Sigma Delta Rho

ΣΔΡ

Honors Science Research Society

ABSTRACTS '88

Yeshiva University, 500 West 185 St., New York, NY 10033

SIGMA DELTA RHO

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Honors Research Society

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Sigma Delta Rho
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ABSTRACTS '88
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Sigma Delta Rho

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Yeshiva College

MESSAGE FROM THE FACULTY ADVISOR

Once again, it is a pleasure to present to the Yeshiva University community "ABSTRACTS 88", a compendium of scientific research performed by students and faculty of Yeshiva College and Stern College. I note with pride the high quality and diversity of research presented in this volume.

Sigma Delta Rho, the parent organization of "ABSTRACTS 88", had a successful second year. During this year we ran a series of research seminars that were both informative and entertaining for all those who attended. This year our seminars included sessions given by current Yeshiva and Stern College students, as well as by world renowned scientists from outside the University.

To those who have performed research, I invite you to share your experiences with the rest of us either at our seminars or in next years' volume. To those who have not yet had the opportunity, come join us, and catch a taste of the excitement.

Carl Feit, Ph.D.
Department of Biology

Message from the President

Fellow students and faculty,

It is the Yeshiva and Stern College students' studious and critical inquiry aimed at the discovery and interpretation of knowledge that inspired the great influx of new members to the Sigma Delta Rho Honors Science Research Society (ΣΔΡ) this year. Sigma Delta Rho was founded on the notion that many science students and much of the faculty of Yeshiva and Stern colleges share a common desire to exchange scientific ideas and gain insight into the extensive array of scientific fields within the framework of our undergraduate colleges.

Sigma Delta Rho is unique because it opens its arms to any student who is seeking placement in a medical school research laboratory or in a hospital for the summer or part time during the school year. During Sigma Delta Rho's second year of existence, distinguished lecturers from all across North America were instrumental in establishing the society as being the most active undergraduate medical society. A student forum, a one-day trip to the Albert Einstein College of Medicine and a highly successful pre-medical shabbaton contributed to Sigma Delta Rho's success. Students were kept informed through posters and the undergraduate college newspapers. Finally, we published "Abstracts '88," the second issue of our annual journal which contains abstracts of research projects from the undergraduate student body and from faculty members who direct research at the undergraduate colleges. We hope that these innovative abstracts will be found interesting and informative.

On behalf of the society, we would like to thank the many people who aided in the running of Sigma Delta Rho. We especially extend our deepest gratitude to Dr. Carl Feit, our faculty advisor and to S. Nahum Goldberg, founder of Sigma Delta Rho. Without their guidance and devotion our society would have not been as successful

as it was. We also wish to thank Michael Levine, president of YCSC, Elaine Witty, president of SCWSC, Leah Bluth, Treasurer of SCWSC, and Alumni Affairs for their financial backing.

I personally wish to thank all the students and faculty who submitted abstracts for our publication. I would further like to express my sincere gratitude to the officers of the society, and the editors of this journal. Good luck to next year's president Marc Scheiner and his incoming board. I wish them the greatest success.

Tamara Carmel

Respectfully yours,
Tamara Carmel
President, ΣΔΡ

BIOCHEMISTRY

Isolating Cytochrome bc_1 Complex

David Inslicht

Dan E. Robertson

University of Pennsylvania
Department of Biochemistry and Biophysics

The study of bioenergetics depends on an ample supply of constituents in the energy-transduction process. One such constituent is ubiquinol-cytochrome c oxidoreductase complex, known as cytochrome bc_1 for short, which transfers electrons from dihydroquinol to cytochrome c. The difficulty in isolating proteins like these is that they naturally occur within a membrane, and when removed from their phospholipid environment they often lose much of their enzymatic activity. A technique has been developed which overcomes this difficulty, to yield cytochrome bc_1 complex which retains almost all of its original activity.

The enzyme was isolated from a species of green photosynthetic bacteria, *Rhodobacter capsulatas*. Grown phototropically in 9-liter bottles of medium, the bacteria were then pressed in a French pressure cell to extract chromatophores. The desired bc_1 complex located in the chromatophore membranes, was solubilized with the detergent dodecyl maltoside. After a series of ultracentrifugation steps, the solution was loaded onto a column containing Sephadex CL-6B, an ion-exchange chromatographic gel. It was then eluted from the column in fractions of 150 drops each by a gradual increase in salt concentration- from 100 mM NaCl up to 500 mM NaCl. The fractions were then assayed spectrophotometrically for the ubiquinol-cytochrome c oxidoreductase complex. Ubiquinol was added to a buffer solution

containing cytochrome c. After inhibiting other enzymes with antimycin, the fraction was added. An increase in the rate of absorbance at 500 nm was taken as a sign of bc_1 activity. The peak fractions were pooled, dialyzed from the NaCl and concentrated using an Amicon membrane. The resulting bright red solution was diluted into 50% glycerol and stored at -20 degrees centigrade.

