjects with RTH, and almost all mutations are located in two "hot spot" regions in the ligand binding domain of the TRβ. We have analyzed one Japanese family with RTH in both clinical and molecular basis. The proband was noted to have elevated serum levels of T3, T4 and TSH at birth. He was metabolically euthyroid and oral administraion of T3 to him induced no thyrotoxic signs. His mother was noted to have goiter at the age of 20 and her laboratory examination showed hyperthyroxinemia with TSH level within normal range. His father and two siblings did not have any abnormal clinical or laboratory findings. Genomic DNA was extracted from leukocytes of family members and exon 3 to exon 8 of TRβ1 gene were analyzed by direct sequencing. A guanine to adenine substitution at nucleotide 1013 replacing Arg for Gln at amino acid 243 of TRβ1 was identified in one of two alleles of the proband and his mother. No other mutations were found in either DNA- or ligand-binding domains. This is the second RTH mutation identified in exon 5 of TRβ gene. To characterize this mutant TRβ (βR243Q), expression vector for βR243Q was prepared by site-directed mutagenesis. Scatchard analysis showed that βR243Q has slightly lower but statistically not significant T3 binding affinity compared to that of wild-type TRβ1 [$(5.25\pm1.31)\times10^9$/M for βR243Q and $(6.32\pm1.12)\times10^9$/M for wild-type TRβ1]. Lee and Mahdavi recently reported (JBC 268: 2021, 1993) that mutant TRα1, of which positively charged amino acids in the D domain (R^{188}-R^{189}-K^{190}) were mutated to neutral amino acids, had normal DNA- and ligand-binding activities, but had impaired transcriptional regulatory functions and showed dominant negative effect. Since R^{243} of TRβ1 corresponds to R^{189} of TRα1, βR243Q may also be devoid of transactivation function and act as a dominant negative repressor as is the case for other mutant TRβ receptors identified in patients with RTH, which could explain why βR243Q causes RTH.

163. HOMO- AND HETERO-DIMERIZATION ABILITY OF TWO DEFECTIVE DOMINANT NEGATIVE MUTANT THYROID HORMONE RECEPTOR β : ARG311HIS AND ^422

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Recent studies suggest that dominant negative inhibition by mutant thyroid hormone receptors (TR) are mediated through a competitive DNA binding of inactive homodimer or heterodimer with RXR. We report here the evidence that homo- and hetero-dimerization is necessary for dominant negative inhibition by mutant receptor but not sufficient. We investigate two different type of defective dominant negative mutant receptors : G.H. and TRβ^422. The G.H. receptor (Arg311His) was cloned from a patient with severe pituitary resistance to thyroid hormone and two individuals with normal phenotype. In contrast to the many other β receptor mutants responsible for the generalized form of thyroid hormone resistance, the G.H. receptor appears unable to antagonize normal receptor function in transient transfection assay, even it has significantly impaired T3 binding activity (M.E.Geffner et al., J.Clin.Invest.91:538-546,1993). TRβ^422 has amino acids insertion in the 9th heptad region which is known to be essential for dimerization. We reported that introduction of ^422 mutation to Mf mutant (Gly345Arg) obliterated the dominant negative inhibition of Mf receptor, suggesting that dimer forming ability is necessary for dominant negative activity. To investigate the ability of homo- and hetero-dimerization of these mutant receptors precisely, we performed gel mobility shift assay using wild type TRβ, G.H.receptor, ^422 receptor and RXRα overexpressed by the baculovirus-Sf9 insect cell system. The G.H. receptor formed homodimers and heterodimers with RXRα on rGH- and LAP-TRE with same efficiency of wild type receptor and T3 disrupted homodimeric binding of wild type receptor but not G.H. receptor. TRβ^422 receptor did not form homodimer or heterodimer on either TREs. These results suggest that homo- and hetero-dimerization is necessary for mutant receptor to exert dominant negative activity but not sufficient. Other unknown factors must be involved in dominant negative inhibition by mutant receptor.

165. IDENTIFICATION OF A RETINOIC ACID RESPONSE ELEMENT IN THE RAT THYROTROPIN BETA SUBUNIT GENE.

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We have recently shown that rat thyrotropin beta subunit (TSHß) mRNA levels are dependent on vitamin A status in vivo, suggesting that TSHß gene expression is regulated by retinoic acid (RA). We have therefore used transient transfection to identify a potential RA response element (RARE) in the rat TSHß gene. A chimeric plasmid containing ~0.8 kb of the rat TSHß 5'-flanking sequence, exon 1 and 150 bp of intron 1 (I150), linked to a luciferase reporter (LUC) was transfected into CV-1 cells. Cotransfection of RA-receptor α (RARα) and RARα+retinoid X receptor ß (RXRß) caused a 2- and 4-fold induction of TSHßLUC expression, respectively. Addition of 5×10^{-7} M all-trans RA (atRA) had no effect on TSHßLUC expression in the presence of RARα, but suppressed induction by RARα+RXRß by 50%. Deletion analysis showed that atRA responsiveness was mediated by sequences between -204 and I150. The rat TSHß gene contains a major negative thyroid hormone response element (TRE) at +11 to +27 and a minor negative TRE at -22 to +8, and many TREs have been shown to also act as RAREs. Interestingly, however, a construct in which the downstream TSHß TRE had been deleted was fully suppressed by atRA. Moreover, expression of a construct containing the downstream TRE inserted into a LUC reporter driven by the mouse mammary tumor virus promoter (MMTVLUC) was unaffected by atRA in the presence of RARα+RXRß. In contrast, MMTVLUC containing the upstream TRE was induced 10-fold by RARα+RXRß and 20-fold by RARα+RXRß + atRA. Consistent with these functional data, electrophoretic mobility shift assays showed that a complex was formed on the upstream TRE with nuclear extracts from CV-1 cells expressing RARα+RXRß but not on the downstream TRE. These data show that the rat TSHß gene contains a negative RARE that binds RARα/RXRß. This sequence element functions as a positive RARE when removed from the context of the TSHß gene.

170. **EFFECTS OF THYROID HORMONES ON THE COLONIC AND BROWN ADIPOSE TISSUE THERMAL RESPONSE TO NOREPINEPHRINE**. N. Negrão, F.L.A.S. Lebrun, A.C. Bianco. Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

Brown adipose tissue (BAT), a major site of facultative thermogenesis in small mammals, contains type II T_4 5'-deiodinase (5'-DII), that is profusely stimulated by norepinephrine (NE). The local synergism between NE- and T3-generated signals results in several fold stimulation of uncoupling protein (UCP) gene transcription and mRNA stabilization, the rate limiting protein of BAT thermogenesis. To study the role of the 5'-DII on the BAT thermal responses to NE, urethan-anesthetized rats (1.2g/kg, ip), maintained at 26±0.5°C, received a constant venous infusion of NE over 20 min, while core and BAT temperatures were measured by means of thermistor probes inserted 5-6 cm into the colon or fixed under the interscapular BAT pad, respectively. Data was plotted against time and the areas under the curves (AUC) were integrated and expressed as AUC units. A dose-response curve plotted with data obtained from 12 intact rats indicated that NE effect was maximal at a dose of ≈5μg/kg/min; this dose was used throughout the experiments. Surgically thyroidectomized rats (Tx; n=5-7/group) kept on 0.03% MMI were also studied alone, after they were treated with only 3 doses of T4 (4μg/kg/12h, sc.), or implanted 5 days prior the experiment with osmotic pellets that deliver a constant dose of T3 (5μg/kg/day). An additional group of T4-treated Tx rats received iopanoic acid (IOP; 50mg/kg/12h, ip.) during the 48h that preceded the NE infusion.

Site	Intact	Tx	Tx+T4	Tx+T4+IOP	Tx+T3
		Basal Temperature (°C)			
Colonic	38.2±0.5	36.3±0.3*	36.3±0.5*	36.6±0.5*	38.1±0.3
		Thermal Response to NE (AUC units)			
Colonic	4.8±1.5	2.2±1.7	3.2±1.6	2.2±2.4	4.3±1.8
BAT	18.7±8.1	7.3±4.6*	15.2±5.4	4.6±4.1*	18.6±3.7
		Thyroid Hormones Serum Concentration (nmol/L)			
T4	22.5±3.9	<2.2*	11.2±6.4*	14.9±4.7*	<2.2*
T3	1.35±0.12	0.72±0.05*	1.41±0.45	0.97±0.34	1.18±0.13

*p<0.05 vs. intact rats

Conclusions: (i) hypothyroidism is associated to a blunted BAT thermal response to NE, that contributes to core hypothermia; (ii) the 5'-DII is functionally operative because replacement with T4 did not correct core hypothermia but fully restored BAT thermal response to NE, an effect that was blocked by IOP treatment; (iii) at least in this experimental model, plasma borne T3 is also important, as seen by the full BAT thermal response observed in the T3-treated Tx rats (supported in part by FAPESP).

174. INDIRECT EVIDENCE FOR A ROLE OF THE 5'-MONODEIODINASE ON THE REGULATION OF GH mRNA LEVELS. C.B.Volpato and M.T.Nunes. Department of Physiology & Biophysics, Institute of Biomedical Sciences, University of São Paulo, Brazil.

Previous studies have demonstrated that triiodothyronine (T_3) stimulates GH gene expression in the GC, GH_1 and GH_3 pituitary tumoral cells. In an attempt to further study the role of T_3 and T_4 in the GH gene expression as well as the participation of 5'MD-II in this process, thyroidectomized rats (Tx; ~200 g), drinking MMI (0.03%), were used throughout.The animals were killed by decapitation at different times after T_3 administration (100 ug/100 g, ip), T_4 administration (5 ug/100 g BW, i.p.) or 30 min after given different T_4 doses: 0.8, 0.4 or 0.2 ug/100 g BW, i.v. The abundance of GH mRNA was estimated by northern blot analysis, using a full length rat GH ^{32}P-cDNA probe. As expected, GH mRNA levels were very low in Tx rats; T_3 injection caused a prompt enhance (2 fold) of mRNA levels (15 min), that increased progressively until 1 h up to 4 fold, and plateaued. The administration of 5 ug of T_4/100 g BW, i.p. did not induce any changes in GH mRNA levels until 2 h of the injection. After a 7-day treatment period, however, GH mRNA levels were enhanced (2.5 fold). On the other hand, when T_4 was given along with propylthiouracil (PTU), to decrease 5'MD-I activity, the GH gene expression was decreased to euthyroid animals levels. In addition, the administration of 0.8, 0.4 and 0.2 ug of T_4/100 g BW, i.v.induced a progressive increase of GH mRNA level. This response was higher (4 fold) when lesser amounts of T_4 (0.2 ug/ 100g, i.v) were administered. The results indicate that the increase in GH mRNA levels detected after T_4 treatment depends on its conversion to T_3. The involvement of the 5'MD-II, whose activity is inversely modulated by thyroid hormones, is strongly suggested considering that: (i) GH mRNA levels were gradually increased while the dose of T_4 administered to Tx rats were progressively decreased and (ii) no alteration in the GH mRNA levels could be detected until 2 h after administration of supraphysiological dose of T_4 (5 ug/100 g BW, ip), condition where 5'MD-II activity is known to be suppressed. (FAPESP, CNPq)

178. T3-DEPENDENT ONTOGENY OF A T3-ACTIVATED STRETCH CHANNEL IN RAT ATRIOCYTES. W.L. Green, C.A. Shuman and V. Chen, Veterans Affairs Medical Center and SUNY/Brooklyn, Brooklyn, NY.