Although I worked with *Rhodobacter capsulatas*, the use of dodecl maltoside and an appropriate ion-exchange column presumably is effective on a variety of species. The method has already been used to isolate bc_1 complexes from the mitochondria of both yeast and bovine heart.

Cytotoxic Potencies of Polynuclear Aromatic Hydrocarbons

H. Babich and E. Borenfreund

Stern College for Women and Rockefeller University

The neutral red *in vitro* cytotoxicity assay was adapted for use with the human hepatocellular tumor cell line HepG2 to detect the cytotoxic potencies of polynuclear aromatic hydrocarbons (PAHs). Using benzo(a)pyrene (BaP) as the representative PAH, it was determined that a 3-day exposure was the most suitable for detecting cytotoxic potency and that pre-exposure to 5 µg/ml Arochlor enhanced the sensitivity of the HepG2 cells to the toxicant. Such enhanced sensitivity probably reflected increased metabolic conversion of the BaP to active metabolites, as shown by a 3-fold increase in the activity of 7-ethoxycoumarin de-ethylase, an indicator of mixed-function oxygenase (MFO) activity, after culturing the cells in the presence of Arochlor. Furthermore, a reduction in sensitivity to BaP occurred when the cells were cultured in the presence of α-napthoflavone, an inhibitor of aryl hydrocarbon hydrolase activity. When Arochlor-induced cells were transferred to

medium lacking Arochlor, the level of 7-ethoxycoumarin de-ethylase quickly declined to basal levels. Arochlor-induced cells were also able to detect the cytotoxic potencies of benzo(k)fluoranthene, benzo(b)fluoranthene, chrysene, benzo(a)anthracene, pyrene, phenanthrene, and fluoranthene; whereas fluorene, anthracene, acenaphthene, and acenaphthylene were not cytotoxic.

Determination of Calcium Concentration of Heart Myocytes under Various External Conditions

Chaim Mandelbaum

Dr. M. Makman

Albert Einstein College of Medicine,
Dept. of Biochemistry

Guinea pig heart myocytes were perfused by mincing the heart in a solution containing collagenase, then resuspended in a solution containing 0.1M NaCl, 4.8mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$-$7H_2O$, 1 mM $CaCl_2$, 0.2% Glucose, 0.6% Na Hepes, at a pH of 7.4 (Solution C). The crude heart myocytes were then fractionated by a discontinuous mixture of stock percoll (pH 7.4, 292-300 mosmo., 1.5% BSA, pure Percoll, 0.75% NaCl) by centrifugation. These cells were then washed three times with Solution C.

The heart mycocytes were then incubated for 45 minutes with a 2 mg/ml Acetoxymethyl derivative solution of Fura-2: 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2'-amino-5'-methyl phenoxy)-ethane-N,N,N´,N´-tetraacetic acid (10 microliters per ml of cells). Esterases within the cell cleave the Acetoxymethyl Fura-2, releasing the free Fura-2 which chelates to intracellular calcium.

Fluorescence emission of the Fura-2 calcium complex was taken at 500nm with excitations at 340 and 380nm to determine the intracellular calcium concentrations. Various substances, e.g. norepinephrine and KCl were added to one 2.5 ml aliquot in order to determine their effects over a period of time (approx. 20 min.). The other aliquot was used as a control. Digitonin was then added to burst open the cells, releasing the Fura-2, which subsequently bonded to excess calcium, yielding the maximum fluorescence of the cells. EGTA (ethylene-bis-(oxyethylenenitrilo)-tetraacetic acid), which has a higher affinity for calcium, is then added to the cells to which they chelate in order to determine the minimum amount of fluorescence in the aliquot.

Actual calcium concentrations were determined by the equation: $224(S)(F-F_{min})/(F_{max}-F)$. F is the fluorescence ratio at 340/380 at a given time. F_{min} and F_{max} are the minimum and maximum fluorescence ratios of 340/380 determined by EGTA and Digitonin respectively. S is the fluorescence at 380 of EGTA divided by that of Digitonin at 380. The procedure was done likewise to rat heart myocytes.

The average basal calcium concentrations for the guinea pig myocytes was 155nm while that of the rat myocytes was at 250nm. In one representative experiment, the addition of 20 microliters of 1mM norepinephrine. increased the intracellular calcium levels of guinea pig myocytes at a rate of 40nm/min for 10 minutes, compared to a control group which was increasing at a rate of 5nm/min. The rat myocytes did not show any significant response. The KCl response (30 microliters of 3.75M KCl) was much greater for both cells. Guinea pig cells increased their calcium level at a rate of 86nm/min for 5 minutes, compared to the control at 31nm/min. The rat mycocytes increased at 36nm/min with the control rate at 10nm/min. The increase in the fluorescent values of the control cells might show a possible leakage of Fura-2 into the surrounding medium (Sol. C), or there may be a possible physiological effect of the medium upon the cells. The experiment, though, seems to show that the rate of possible leakage is relatively small for the first 10-15 minutes but seems to increase somewhat exponentially thereafter. This might show that there is some physiological effect taking place.