We earlier described ion channels in neonatal rat atriocytes that open following a stretch stimulus, and that are more active when T3 is added to the incubation medium. To assess responses in hypothyroid rat neonates, dams received methimazole (MMI) from day 10 of pregnancy, following the protocol of Näntö-Salonen and Rosenfeld (Endocrinology 131:1489, 1992); hypothyroidism in the pups was confirmed by T4 assay. Pups were sacrificed at 0-3, 12, or 18 days; some were continued on MMI until sacrifice. Isolated atriocytes were examined by cell-attached patch clamp techniques, with varying amounts of T3 in the recording pipette. To create a stretch stimulus, suction was applied to the recording pipette. As before, atriocytes from euthyroid pups showed little response to stretch without T3, but responded with frequent channel openings when T3 was present; a T3 effect was seen at 1nM T3 and was maximal at 1 μM T3. Atriocytes from hypothyroid neonates were usually unresponsive to stretch, even at 1 μM T3, and the response did not appear at 12 or 18 days in the group maintained on MMI. However, in atriocytes obtained at 12 or 18 days from the initially hypothyroid pups that did not receive MMI, the channel activated by T3 plus stretch was usually present. We previously speculated that known actions of T3 on the atrium, including augmented secretion of atrial natriuretic peptide (ANP) and positive chronotropism, might be mediated by increased sensitivity to stretch stimuli. The report by Zamir et al (Horm Metab Res 25:152, 1993), that stretch-induced ANP secretion is blunted in hypophysectomized rats and restored by T4 treatment, is evidence for such a mechanism. The absence of a T3- activated stretch channel in hypothyroid pups, and its presence after return to euthyroid status, suggests that development of this ion channel may also be T3 dependent. Thus, T3 may act in two ways to modify responses to stretch: by stimulating development of stretch channels and by acutely regulating their activity.

179. DIFFERENTIALLY EXPRESSED RXRS MODULATE THYROID HORMONE ACTION IN PRIMARY HEPATOCYTE CULTURES. T. Nagaya, Y. Murata, M. Menjo, A. Sugawara, and H. Seo, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan, and Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Thyroid hormone action is mediated through its nuclear receptors (TRs), which form a homodimer or a heterodimer with thyroid hormone receptor auxiliary proteins (TRAPs) such as RXRs. The heterodimer complex is considered as a major form of T3-dependent transcriptional activation. We have previously shown that T3 responsiveness is distinct in two different types of primary hepatocyte cultures (monolayer or spheroid). For example, T3-dependent increase of 5'-deiodinase mRNA is blunted in the conventional monolayer cultures, whereas it is well preserved in spheroids. Electrophoretic mobility shift assays (EMSA) and transfection assays were performed to study the mechanism involved in the altered T3 responsiveness under the two culture conditions. Isolated rat hepatocytes were grown on collagen-coated dishes for monolayer or on positively charged polystyrene dishes for spheroid cultures. Three days after plating, cells were harvested and the nuclear extracts were prepared. For EMSA, the nuclear extract was incubated with ^{32}P-labeled TRE (Pal, Lap or DR4) in the absence or presence of *in vitro* translated hTRβ. The nuclear extracts themselves did not show apparent binding. Co-incubation with TRβ exhibited retarded receptor DNA complexes. Two retarded bands were observed in the nuclear extract from spheroid cultures. The slower band migrated similarly to TR-RXRα heterodimer. Another band migrated faster than TRβ homodimer. These two bands were considered as TR-TRAP heterodimers because they were not dissociated by T3. Similar TRAP activities were observed in the normal rat liver nuclear extract. Only a faster migrating band was demonstrated in the nuclear extract from monolayers. Incubation with the antibody against the hinge region of RXRα supershifted both the slower and the faster migrating complexes. However, the antibody against the amino-terminus of RXRα only recognized the slower migrating band. The similar mobility with TR-RXRα heterodimer and the reactivity with antibodies suggest that the slower migrating complex includes full length RXRα, and that the faster migrating one would be derived from truncated RXRα. Adenovirus-mediated transfection of 3x Pal TK Luc reporter gene demonstrated 1.3-fold activation by T3 in monolayer and 2.9-fold in spheroid cultures, respectively. These results indicate that two types of RXRα are differentially expressed under different culture conditions. Thus, the presence of intact RXRα may participate in the T3-dependent transcriptional activation in spheroid cultures, and possibly in hepatocytes *in vivo*.

187. CHRONIC TREATMENT WITH VITAMIN A DOES NOT MODIFY CLINICAL AND BIOCHEMICAL FEATURES OF PATIENTS WITH THYROID HORMONE RESISTANCE. P. Beck-Peccoz, D. Cortelazzi, L. Persani, D. Preziati and *V. K. K. Chatterjee, *Institute of Endocrine Sciences, University of Milan, Milan, Italy, and *Department of Medicine, University of Cambridge Clinical School, Cambridge, UK.*

Early studies on the effects of Vitamin A on thyroid economy in both animals and humans showed that large doses of retinoids reduce metabolic rate and thyroid gland size, and decrease total thyroid hormone (TH) concentrations without affecting their circulating free fractions. The reasons for these changes were unclear and explained on the basis of the complex interrelationship between TH and retinoid serum transport proteins. More recently, it was demonstrated that retinoids and their receptors (RAR, RXR) play an important role in modulating the effects of TH and their receptors (TR) on the induction of target gene expression. In particular, RXR heterodimerizes with TRs, thus acting as a T3 receptor auxiliary protein (TRAP) in enhancing the binding of TRs to thyroid response elements (TREs). Moreover, TRE-containing target genes show amplification of TH responses with 9-*cis*-retinoic acid the natural RXR ligand, which is a product of Vitamin A.

The aim of this study was to evaluate the effects of chronic treatment with Vitamin A (Arovit® oral drops: 20,000 U/day for one month) on several clinical and biochemical indices in patients with thyroid hormone resistance (RTH) and in their unaffected relatives. It is well known that mutations in the T3 binding domain of TRβ gene account for the expression of RTH and that the mutant proteins are able to inhibit the function of the normal receptors in a dominant negative manner. Although the precise mechanisms involved in the dominant negative effects of mutant TR are still unclear, it is conceivable that augmenting transactivation by normal or mutant TR with retinoids may have beneficial effects in RTH patients. We treated 3 RTH patients and one unaffected relative of the same family harboring a R316H mutation, as well as one sporadic case without documented TR mutation and her unaffected daughter. We measured serum TSH, FT4, FT3 and Tg concentrations weekly, as well as some parameters of peripheral thyroid hormone action, such as sex hormone-binding globulin, osteocalcin and angiotensin-converting enzyme. Before and following one month of treatment, serum TSH, its bioactivity (quantified as cAMP accumulation in FRTL5 cells), FT4 and FT3 levels were measured under both basal and TRH-stimulated conditions. No changes in the above indices, including TSH bioactivity, were recorded during the period of Vitamin A administration either in affected or unaffected subjects. No adverse reactions were recorded and the clinical symptoms in the various subjects were unchanged.

In conclusion, pharmacological doses of Vitamin A do not modify any of the clinical and biochemical findings of patients with RTH, suggesting that the enhanced availability of retinoids may not modulate resistance to TH action in this disorder.

191. **SEX DIFFERENCES IN THE ACTION OF TRH ON RAT PITUITARY TSH AND PRL SECRETION IN VIVO AND IN VITRO.** Xiangbing Wang, Monte A. Greer, and Susan E. Greer, Section of Endocrinology, Department of Medicine, Oregon Health Sciences University, Portland OR 97201.

TRH stimulates both TSH and PRL secretion and may be a major physiologic stimulator of PRL secretion. In the human, the PRL response to TRH stimulation is similar in men and women. However, in rats in vivo TRH induces PRL secretion only in females but stimulates TSH secretion similarly in both sexes. This is due to specific conditions existing in vivo since estrogen treatment of male rats produces PRL secretory responsivity to TRH. Within 90 min after removing and dispersing rat pituitary cells with collagenase, TRH stimulates both PRL and TSH secretion from cells of both sexes. In the present study, we compared the effect of TRH on PRL and TSH secretion in female and male rats in vivo and in vitro with the specific aim of evaluating what sex differences might be present after acute removal of the pituitary cells from the in vivo environment. Two-month-old male and female Sprague-Dawley rats were injected iv with 200 ng TRH/100 g b.w.; plasma samples were collected at 0 and 60 min. Collagenase-dispersed anterior pituitary cells from similar rats were perifused with Krebs-Ringer-Hepes medium gassed with 95% O_2 : 5% CO_2 in 0.2 ml chambers containing 1-2x10^6 cells at a flow rate of 0.5 ml/min; fractions were collected each min. PRL and TSH in the same plasma or perifusion effluent samples were measured by RIA. In vivo the basal plasma TSH was 2X higher in males than in females while basal PRL was similar in both sexes. TRH induced a 4-fold increase in TSH secretion in both sexes; it stimulated PRL secretion 3-fold in females but had no effect on PRL secretion in males. In vitro, 2-min perifusion of graded concentrations from 0.1-1000 nM TRH were compared. There was a TRH concentration-correlated secretion of PRL and TSH by cells from both females and males but there were differences in PRL secretion between the sexes. 1) Basal PRL secretion was 3-fold higher and the relative TRH-induced PRL secretion above baseline was 2-fold higher in female than in male cells; 2) Induced PRL secretion reached a maximum plateau at 10 nM TRH in male cells but the response was still in the ascending phase at 1000 nM TRH in female cells. **Conclusions:** A sex difference in TRH stimulation of PRL secretion is present in rats both in vivo and in vitro, but is much more marked in the former. Androgen or estrogen concentration may be factors responsible for the absent male response to TRH in vivo but the inhibition is rapidly dissipated after removal of the cells from plasma. A higher TRH receptor density in female rat lactotrophs may be responsible for the differences in vitro.

192. **OSMOTIC CELL SWELLING STIMULATES HYPOTHALAMIC MEDIAN EMINENCE TRH SECRETION WHICH IS INDEPENDENT OF Ca^{2+} INFLUX.** M.A. Greer, S.E. Greer and X. Wang. Oregon Health Sciences University, Portland, OR.

Cell swelling caused by extracellular hyposmolarity or isosmolar permeant molecules (e.g. urea, glycerol, ethanol) evokes a prompt hormonal secretory burst from rat pituitary cells. A variety of studies has established that this secretion is induced through an intracellular transduction chain and not because of non-specific damage to the cell resulting in a passive loss of hormone. Secretion may be initiated by stretch-activated receptors in the plasmalemma. We have evaluated whether cell swelling will also stimulate TRH secretion by hypothalamic neurons. Osmotic fluctuations such as we employed have been shown by other investigators to cause reversible, non-toxic neuronal swelling. We used perifused median eminence (ME) tissue. For each experiment 6 young adult male Sprague-Dawley rats were decapitated, their ME rapidly removed, kept at 4 C, and finely minced in pH 7.4 300 mOsm Krebs-Ringer-HEPES buffer (KRH) gassed with 95% O_2-5% CO_2. The minced tissue was placed in a 0.2 ml chamber over a thin layer of Cytodex-3 beads and perifused with gassed KRH at 37 C. Stimuli were 30% hyposmolarity and 30 mM K^+; exposure to each stimulus was for 5 min. The flow rate was 0.5 ml/min and each fraction was collected in 4 ml methanol at 1-min intervals to minimize TRH degradation. TRH was measured by RIA. Because of the low concentration of TRH in the effluent, 5 consecutive fractions were pooled just before (basal) and just after (stimulated) application of the stimulus to the perifused tissue. The methanol was evaporated at 37 C under a stream of nitrogen and the residue of each pool dissolved in 100 μl of the RIA buffer. Each stimulus was compared at least 3X in the same cells in normal or Ca^{2+}-depleted KRH. With normal Ca^{2+} KRH, both 30% hyposmolarity and 30 mM K^+ stimulated TRH secretion 2-7-fold (means 451 and 313% of basal secretion, respectively). Induced secretion promptly returned to baseline after withdrawing the stimulus. As is the case with hormone secretion by normal pituitary cells, in Ca^{2+}-depleted media osmotic stimulation of TRH secretion was not decreased (mean 524% of basal) but stimulation by 30 mM K^+ was abolished. Many different conditions can contribute to osmotic pressure fluctuations in brain neurons, including alimentary ingestion, intracellular metabolic activity, head trauma, and stroke. It is possible that changes in thyrotropin secretion under such conditions might be related to altered secretion of TRH or other stimulatory or inhibitory central neurotransmitters.

Conclusions: Osmotic cell swelling stimulates TRH secretion from median eminence tissue as it does hormone secretion from normal pituitary cells. In both, Ca^{2+} influx is not required for the stimulation of secretion.

193. STRUCTURE-FUNCTION RELATIONSHIP OF AMIODARONE ANALOGUES IN THE INHIBITION OF THYROID HORMONE BINDING TO α_1 AND β_1 T_3 RECEPTORS. W.M. Wiersinga, H.C. van Beeren and O. Bakker, Department of Endocrinology, University of Amsterdam, The Netherlands.

We have previously shown that desethylamiodarone (DEA), the major metabolite of the potent antiarrhythmic drug amiodarone (AM), inhibits T_3 binding to its nuclear receptor: it acts as a competitive antagonist to the α_1 thyroid hormone receptor (T_3R) and as a noncompetitive antagonist to the β_1 T_3R. The aim of the present study was to evaluate whether other potential metabolites of the drug act in a similar way.

Methods. The chicken α_1 and rat β_1 T_3R (expressed in an E. Coli system) were incubated in the absence or presence of AM analogues with [^{125}I] T_3 for 2h at 22°C in TRIS buffer containing 0.025% Triton X-100 and 0.05% BSA in order to solubilize the AM analogues. Scatchard plots (by adding increasing amounts of nonradioactive T_3) of T_3 binding were constructed at AM analogue concentrations around IC50 values, followed by Lineweaver-Burke analysis to study the mode of inhibition.

Results. 1) Desdiethylamiodarone (LB33536) (tested at 0, 0.1 and 0.25 x 10^{-4}M) acted as a competitive antagonist with respect to T_3 binding to the α_1 T_3R but as a noncompetitive antagonist to the β_1 T_3R; 2) Desdiiodoamiodarone (L3937) (tested at 0, 0.3 and 0.6 x 10^{-4}M) acted as a noncompetitive antagonist to the α_1 T_3R and the β_1 T_3R; 3) The 2-butyl-3 (4- hydroxy-benzoyl) benzofuran derivate L3373 (still containing two iodine atoms) (tested at 0, 0.1 and 0.25x 10^{-4}M) acted as a competitive antagonist to the α_1 T_3R and the β_1 T_3R.

Conclusions. 1) Desethylation of AM results in competitive inhibition to the α_1 T_3R and noncompetitive inhibition to the β_1 T_3R; 2) Deiodination of AM results in (less potent) noncompetitive inhibition to the α_1 and β_1 T_3R; 3) The competitive inhibition of the iodinated compound L3373 to the α_1 and β_1 T_3R disappears upon deiodination. It appears that the presence of iodine atoms and the accessibility of nitrogen (in the desethylated analogues) or hydroxylgroup (in the L3373 compound) determine the fit of amiodarone analogues in the "α/β barrel" of the T_3 receptor and thereby the nature of inhibition of T_3 binding.

224. THE EFFECT OF RETINOIC ACID (RA) ON T_3-INDUCED RESPONSES IN HEPG2 CELLS.
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We investigated the effects of RA on T_3-induced responses in human cells using the HepG2 hepatoma cell line. Cells were incubated for 4 days in serum-free medium with T_3 and/or RA or 9-cisRA. Effects of T_3 were measured as stimulation of secreted sex-hormone binding globulin (SHBG, by RIA) or inhibition of secreted thyroxine binding globulin (TBG, by change in B/F ratio of $[^{125}I]$-T_4 and corrected using K_d 71 pM, charcoal separation). Relative SHBG and TBG mRNAs by Northern analysis were also measured.

T_3 induced a dose-responsive increase in SHBG secretion (basal 25-60 nM) with maximal effect (177±25% untreated, ±SD, n=8) at 3 nM. Half-maximal stimulation occurred at 30 pM bioavailable T_3. After 24-48 h exposure to T_3, SHBG mRNA levels rose and were followed 3-4 days later by a rise in SHBG secretion. With 100 nM RA or 9-cisRA, SHBG secretion was reduced slightly to 77±12% and 88±11% of control respectively. When cells were incubated with T_3, 0-10 nM, together with RA, 10 nM or 100 nM the rise in SHBG secretion was abolished. With 100 nM 9-cisRA, the T_3 dose-response curve was shifted to the right such that half-maximal stimulation occurred at 1.4±0.7 nM total T_3 (±SD, n=3) compared with 0.23±0.09 nM with T_3 alone ($p<0.001$). In the presence of 10 nM T_3, increasing concentrations of RA or 9-cisRA were able to inhibit SHBG secretion with half-maximal effects at 0.3 and 200 nM respectively.

Conversely, TBG secretion and TBG mRNA production were reduced to 47±14% (n=8) of control with 10 nM T_3. RA alone did not affect TBG secretion but antagonised the effect of T_3. In the presence of 10 nM RA, half-maximal effect of T_3 was 10 nM, compared with 0.2 nM without RA. Surprisingly, the maximal effect of T_3 in the presence of RA was greater than the maximal effect of T_3 alone suggesting a complex, concentration-dependant interaction between T_3 and RA. T_3 and RA alone or in combination had no effect on either nuclear T_3 binding, secreted total protein or albumin.

These results show that (i) RA, and to a lesser extent 9-cisRA, are antagonists which are able to inhibit both stimulation and repression by T_3 in HepG2 cells; and (ii) RA has only minor direct effects on SHBG and TBG secretion. These findings suggest that RA may influence T_3 action in human cells.

230. THYROID HORMONE REGULATION OF MITOCHONDRIAL ADENINE NUCLEOTIDE TRANSLOCASE AND GLYCEROL-3-P DEHYDROGENASE. K. Dümmler[#], S. Müller[#] A. Harneit[#] F. Buck[¶] and H.-J. Seitz[#],[#]Inst. Physiol. Chemie,[¶]Inst. Zellbioch., Univ.-Krankenhaus Eppendorf, 20246 Hamburg, Germany

It is well-known that administration of thyroid hormones results in increases in mitochondrial oxygen consumption. However, the underlying mechanisms are poorly understood. The hormone mediated effect seems to be related not only to changes in the activity of respiratory chain enzymes, but also in enzymes linked to mitochondrial oxidative metabolism, connecting cytosolic and mitochondrial pathways. The adenine nucleotide translocase (ANT) an integral inner mitochondrial membrane protein is responsible for ADP/ATP exchange between cytosol and mitochondria. After T3 application accelerated ANT exchange is observed in rat liver mitochondria. We examined T3 dependent ANT gene expression in different rat tissues. For this we used human ANT isoform specific oligonucleotides and, in addition, cloned and sequenced a full length rat ANT2 cDNA. In Northern blot analysis, we found that two different isoforms are expressed in rat. ANT1 solely in heart and skeletal muscle, ANT2 in all tissues examined: liver, heart, kidney, muscle and brain. Expression of ANT1 mRNA is independent of hormone status, whereas the expression of ANT2 is dramatically increased after T3 application in liver and heart within 6-24h, but not in kidney and brain. The mitochondrial glycerol-3-P dehydrogenase (mGPDH) is part of the glycerophosphate shuttle, where it works in conjunction with cytoplasmatic GPDH to transfer reducing equivalents from cytosol to mitochondria. mGPDH activity in rat liver was found to be dramatically increased after T3 application, and thus this enzyme became a classical marker for thyroid hormone action on liver metabolism. To elucidate the T3 regulated gene expression of mGPDH we cloned a cDNA for this enzyme from rat liver. The cloned gene encodes a protein of 727 amino acids, including a putative mitochondrial targeting signal. The alignment with yeast mGPDH shows an identity of 34% with the highest homology occuring at the N-terminus, where the FAD-binding site is located. mRNA for this gene was detected in liver, heart, muscle, brain, testes and pancreas. With the exception of testes, basal expression levels were very low in all tissues examined. However, application of thyroid hormones led to a 10-15 fold increase in liver mGPDH mRNA within 4h, whereas hypothyroidism further decreased the mRNA level. Enzyme activity correlated very well with these mRNA data. Our results strongly suggest that mGPDH and ANT2 are regulated by T3 at the transcriptional level. This up-regulation may contribute to the increased oxygen consumption of mitochondria under thyroid hormone influence.

-Supported by Deutsche Forschungsgemeinschaft, SFB 232 -

2:00 P.M. 19. ESTABLISHMENT OF AN ANTI-HUMAN THYROTROPIN RECEPTOR (TSHR) SPECIFIC CD4+ T CELL LINE FROM A PATIENT WITH GRAVES' DISEASE : EVIDENCE FOR MULTIPLE T CELL EPITOPES ON TSHR

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Graves' disease is involved with an autoimmune response against the TSHR. Anti-TSHR antibodies cause hyperthyroidism and they have been the subject of intense research efforts. Recently, investigation of B cell epitopes has advanced since the cloning of the receptor gene. However, little is known about T cell epitopes on TSHR. Determination of definite epitopes or molecular studies on T cell receptor gene have not been achieved.

From peripheral lymphocytes of patients with Graves' disease, we established a T cell line using a pool of 49 synthetic peptides corresponding to the entire human thyrotropin receptor (TSHR). This T cell line showed a specific response to the pool of peptides in the microproliferation assay (stimulation index: 4.8). Flow cytometry analysis revealed that the cell surface markers were $CD4^+CD8^-$, T cell receptor (TcR) $\alpha\beta^+$ and $TcR\gamma\delta^-$. To investigate T cell epitopes on TSHR, 49 synthetic peptides were divided into 6 groups of peptides and the responses of the T cell line to each group were tested. As a result, the T cell line reacted well against at least three groups: the N-terminal (amino acids 31-169) and C-terminal (338-420) regions of the extracellular domain and the N-terminal half (441-661) of the transmembrane domain of the receptor. This result suggests a multiplicity of T cell epitopes on TSHR and was supported by the TcR gene analysis of the cell line. Thus, the TCR gene analysis showed the expression of 5 Vα genes in the cell line: Vα-1, -2, 10, -20 and -w25. Interestingly, Davies et al. reported that usage of all these five genes was rather restricted in intrathyroidal lymphocytes obtained from patients with Graves' disease [J Clin Endocrinol Metab 76:660, 1993]. In conclusion, a multiplicity of T cell epitopes on TSHR were indicated.

2:15 P.M. 110. THYROID ATROPHY WITH EXTENSIVE FAT REPLACEMENT AND HYPOTHYROIDISM IN MICE IMMUNIZED WITH THE EXTRACELLULAR DOMAIN OF THE HUMAN TSH RECEPTOR. V. C. Guimaraes, Y. Hidaka, J. Quintans, F. H. Strauss, Y. Okamoto, G. Medeiros-Neto and L. J. DeGroot. Thyroid Study Unit, The University of Chicago, Section of Endocrinology of Osaka City General Hospital, and Hospital das Clinicas, Univ. of San Paulo Medical School, Brazil.

We studied the immune response to the rec-h-TSH-R-ECD (aa 19-417), h-TSH-R-ECD derived peptides (aa 30-49; aa 227-242; aa 258-277; aa 315-334) and a peptide predicted by the mRNA of the TSH-R Variant(aa 243-253). 48 female mice from two different strains, DBA/1j (H-2q) and C57BL/6j (H-2b), were immunized on days 0 , 15, 30, 45 and sacrificed on day 90. T3 levels, anti-Tg antibodies, thyroid histology , TSAb, and TSBAb activities were evaluated. Depending on the antigen used, a surprising finding of thyroid atrophy with diminished number of follicles and extensive fat replacement was observed in the (H-2q) strain. This change was not seen in the control animals which received only complete Freund's adjuvant + saline or in (H-2b) mice immunized with TSH-R-ECD. A negative correlation ($r= -0.819$ and $p<0.001$) was found between serum T3 levels and degree of thyroid atrophy particularly in animals with fat replacing more than 20% of the thyroid. All groups, including the control group which received only CFA, showed TSBAb activity and at least one animal from each group developed anti-Tg antibodies. No correlation was seen between antibody titers and thyroid atrophy. Our results suggest: a) CFA by itself is able to induce anti-Tg ab and blocking antibodies against TSH-R-ECD, but does not cause thyroid atrophy and hypothyroidism. b) In DBA/1j (H-2q) mice thyroid atrophy was observed in different degrees according to the antigen used, being most remarkable with preparations containing the rec-h-TSH-R-ECD (aa 19-417) and peptide aa 315-334. According to our data, the extracellular domain of the h- TSH-R is antigenic and pathogenic in DBA/1j(H-2q) mice. The cause and meaning of fat replacement without lymphocytic infiltration is unclear. It may represent the end stage of thyroiditis with antibodies playing an ambiguous role. TSBAb may functionally block the thyroid epithelial cell and cause cell death. Possibly TSBAb (or other unrecognized antibody) stimulate fat cells and cause hyperplasia, since fat cells are known to have TSH receptors and to be normally present in the mouse thyroid.

2:30 P.M. 114. NATIVE GEL ELECTROPHORESIS OF THE 64 kDa PROTEIN SHOWS THAT IT IS EYE MUSCLE SPECIFIC AND AN IMPORTANT TARGET FOR AUTOANTIBODIES IN PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY. J.R. Wall, D. Scalise, M. Hayes, C. Stolarski, M. Sato, M. Salvi and V. Nebes. Thyroid Center, Allegheny-Singer Research Institute, Pittsburgh, PA.

Although SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting are widely used to detect serum antibodies in patients with autoimmune disorders this procedure unfolds and denatures proteins and may alter antibody binding sites. We have used a gentle protocol for the preparation and purification of eye muscle (EM) membrane antigens. Pig EM membrane proteins were solublized by extraction in PBS. Such "native" membrane proteins" (NM_{prot}) were electrophoresed on an 8.5% polyacrylamine gel in the absence of detergents, reducing agents or urea, transferred to PVDF paper and incubated with sera from patients with thyroid-associated ophthalmopathy (TAO) or normals. Thirteen bands of reactivity of different relative mobilities were identified. In order to identify the target antigens proteins from individual bands were eluted, applied to standard SDS-PAGE and immunoblotted with sera from patients with TAO. A prominent 64 kDa protein was recognized by autoantibodies in sera from 35% of patients with TAO but only 4% of normals. To further purify the 64 kDa protein and increase sensitivity of immunoblotting crude NM_{prot} were applied to an isoelectric focusing (IEF) "Rotofor" apparatus and separated according to their isoelectric point (pI). Individual fractions were collected and applied to SDS-PAGE and Western blotting and probed with TAO patient sera. The 64 kDa protein appeared in IEF fraction #7 and had a pI of 6.2. Similar results were found for human EM membranes. Albumin, which separated in fraction #6, had a pI of 5.2 and MW of 66 kDa and $_1$D, cloned from a thyroid expression library by M. Ludgate et al. and extensively studied in our laboratory, had a pI of 9.2. and MW of ~ 70 kDa. The 64 kDa protein was not present in other skeletal muscle. Sera from groups of patients and normals were then tested in immunoblotting against the pig EM 64 kDa protein prepared from NM_{prot} and separated in IEF. Tests were positive in 8 of 12 (67%) patients with TAO, in 3 out of 8 (37.5%) patients with Graves' hyperthyroidism (GH) without eye disease, one out of 9 (11%) with Hashimoto's thyroiditis without eye disease and in one out of 11 (9%) normal subjects. In conclusion, we have confirmed that the 64 kDa protein is expressed in EM but not other skeletal muscle and that there is a close relationship between the corresponding antibodies and ophthalmopathy in patients with GH. From microsequence information oligonucleotides can be prepared to screen an EM expression library. With the availability of recombinant 64 kDa protein simple antibody tests can be developed to screen patients with GH before the onset of eye disease.

2:45 P.M. 117. **HUMAN AUTOANTIBODIES TO THE TSH RECEPTOR: POLYCLONAL RECOGNITION OF LINEAR, FOLDED AND GLYCOSYLATED RECOMBINANT EXTRACELLULAR DOMAIN.** H. Vlase, P. N. Graves, R.Magnusson and T.F. Davies, Departments of Medicine and Pharmacology, Mount Sinai School of Medicine, New York, New York.

The extracellular domain of the human TSH receptor (hTSHR-ecd) is the primary immune target in Graves' disease. To examine the clonality of autoantibodies to the hTSHR (hTSHR-Abs) we evaluated their recognition of prokaryotic recombinant hTSHR-ecd in an unfolded (linear) and folded (non-linear) state and their recognition of eukaryotic glycosylated hTSHR-ecd expressed in High Five insect cells. Recombinant products were characterized by specific binding to monoclonal antibody (MAb A-10) recognizing amino acids 1-35 of the hTSHR-ecd (Dr. P. Banga, University of London) and two rabbit polyclonal antisera recognizing amino acids 37-71. After purification and refolding of the prokaryotic hTSHR-ecd, non-reducing PAGE/SDS resolved a 54 kd unfolded monomer, a 58 kd folded monomer and a 230 kd tetrameric form. Glycoprotein analogs from the eukaryotic system were a 66 kd unfolded monomer, 74 kd folded monomer, and 270 kd tetrameric form. Twenty sera from patients with Graves' disease and TSH receptor autoantibodies, as measured by TSH competition assay, were tested against each of the 6 antigenic preparations. Twelve (60%) bound folded prokaryotic hTSHR-ecd monomer, 7 (35%) bound to the unfolded monomer, and 3 (15%) bound to the tetrameric species. Certain sera showed a higher affinity for the folded rather than unfolded monomer while MAb A-10 and the rabbit antisera showed no preference. When reacted against the glycosylated insect cell hTSHR-ecd, 9 (45%) sera recognized the unfolded monomer and 8 of these 9 also recognized the folded monomer. N-deglycosylated hTSHR-ecd was not recognized by any of the human antisera. The recognition pattern was heterogeneous even within the same serum sample recognizing linear, folded and glycosylated hTSHR-ecd monomers. We conclude, therefore, that patients with Graves' disease have multiple autoantibodies to the hTSHR-ecd and that this polyclonality indicates that hTSHR-Ab induction is a secondary phenomenon in autoimmune thyroid disease.

2:00 P.M. 42. **TRH STIMULATES TSHB PROMOTER ACTIVITY BY TWO DISTINCT MECHANISMS INCLUDING Ca^{2+} UPTAKE THROUGH L-TYPE Ca^{2+} CHANNELS AND ACTIVATION OF PROTEIN KINASE C.** M. A. Shupnik, P.M. Hinkle. Dept. of Internal Medicine, Univ. of Virginia Health Sciences Center, Charlottesville, VA and Dept. of Pharmacology, Univ. of Rochester Medical School, Rochester, New York

TRH stimulates the rTSHβ gene at two DNA response elements, which are also stimulated by Protein Kinase C activators. We investigated the dependence of TRH-stimulated transcription of the TSHβ gene on a rise in intracellular Ca^{2+} ($[Ca^{2+}]i$), and on the necessity for Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels in two transfected cell lines and in normal thyrotropes. In GH_3 cells 1nM TRH treatment resulted in a burst followed by a sustained increase in $[Ca^{+2}]i$. The initial burst was blocked by depleting internal $1P_3$-sensitive stores with thapsigargin, and the sustained phase was blocked by chelating external Ca^{2+} with EGTA or by inhibiting L-type Ca^{2+} channels with 500nM nimodipine. To test for transcriptional activation by TRH, constructs of the 5'-flanking region of the TSHβ gene fused to the luciferase reporter (TSHβLUC) were transfected into GH_3 cells. TSHβLUC was stimulated 2- to 5-fold by 1nM TRH or 100nM Bay K8644, a potent L-channel agonist, and the TRH effect was nearly abolished by nimodipine or chelation of external Ca^{2+}. Constructs containing Isolated TRH-responsive elements fused to a heterologous promoter responded similarly. In GH_3 cells, the protein kinase C activator PMA (100nM), also stimulated TSHβLUC transcription, but this effect was not inhibited by nimodipine. A stable heterologous cell line containing the mouse TRH-R was constructed by transfection of 293 cells, a human embryonal kidney line. The resulting 293-TRHR cells contained 1.4 pmole TRH-R/mg protein, similar to GH_3 cells. In these cells, TRH treatment resulted in a burst of $[Ca^{2+}]i$ that was thapsigargin sensitive, but in contrast to GH_3 cells, Bay K8644 did not increase $[Ca^{2+}]i$, and nimodipine did not inhibit the TRH effect. IN 293-TRHR cells, transfected TSHβLUC activity was increased 2- to 3-fold by TRH or PMA; nimodipine did not block stimulation by either treatment, and Bay K8644 had no effect. The transcriptional response did not require external calcium. The transcription rate of the homologous TSHβgene in normal thyrotropes was measured by nuclear run-off assays. Bay K8644 stimulated TSHβ gene transcription 6-fold and TRH stimulation of TSHβ gene transcription was partially blocked by nimodipine (4-fold to 2-fold), but PMA-stimulated transcription (3-fold) was not. Transfected TSHβLUC activity was stimulated by 3-fold TRH, and this was partially blocked by nimodipine. We conclude that TRH-stimulation of TSHβ gene transcription require Ca^{2+} release from IP_3-sensitive stores and Ca^{2+} influx via L type calcium channels in GH_3 cells, but in 293 TRH cells activation of protein Kinase C plays a predominant role in activating TSHβ. Both mechanisms appear to be operative in normal thyrotropes.

2:15 P.M. 125. MSX-1 and OCT-1 interact with 5' flanking sequences important for thyrotropic α-subunit gene expression.

The mouse α-subunit gene 5' flanking region contains sequences between -480 and -381 relative to the transcriptional start site that are important for expression in thyrotrope cells, both the TSH-producing TtT-97 tumor cells and the αTSH cell line that only produces the α-subunit of TSH. Both of these cell lines contain nuclear proteins that bind to that region of the α-subunit gene. To determine what factors interact with that region we used a fragment of the mouse α-subunit gene extending from -490 to -310 to ligand-screen an αTSH λEXlox protein expression library. A number of clones were carried through tertiary screening and were sequenced. One of these clones contained a fragment of the MSX-1 gene, that is related to the Drosophila Msh homeobox-containing gene. Expression of this gene has been described early in embryogenesis in regions of epithelial-mesenchymal interaction and its expression has been detected in the developing brain and anterior pituitary. The function of MSX-1 has not been determined and its target genes have not yet been described. The cloned MSX-1 gene contained the homeobox region and slightly differed from the published sequence by a 3 base pair substitution at the 5' end of the homeobox region. MSX-1 transcripts were detected by Northern blot analysis of thyrotropic (αTSH and TtT-97) and hypothyroid mouse pituitary mRNA. The cloned MSX-1 gene was expressed in BL21LysE cells and produced a fusion protein of the appropriate size. Gel mobility shift assays demonstrated that this protein bound to fragments of the α-subunit promoter from -484 to -446, from -449 to -421 and from -417 to -373, regions that are footprinted by nuclear extracts from αTSH cells and TtT-97 tumors. Competition studies with cold homologous oligonucleotides showed that this interaction was specific. Other clones obtained in the ligand-screening contained fragments of the transcription factor OCT-1. These clones contained the POU-HOMEO box region but not the HOMEO-specific regions of OCT-1. Two different OCT-1 clones were obtained, corresponding to two splice variants OCT-1b and OCT-1c. There is an imperfect octamer sequence, ATGAAAAT, from -356 to -349 of the mouse α-subunit gene, that is a potential binding site for this factor. In summary, two transcription factors, MSX-1 and OCT1 (b and c isoforms) are expressed in thyrotropes and interact with regions important for thyrotrope-specific expression of the mouse α-subunit gene.

2:30 P.M. 126. CONSTRUCTION OF A 3-DIMENSIONAL MODEL OF THE THYROTROPIN-RELEASING HORMONE RECEPTOR BINDING SITE AT AN ATOMIC LEVEL. J.H.Perlman,L.Laakkonen, R.Osman and M.C.Gershengorn,Cornell Univ Med Coll and Mt Sinai Med Ctr,NY,NY.

We have previously shown that the hydroxyl group of Tyr106 in the third transmembrane (TM) helix of the thyrotropin-releasing hormone receptor (TRH-R) binds the ring carbonyl group of the pyroglutamyl (pyroGlu) moiety of TRH (pyroglutamyl-histidyl-prolinamide). Based on this finding, homology to other seven transmembrane-spanning G protein-coupled receptors (GPCRs) and preliminary experimental results, we developed a 3-dimensional model for the binding site of TRH-R using a series of software programs on a Silicon Graphics system. In addition to the bond between Tyr106 and TRH pyroGlu, the proposed model includes the following: Asn110 (TM-3) interacts with the ring N-H of the pyroGlu moiety of TRH; Gln105 (TM-3) and Arg306 (TM-7) stabilize the bond between Tyr106 and TRH pyroGlu; Arg283 (TM-6) interacts with the TRH prolinamide. We found that the affinities of WT, N110A (in which Asn at position 110 was substituted by Ala) and N110S TRH-Rs for N-τ-methylhistidylTRH (MeTRH) were 1.5, 48, and 130 nM, respectively, demonstrating the importance of Asn110 for binding. The affinities of WT, N110A and N110S TRH-Rs for α-hydroxyglutaryl-γ-lactone^{1}TRH, an analog of TRH in which the pyroGlu ring N-H was replaced by O, were 3200-, 580-, and 380-fold lower than for MeTRH. The lack of full additivity resulting from the effects of mutating Asn110 and substituting the pyroGlu ring N-H is evidence of an interaction between Asn110 and the pyroGlu N-H. Compared to WT TRH-R, the affinity of Q105A TRH-R for MeTRH was 3-fold lower consistent with an indirect effect of Gln105 on binding. Preliminary data indicated the affinities of R306A and R283A to be greater than 1 uM. The proposed model is, therefore, consistent with the experimental data and, importantly, hypothesizes other interactions to be tested experimentally. This approach emphasizes the utility of substituting residues in the receptor and in the ligand, and integrating experimental and theoretical methods, for the modelling of the receptor binding site at an atomic level of detail. Our model of the TRH-R binding site places the binding pocket for TRH within the transmembrane domains of the receptor and is the most detailed model to date for the interaction of any GPCR with a peptide ligand.

2:45 P.M. 140. **ROLE OF THE CARBOXYTERMINUS OF THE ALPHA SUBUNIT IN THE EXPRESSION AND BIOACTIVITY OF HUMAN TSH: IDENTIFICATION OF DELETION MUTANTS THAT ACT AS COMPETITIVE ANTAGONISTS.** M. Grossmann, M. W. Szkudlinski, H. Zeng*, R. N. Thotakura, J. E. Tropea, T. H. Ji* and B. D. Weintraub, MCEB, NIDDK, National Institutes of Health, Bethesda, MD 20892, and *Department. of Molecular Biology, University of Wyoming, Laramie, WY 82071.

The glycoprotein hormones TSH, LH, CG and FSH are heterodimers consisting of a hormone specific β subunit and a common α subunit. The carboxyterminal end of the α subunit (amino acids 89-92) has been shown to be important for the *in vitro* bioactivity of FSH and CG, but its role for TSH and the more closely related LH is not clear. The aim of the present study therefore was to investigate the importance of this segment for human TSH expression and bioactivity, and to identify possible antagonists. We thus successively truncated the human α subunit cDNA by site directed-mutagenesis, creating mutants that lack amino acids 92, ($\alpha\Delta92$), 91-92 ($\alpha\Delta91$-92), 90-92 ($\alpha\Delta90$-92), and 89-92 ($\alpha\Delta89$-92). Wild type (wt) or mutant αcDNAs were coexpressed transiently with a human TSHβ minigene in CHO-K1 cells. Expression of $\alpha\Delta92$/TSHβ was only 20% of wt TSH as measured by two different TSH immunoassays. Additional truncation, however, did not further decrease expression levels. Next, cAMP stimulation by the mutant TSH variants was compared to wt TSH in CHO cells stably transfected with the human TSH receptor (IP09, kindly provided by Dr. G. Vassart, Belgium). Wt TSH dose-dependently increased cAMP production (EC50 = 4.5 ng/ml), reaching maximal stimulation at 140 ng/ml. Deletion of amino acid serine at position 92 led to a pronounced decrease of bioactivity, and maximal cAMP levels of the mutants $\alpha\Delta92$/TSHβ and $\alpha\Delta91$-92/TSHβ were 60% of wt TSH (EC50 = 10 ng/ml). Further truncation of histidine 90 almost completely abolished cAMP production. At 140 ng/ml, cAMP production of mutants $\alpha\Delta90$-92/TSHβ and $\alpha\Delta89$-92/TSHβ was only 4.6% and 3.1% of wt TSH, respectively (EC50 not measurable). In coincubation experiments with fixed concentrations of wt TSH, mutants $\alpha\Delta90$-92/TSHβ and $\alpha\Delta89$-92/TSHβ, but not $\alpha\Delta92$/TSHβ or $\alpha\Delta91$-92/TSHβ, dose-dependently inhibited cAMP production. In the presence of 2 ng/ml wt TSH, 50% inhibition was achieved at 70-fold molar excess, and with 10 ng/ml wt TSH, inhibition was 38% at 14-fold molar excess. In summary, these data show that, as in CG and FSH, the carboxyterminus of the α subunit is crucial for the *in vitro* bioactivity of TSH. However, the relative importance of individual amino acids differs in these glycoprotein hormones. In TSH, amino acids serine 92 and histidine 90 are especially critical, whereas, by contrast, in CG and FSH, serine 92 is dispensable, and lysine 91 and histidine 90 are important. In addition, serine 92 is needed for effective expression of TSH. The mutants $\alpha\Delta90$-92/TSHβ and $\alpha\Delta89$-92/TSHβ, which are almost completely devoid of intrinsic activity, act as partial competitive TSH antagonists.

3:30 P.M. 58. MOLECULAR MODELING AND SITE-DIRECTED MUTAGENESIS OF THE LIGAND BINDING DOMAIN OF THYROXINE-BINDING GLOBULIN. C. Büttner, B. Treske and O.E. Janssen, Medizinische Klinik Innenstadt, Ludwig-Maximilians-Universität, Munich, Germany.

Thyroxine-binding globulin (TBG) and corticosteroid-binding globulin (CBG) belong to the serine protease inhibitor (SERPIN) superfamily of proteins. They differ from "true" SERPINs like α_1-antitrypsin (AT) by not inhibiting serin proteases and by binding a small ligand with high affinity. No structure model of TBG or CBG has been obtained so far, most likely due to their microheterogeneic glycosylation. However, the crystallographic structure of AT has been determined and was found to represent the archetype of the SERPINs[1]. The AT structure contains a characteristic barrel of ß-strands, compatible with the ligand-binding domain of TBG and CBG previously identified by chemical crosslinking[2,3]. This model has been used for structure-function correlations of TBG. We have previously shown that introduction of a new glycosylation site (TBG-Leu246Thr) within this region abolishes thyroxine (T_4) -binding of TBG without affecting the integrity of the molecule. Interestingly, most of the other SERPINs are glycosylated at this position, a possible explanation for the specific binding of T_4 by TBG. In CBG, glycosylation of this site has even been shown to be required for cortisol binding[4]. We now present a refined model of the T_4-binding site and further characterization of TBG mutants expressed in *Xenopus* oocytes and characterized by a resin T_4-binding assay and immunoprecipitation. Exchange of the positively charged lysine-253 with a neutral alanine or a negatively charged aspartate reduces T_4-binding by 17 or 30 %, respectively. This residue at the entrance to the putative ligand-binding pocket is the one that can be cross-linked with T_4[2]. It may interact with the 4'-hydroxyl- or the carboxyl-group of the T_4 molecule, however, spatial, electric and hydrophobic factors favor the latter orientation. Both TBG mutants were synthesized and secreted by the *Xenopus* oocytes and appeared to be in the native conformation when analyzed by immunoprecipitation. CONCLUSION: The charge of residue 253 appears to be important for the high affinity binding of T_4 to TBG. 1) Huber & Carell (1989) Biochemistry 28, 8951; 2) Tabachnik & Perret (1986) Biochem. Int. 15, 409; 3) Kahn & Rosner (1977) J. Biol. Chem. 252, 1895; 4) Avvakumov, Warmels-Rodenhiser & Hammond (1993) J Biol Chem 268, 862.

3:45 P.M. 69. EFFECT OF 6-ANILINO-2-THIOURACIL, A POTENT INHIBITOR OF HEPATIC 5' D-I ACTIVITY, AND SELENIUM DEFICIENCY ON THYROID HORMONE ECONOMY IN THE NEONATAL RAT. I.E. Veronikis, S. Alex, C.H. Emerson, S.L. Fang, G. Wright, and L.E. Braverman. University of Massachusetts Medical School, Worcester, MA.

Selenium (Se) deficiency markedly reduces hepatic 5' deiodinase (5'D-I) activity but does not alter the neonatal T3 surge (Endocrinology 133:2604, 1993). We now studied the effects of 6-anilino-2-thiouracil (ATU), a 5'D-I inhibitor which does not affect thyroid hormone synthesis, on thyroid hormone economy in the neonatal rat. We also determined the effect of ATU treatment, and Se deficiency, on skin 5 deiodinase (5D) activity. Pregnant rats were fed 0.1% ATU in the chow from conception through 28 d post partum. ATU was present in breast milk and neonatal serum as assessed by HPLC. Pups were sacrificed at 14 and 28 d of age and serum thyroid hormone, TSH concentrations , hepatic 5'D-I and skin 5 D activities were measured.

Age, days	Rx	T4, µg %	T3, ng %	rT3, pg/ml	TSH, U/l	Liver 5'D, % Control	Skin 5D, % Control
14	Control	4.2 ± 0.2	100 ± 8	236 ± 16	54 ± 4	-	-
	ATU	3.4 ± 0.6	77 ± 5	1018 ± 84*	57 ± 4	26*	111
28	Control	2.3 ± 0.1	185 ± 13	91 ± 7	35 ± 1	-	-
	ATU	3.4 ± 0.2†	135 ± 9†	215 ± 16*	38 ± 2	52*	200†

† $p < 0.01$; * $p < 0.001$; All results as Mean ± SEM

As shown, ATU treatment did not abolish, but did blunt, the neonatal/prepubertal T3 surge and markedly increased serum rT3, decreased liver 5'D and had lesser effects on serum T4. Skin 5D activity was actually increased at 28 days but the effect on skin 5D did not correlate with the changes in serum rT3. When Se deficiency was induced, throughout gestation and postpartum, hepatic 5'D decreased by > 90 %. Similar effects on serum T4 and T3, as observed with ATU, were noted. The effects of Se on serum rT3 were markedly less than those of ATU, however, Se deficiency did not affect skin 5D. Conclusions: The neonatal/prepubertal surge in serum T3 in the rat does not appear to be due to changes in peripheral deiodination. The disparity between the effects of ATU and Se deficiency on serum rT3 suggests that impaired hepatic 5'-deiodination of rT3 is not the only mechanism for the increase in serum rT3 in ATU treated rats.

4:00 P.M. 101. **THE TYPE III 5-DEIODINASE IN *RANA CATESBEIANA* (RC) TADPOLES IS ENCODED BY A THYROID HORMONE-RESPONSIVE GENE.** V. A. Galton, M. J. Schneider, K.B. Becker and J. C. Davey, Dartmouth Medical School, Lebanon, NH 03756.

We have recently reported that XL-15, a thyroid hormone (TH)-responsive gene isolated from a *X. laevis* tadpole tail cDNA library (Wang & Brown, J. Biol. Chem. 268:16270, 1993) encodes a selenoprotein which is a type III 5-deiodinase (5D) (St. Germain, *et al.* Proc. Endocr. Soc., 1994). This enzyme metabolizes TH to inactive metabolites. In order to study the regulation of 5D in developing RC tadpoles, an RC tadpole tail cDNA library was screened for a 5D cDNA using XL-15 as a probe. A partial cDNA (RCD10) was isolated which has 80% nucleotide identity to XL-15, including a conserved TGA codon that encodes selenocysteine. RCD10 and XL-15 both hybridize to the same 2.2 Kb mRNA species in RC tail, skin, liver and eye. Tissue expression of the RCD10 gene in RC tadpoles has been determined using a sensitive reverse-transcription/PCR technique. In nine tissues (leg, tail, kidney, eye, brain, intestine, liver, heart, and skin) from untreated and T3-treated early prometamorphic tadpoles (stage XII), the levels of RCD10-related mRNA transcripts correlated closely with 5D but not 5'-deiodinase (5'D) activity. Thus RCD10 mRNA levels and 5D activity were detected in all the tissues from untreated tadpoles and the levels of both were greatly increased in tadpoles exposed to T3 (10^{-8}M) for 3 days. In contrast, 5'D activity, which was detectable only in leg, skin, intestine, eye and brain at this stage of development, was not enhanced by the 3-day exposure to T3. It is notable that RCD10 mRNA was readily detected in liver and kidney, tissues in which 5'D activity has been shown to be absent at all stages of development. TH-responsiveness of this gene occurs very early in development. Comparable studies with young tadpoles (stage III) revealed that 5D activity was already present in kidney, eye, brain and skin; following exposure to T3, activity was greatly increased in these tissues, and was induced in tail, limb bud, and liver.

It is concluded that RCD10 is the RC homologue of XL-15 and encodes a type III 5D. Young tadpoles are subject to uncoordinated development and eventually death in the presence of high levels of exogenous TH. The finding that the 5D gene is expressed in some tissues soon after hatching and in all major tissues by prometamorphosis, and is up-regulated by TH, suggests that the 5D system plays a major role in coordination of metamorphic processes.

4:15 P.M. 102. **IDENTIFICATION OF A cDNA FOR THE RAT TYPE III IODOTHYRONINE 5-DEIODINASE (5DIII).** W. Croteau, S. L. Whittemore, M. J. Schneider and D. L. St. Germain, Departments of Medicine and Physiology, Dartmouth Medical School, Lebanon, New Hampshire.

The 5DIII metabolizes the active thyroid hormones T4 and T3 to inactive compounds and is the predominant deiodinase activity present in rat brain, skin, placenta, and during early fetal development. Recently, we have characterized a *X. laevis* cDNA (XL-15) and demonstrated that it encodes a selenoprotein with 5DIII activity (76th Annual Meeting of The Endocrine Society, 1994). Although little structural information is known about the mammalian 5DIII, prior studies demonstrating (1) an insensitivity of rat 5DIII to inhibition by propylthiouracil and aurothioglucose, (2) an inability to label a candidate 5DIII with ^{75}Se in rat tissues, and (3) maintenance of 5DIII activity in rat tissues in the face of nutritional selenium deficiency, have been interpreted as indicating that rat 5DIII is not a selenoprotein. In previous studies using the *X. laevis* oocyte translational assay system, we have demonstrated that 5DIII mRNA is highly expressed in rat neonatal skin (rNS), but is essentially undetectable in skin from post-partum dams. With the isolation of a cDNA for the rat 5DIII as a goal, we prepared a cDNA library in the λZapII vector using rNS mRNA as template and screened the library at low stringency with a portion of the XL-15 cDNA that included the coding region. Among the clones isolated was one (rNS27-1) which contained a 1.6 kb cDNA insert that manifested significant homology to the XL-15 and the G21 rat type I 5'-deiodinase (5'DI) cDNAs. Homology to these cDNAs based on 572 bp of nucleotide sequence from the presumptive coding region of the rNS27-1 is as follows:

cDNA	*Nucleotide Homology*	*Amino Acid Homology*
XL-15 - amphibian type III	67% identity	62% identity
G21 - rat type I	54% identity	46% identity

Included in rNS27-1 in a region of high homology with XL-15 and G21 is an in-frame TGA codon which presumably encodes selenocysteine. On Northern analysis, rNS27-1 hybridizes to a 2.2 kb RNA species present in rNS and in rat placenta, but fails to hybridize to RNA derived from rat post-partum skin. Based on these findings, we propose that rNS27-1 encodes the rat 5DIII. (Because rNS27-1 lacks a translational start site, functional data and the predicted mass of the encoded protein are not available.) In conclusion: (1) the 5DIII and 5'DI constitute a family of selenoenzymes which share certain structural features, and (2) the traditional criteria, as cited above, to identify a deiodinase as a selenoprotein are not reliable.

4:30 P.M. 133. COMPARISON OF AMINO ACID REQUIREMENTS FOR 5′ AND 5 DEIODINATION OF IODOTHYRONINES BY TYPE 1 DEIODINASE (DI-1).

N. Toyoda, P. R. Larsen, M. J. Berry, J. W. Harney, C. Horst#, and T. J. Visser*. Brigham and Women's Hospital, Boston, MA, #MMDRI, Henning, Berlin, Germany and *Erasmus University, School of Med, Rotterdam, The Netherlands.

DI-1 catalyzes 5′ deiodination (5′D) of reverse T3 (rT3) and 5 deiodination (5D) of T3 SO_4 (T3S). The following studies were performed to compare the structural requirements for 5′ and 5D by this enzyme. 5′D of rT3 and 5D of T3S (using 3,5 ^{125}I-T3S) were compared in dog (d) and human (h) hepatic microsomes. The Km of dDI-1 for rT3 (5 μM) was 20 fold that of hDI-1 while that for T3S (8 μM) was only 4 fold higher. Vmax/Km ratios showed that T3S was the preferred substrate for dDI-1 and rT3 for hDI-1. These 2 enzymatic functions were further compared using transiently expressed wild type and site-directed mutants of h and dDI-1 cDNAs.

	rT3 5′D			T3S 5D		
COS cell sonicate	Km (μM)	Vmax (pmol/min/mg)	Vmax/Km	Km (μM)	Vmax (pmol/min/mg)	Vmax/Km
hDI-1	0.34	74	211	1.2	219	180
Hm 6	12	21	2	1.4	298	213
dDI-1	9.0	17	1.9	6.9	1058	153
Dm 7	0.45	67	150	5.1	431	86

Hm 6 is a mutant hDI-1 in which amino acids (aa) 45, 46, and 65 were changed to those of dog. Dm 7 is a mutant dDI-1 in which aa 45, 46, 65 and 66 were changed to those present in the human. These results demonstrate that mutations in the human or dog DI-1s which have marked effects on rT3 5′ D do not affect 5D of T3S. Thus, amino acids critical for rT3 binding in DI-1 can be clearly distinguished from those important for T3S binding.

4:45 P.M. 135. STRUCTURE AND FUNCTION OF NORMAL AND DEFECTIVE MOUSE TYPE I DEIODINASE (*dio1*) GENES. A.L. Maia, M.J. Berry, R. Sabbag, J.W. Harney and P.R. Larsen, Brigham and Women's Hospital, Boston, MA.

Type 1 deiodinase (DI-1) provides the major portion of circulating T_3 in vertebrates. In C3H and related mice, liver DI-1 activity is lower than in the common phenotype, C57. The lower activity is paralleled by decreased normal-sized *dio1* mRNA and hyperthyroxinemia. Low activity cosegregates with a restriction fragment variant (RFLV) in recombinant strains indicating it is due to differences in the *dio1* gene. To identify this difference, *dio1* genes were isolated from C3H and C57 genomic libraries. The exonic structure and sequence were the same in both. The RFLV difference is due to a 150 bp expansion of repetitive sequences in the second intron of the C3H *dio1* gene. To assess if this difference caused lower expression in C3H, we subcloned fragments containing these sequences from both genes into a TK-GH intron and transiently expressed these in COS cells. There was no difference in GH expression, indicating that the RFLV is likely to be only a marker for the abnormal gene. Using S1 mapping and RACE, we identified an identical transcriptional start site in both strains. We have sequenced 1 Kb of promoter and 5′ flanking region of both genes and found only a few differences between C3H and C57. Neither has a TATA box. However, in preliminary functional assays using CAT promoter/constructs in transiently transfected Hep-G2 cells, the C3H gene is less potent than that of C57. These results suggest that a difference in *dio1* gene transcription may be the cause of the low DI-1 activity and elevated free T_4 in C3H mice.

10:30 A.M. 1. THE EFFECT OF THYROID HORMONE RECEPTORS ON THE 9-CIS RETINOIC ACID RESPONSIVENESS OF RETINOID X RECEPTORS IS CELL TYPE AND ISOFORM-SPECIFIC.
Amy L. O'Donnell and Anna Burakowski, Dept. of Medicine, VAMC and SUNY, Buffalo, NY.

It is becoming increasingly apparent that hormonal responses and interactions between thyroid hormone receptors (TRs) and retinoid X receptors (RXRs) may vary depending on the cell line used in transfection studies. The objectives of these studies are to further characterize the effects of TRs on the ability of RXRs to *trans*-activate target genes. We have previously found that TRß1 completely inhibits the 9-*cis* retinoic acid (9-*cis* RA) responsiveness of RXRα and RXRγ through the retinoid X response element (RXRE) from the cellular retinol-binding protein type II (CRBPII) gene in JEG-3 cells. Others have shown that TRα1 inhibits the 9-*cis* RA responsiveness of RXRα in NIH3T3 cells using the same RXRE. In our current experiments, COS cells were transiently transfected with a CAT reporter plasmid containing either one or two copies of the RXRE from the CRBPII gene. Transfections also included expression vectors for TRß1 and/or RXRα or RXRγ. With one copy of the RXRE in the reporter plasmid, cotransfected RXRγ had a 7.0±2.9 fold response to 100 nM 9-*cis* RA. When TRß1 was included in the transfection, the 9-*cis* RA response was decreased to 4.1±1.4. In contrast to our previous studies in JEG cells, the TR alone did not exhibit T3-responsiveness with this RXRE in the COS cells. There was also only a 40% inhibition of the 9-*cis* RA response in the COS cells compared to a complete inhibition in the JEG cells. The experiments with two copies of the RXRE in the reporter plasmid were even more informative, since the overall 9-*cis* RA response was higher. In these studies, there was a similar 54% inhibition of the 9-*cis* RA responsiveness of the RXRγ by TRß1 (36.8 fold response with RXRγ alone vs. 16.9 fold response with both RXRγ and TRß1.) Surprisingly, however, the RXRα response to 9-*cis* RA was not inhibited, but enhanced by the presence of TRß1. The response to 9-*cis* RA was 4.8±2.1 fold in the presence of RXRα alone, and rose to 20.5±9.1 fold in the presence of cotransfected TRß1. In summary, these results show that the effect of TRs on the 9-*cis* RA responsiveness of RXRs is dependent on the cell type and on the RXR isoform used in the studies. In JEG cells, the 9-*cis* RA responsiveness of both RXRα and RXRγ were completely inhibited by the presence of TRs, whereas in COS cells, the RXRα response was not inhibited, but enhanced by the presence of TRs, and the RXRγ response was still inhibited although to a lesser extent.

10:45 A.M. 47. **Interaction of the Thyroid Hormone Receptor with a Novel Oncoprotein Associated with A Pediatric Malignancy.**
Wongi Seol and David D. Moore, Dept. Molecular Biology, Massachusetts General Hospital, Boston, MA

We have used the yeast two-hybrid system to isolate a series of cDNAs encoding human proteins that show specific functional interaction with the β isoform of the thyroid hormone receptor. One of particular interest is a putative transcription factor that is the product of a gene called ALV or FKHR. In the t(2;13)(q35;q14) translocation characteristically observed in alveolar rhabdomyosarcoma, a childhood tumor of skeletal muscle, the 3' portion of the ALV gene is joined to the 5' portion of the PAX3 gene[1,2]. PAX3 is a protooncogene that encodes a developmentally regulated transcription factor. In the PAX3-ALV fusion protein, the N-terminal and DNA binding domains of PAX3 are joined to the C-terminal portion of ALV, which is thought to contain a transcriptional activation function. As demonstrated for similar translocations in other tumors, it is thought that the aberrant transcriptional regulatory properties of the PAX3-ALV fusion protein contribute directly to tumorigenesis. When expressed in yeast, the C-terminal region of the ALV protein interacts specifically with the ligand binding domains of TR or other members of the nuclear hormone receptor superfamily. The interaction with TR is strongly stimulated in cells grown in the presence of thyroid hormone. The consequences of the interaction of TR and its relatives with the putative PAX3-ALV oncoprotein are under investigation.

1. D. N. Shapiro, et al., (1993) Cancer Res. 53;5108-5112.
2. N. Galili et al., (1993) Nature Genetics 5;230-235

11:00 A.M. 99. RELATIONSHIP OF ISOFORM-SPECIFIC T3 BINDING CAPACITIES TO mRNA LEVELS IN RAT PITUITARY AND EXTRAPITUITARY TISSUES.

We have recently developed a method for determining the contribution of T3 receptor (TR) isoforms to total T3 nuclear binding capacity of tissues by immunoprecipitating nuclear extracts with specific IgG's to distinctive epitopes of TRα1, TRβ1, and TRβ2. This has allowed us to assess the effect of thyroidal status on total and isoform-specific binding capacities of rat anterior pituitaries and to compare the isoform-specific binding capacities and mRNA levels in euthyroid pituitary and extrapituitary tissues.

[1] Total pituitary T3 nuclear binding capacity and constituent isoform-specific binding capacities (pmol/mg DNA) in hypo-, eu-, and hyperthyroid rats (Total, α1, β1, and β2): Hypo: 2.11, 0.58, 0.35, 1.18); Eu (1.59, 0.41, 0.26, 0.92); and Hyper (1.78, 0.49, 0.41, 0.88) (Average of 2 pools of 45 pituitaries each). Of interest were the marginal changes in the total and constituent T3 isoform nuclear binding capacities of pituitaries in the transition between the hypo- and hyperthyroid states. These findings contrasted with a report by Hodin et al. (J Clin Invest: 85:101, 1990), confirmed by us, that TRβ1 mRNA increased 3-fold and TRβ2 mRNA fell by 40% in this transition.

[2] We were surprised by readily demonstrable TRβ2 binding capacity in liver, kidney, heart, and cerebrum in the absence of TRβ2 mRNA by northern analysis. PCR-based studies, however, demonstrated the presence of TRβ2 mRNA but only after Southern blot analysis of DNA product. Densitometric comparison of northern blots of progressively diluted pituitary RNA and determination of RNA/DNA ratios of specific tissues allowed us to show that the ratio of TRβ2 mRNA/mg DNA in extrapituitary tissues (cerebrum, liver, kidney, and heart) to pituitary mRNA/mg DNA were uniformly less than 1/100. In contrast, results of northern analyses using comparably sized probes suggest that the levels of TRβ1 mRNA/mg DNA in the pituitary and the extrapituitary tissues analyzed are of the same order of magnitude. We conclude: (a) that the content of TRβ2 mRNA in the extrapituitary tissues is extraordinarily low both as compared to the level of TRβ2 in the pituitary and the level of TRβ1 in all tissues analyzed and (b) that in the extrapituitary tissues there is a massive step-up in the receptor protein/mRNA ratio for TRβ2.

Although the mechanisms leading to these differences in mRNA/protein relationships remain unclear, our results amply illustrate the danger of basing physiological inferences exclusively on changes in mRNA levels.

11:15 A.M. 105. CHARACTERIZATION OF AN ELEMENT IN THE 5' FLANKING REGION OF A PURKINJE CELL GENE (PCP2) WHICH ABOLISHES TRIIODOTHYRONINE (T3) REGULATION IN TRANSIENT TRANSFECTION STUDIES. G.W. Anderson, K.A. Strait and J.H. Oppenheimer, University of Minnesota, Minneapolis, MN.

We have recently identified in the 5' flanking region of the rodent PCP2 gene an element designated A1 (bp -295 to -268) with three half-sites which behave as a T3 response element (TRE) when inserted in the long terminal repeat of the MMTV-CAT construct (Zou, L. et al J Biol Chem, in press). In transient transfections of CHO cells T3 induced a 10 to 15-fold increase in reporter gene expression and gel retardation assays showed specific binding of A1 to T3 receptors and RXRβ. Since T3 stimulates the rate of PCP2 gene expression in cerebellar Purkinje cells of hypothyroid rat neonates we inferred that the A1 TRE could mediate this effect. PCP2 expression in the adult rat, however, is not influenced by thyroid status. We were therefore most interested to find that the effect of T3 on PCP2 gene expression was abrogated in transient transfection studies with MMTV-CAT constructs in which the A1 TRE was ligated to the adjacent downstream 69 bp sequence of PCP2. The specificity of this silencing function became apparent with the demonstration that a random 75 bp sequence ligated 3' to the A1 TRE allowed the full range of T3 stimulation in transient transfections. We have begun to further characterize the attenuating sequence. The 69 bp sequence also fully suppresses transcriptional stimulation by T3 when the palindromic TRE is substituted for the A1 sequence. The sequence, however, does not act as a general transcriptional suppressor since it does not influence the basal activity of the MMTV or thymidine kinase promoters. Accordingly, we suggest that the 69 bp sequence contains a "response silencer element" (RSE). Deletion studies to date show that a residual 15 bp sequence of the RSE (bp - 267 to -253) still reduces T3 transcription stimulation of both A1 and yet another TRE, the myelin basic protein inverted repeat TRE, by 50%. The precise extent of the RSE remains to be defined. We considered the possibility that the most downstream of the three half sites of A1 might allow dimerization of transacting factors associated with it and the adjacent 5' region of the RSE. However, this appears not to be the case since specific mutation of GG to TT in the downstream half site of A1 did not impair the capacity of the RSE to silence the transcriptional response to T3 mediated by the residual two half sites of the A1 TRE. We speculate that in the adult rat a cognate transacting factor bound to the RSE inhibits the transcriptional response of the PCP2 gene to T3. If so, similar arrangements for other genes involved in neonatal brain development could explain their apparent lack of response in the adult animal.

11:30 A.M. 112. **NEGATIVE THYROID HORMONE RESPONSE ELEMENTS ARE DISTINGUISHED BY THEIR INTERACTIONS WITH THE RETINOID X RECEPTOR (RXR).** A.N. Hollenberg, T.R. Flynn, and O. Cohen. Thyroid Unit, Beth Israel Hospital and Harvard Medical School, Boston, MA.

Thyroid hormone feedback on its central regulators, thyrotropin-releasing hormone (TRH) and thyrotropin (TSH), is essential for normal thyroid gland function. Negative thyroid hormone regulation of these hormones is mediated at the transcriptional level. However, the negative thyroid hormone response elements (nTREs) of these genes are different. We have recently characterized the nTREs of the TRH promoter and found that they consist of three separate thyroid hormone receptor (TR) binding half-sites between -150 and +55 bp (Sites 4,5 and 6), all of which participate in the negative regulation of this promoter. Gel-mobility shift assays demonstrate that Site 4, between -52 and -57, interacts with surrounding sequences and binds both TR homodimers and TR-RXR heterodimers, while Sites 5 and 6 bind only TR monomer. Co-transfection of RXR isoforms, with TR and T_3, leads to enhanced negative regulation of the TRH promoter in heterologous cells. Site-directed mutagenesis of Site 4 leads to a loss of this RXR enhancement and reduces negative regulation in functional assays. Unlike Site 4, gel-mobility shift assays on Sites 5 and 6 show only monomeric TR binding which is lost when RXR is added. These data are consistent with TR-RXR heterodimer formation in solution. In contrast to the TRH promoter, the TSH-ß nTRE consists of only two monomeric TR binding sites in Exon 1 spaced by 24 bp. Co-transfection of RXR isoforms, in the presence of TR and T3, leads to decreased negative regulation of this promoter consistent with decreased monomeric TR binding. This is supported by gel-mobility shift assays which show decreased monomeric TR binding to the TSH-ß nTRE in the presence of RXR. These data demonstrate for the first time, that like positive TREs, nTREs differ structurally and functionally. In conclusion: 1) thyroid hormone dependent feedback inhibition of gene expression is modulated differentially by RXR depending on the structure of the nTRE; and 2) nTREs can be classified as monomeric or heterodimeric based on their response to RXR.

11:45 A.M. 138. BROWN ADIPOSE TISSUE $ß_3$-ADRENERGIC RECEPTORS ARE DOWN-REGULATED BY THYROID HORMONE. A. Rubio, A-L. S. Maia, A. Raasmaja and J. E. Silva. Division of Endocrinology, Jewish General Hospital, McGill University, Montreal, Canada.

We have reported that brown adipose tissue (BAT) $ß_1/ß_2$ adrenergic receptors (AR) are reduced in hypothyroidism and that this reduction does not limit BAT acute responses to norepinephrine. $ß_3$-adrenergic receptors ($ß_3$AR) are abundantly expressed in brown and white adipose tissue. These receptors are considered essential for BAT thermogenic responses. Since there is no selective $ß_3$AR labeled ligand, we have used the non-selective β-ligand [^{125}I]-cyanopindolol ([^{125}I]-CYP), which binds to $ß_1/ß_2$AR with a Kd $\approx$ 40pM and to $ß_3$AR with a Kd $\approx$ 0.8nM. Binding to high affinity sites was 1.5-2.7 fold greater in euthyroid than in hypothyroid BAT membranes. In contrast, low affinity sites were prominent in hypothyroid rats but barely discernible in euthyroid animals. Displacement of [^{125}I]-CYP by Propranolol (PROL) also revealed two sets of binding sites: 1) high affinity sites, fully occupied by 1μM PROL ($Kd_{PROL} \approx 1.5$nM), corresponded to $ß_1/ß_2$AR and were 27 $\pm$ 4% more abundant in eu- than in hypothyroid BAT membranes ($p < 0.001$, 6 expts); and 2) low affinity sites, revealed by $>1\mu$M PROL, which were 60% relatively more abundant in hypothyroid rats. Thus, at 5μM PROL, [^{125}I]-CYP binding was 25.2 $\pm$ 4.3% greater in hypo- than in euthyroid BAT membranes ($p < 0.005$, 8 expts). Similarly, with CGP12177, at concentrations which saturate $ß_1/ß_2$AR ($\geq 0.1\mu$M), [^{125}I]-CYP binding was 1.5-2 fold greater in hypothyroid BAT membranes. Non-specific [^{125}I]-CYP binding (100μM PROL or 50 μM CG12177) was $\leq$0.5%. According to data obtained with selective expression of $ß_3$AR in transfected cells, these results suggest an increase in the abundance of $ß_3$AR in hypothyroid BAT. Acute administration of receptor saturating doses of T_3 restored $ß_1/ß_2$ [^{125}I]-CYP binding but reduced or made disappear the low affinity [^{125}I]-CYP binding sites. In agreement with these findings, $ß_3$AR mRNA was 4-5 fold more abundant in total BAT RNA from hypothyroid rats. A receptor saturating dose of T_3 (50μg/100g) given to hypothyroid rats reduced $ß_3$AR mRNA to 5% of the untreated controls and 25% of the euthyroid levels within 24 h. This effect nearly disappeared in 3 days. T_4 replacement of hypothyroid rats (1μg/100g/d) brought $ß_3$AR mRNA levels down to normal in 4 days. Thus, thyroid hormone directly reduces the expression of $ß_3$AR in BAT contrasting with the opposite effect on $ß_1$AR. This effect of thyroid hormone may be a mechanism to reduce facultative thermogenesis in thyrotoxicosis.

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