Chemiluminescence by Activated Human Neutrophils (PMNs): Inhibition by Beta Carotine, Canthaxanthin, and Retinyl Palmitate.

S. Nahum Goldberg

G. Szilagyi and Eli Seifter.

Albert Einstein College of Medicine, Yeshiva University, Bronx,NY

We have reported that incubation of human PMNs with β-carotene enhances their microbiocidal activity. Oxidative killing of bacteria by PMNs involves generation of H_2O_2 and other oxidants and prooxidants. A convenient assay for peroxide(s) is chemiluminescence. We determined the effect of β-carotene, canthaxanthin, and retinyl palmitate on light production using opsonized zymosan and luminol. Light generated in control samples was > 5×10^4 RLU / 10^3 PMNs.

Mean % Reduction in Emitted Light
(Compared to Matched Diluent Blanks)

Treatment/Time:	0′	20′	40′	60′	90′
β-carotene 6γ/ml	2.7	42.6	27.3	23.7	32.1
Canthaxanthin 6γ/ml	2.5	25.7	38.5	37.8	45.0
Retinyl Palmitate 2γ/ml	-1.7	17.1	39.9	25.0	47.7

Emitted light reduction due to treatment occured in every assay. Thus, β-carotene may protect tissues against oxidant damage while improving microbiocidal activity.

In Vitro Microbicidal Action of Human Neutrophils (PMN): Enhancement by Beta Carotene (BC).

S. Nahum Goldberg

E. Seifter, R. Horowitz, J.D. Kanofsky, E.R. Burns

Albert Einstein College of Medicine,
Bronx, N.Y.

Dietary BC enhances inflammatory and immune reactions due to BC itself or due to derived Vitamin A. To study possible BC effects, PMN and autologous sera were prepared from blood of 10 healthy donors. Killing of serum-opsonized *Candida albicans* by PMN was studied in standard assays. BC, 600 µg + 6 mg vehicle (V), or only 6 mg V/dl was present under the following conditions: a) during opsonization of Candida, b) only after opsonization, c) throughout the experiment, (a,b, and c refer to BC treatments; a', b', and c' refer to V). Data were analyzed by Wilcoxon Rank Sum test for paired differences between BC and V (i.e., BC-V) and by the Friedman F_R statistic:

Condition	Mean Kill Rate	Observation	Frequency	Rank Sum
a,b,c, (BC)	49.2 %	(BC-V) = pos	29/30	163
a',b',c' (V)	20.6 %	(BC-V) = neg	1/30	2

Friedman test: no evidence that a,b, or c differ, neither do a',b' or c'; however, a differs from a', etc. Wilcoxon: greatly enhanced killing rate is due to BC, P < .01. Thus, we conclude that BC may be useful in therapy of some microbe-caused diseases.

Beta Carotine (BC) as an Opsonin in the Phagocytic process of Human Neutrophils (PMNs)

S. Nahum Goldberg

R. Horowitz and E. Seifter

Albert Einstein College of Medicine, Bronx, New York

We showed that BC increases microbiocidal and phagocytic indices of PMNs compared to pair matched controls and observed decreased peroxide production by these cells. We sought to determine the mechanisms for this phenomenon. We established that BC requires serum to be added to the phagocytosed particle to enhance microbiocidal activity. BC stained *Candida* which persisted even after engulfment by neutrophils.

Treatment (n=10)	% Kill/6γ BC	% Kill Control	p-value
Opsonized Candida	58.7 + 14.5	37.3 + 6.8	<.001
Unopsonized Candida	7.0 + 4.2	3.9 + 4.3	>.05
Cells incubated in BC	55.8 + 8.8	38.0 + 4.9	<.001

The data suggest that BC can function as a cofactor in opsonization, and that observed increases in kill are directly related to the increased numbers of particles ingested.

Beta Carotene (BC) in Neutrophils (PMN): Effector of Selective Toxicity?

S. Nahum Goldberg

E. Seifter, J. Padawar, J. Kanofsky

Albert Einstein College of Medicine,
Bronx, N.Y.

BC is present in leukocytes (Mathews-Roth) and in marrow

fat nurturing progenitor cells (E.S.). Uptake of BC by healthy donor PMN enhanced killing of *Candida*. Microbiocidal activity results from increased PMN uptake of O_2 followed by production of reactive germicidal oxidants (H_2O_2, O_2^-, $HO^\bullet$, $1O_2$, $3O_2$, ClO^-, IO^-). Also, these oxidants cause host toxicity (inflammation, hyperplasia, genotoxicity) and severe catabolic and antiimmune reactions due to PMN-liberated ACTH-RF like peptides that uncouple inflammatory from immune reactions. O_2 damage also occurs when much more O_2 or oxidants are present than can be reduced to OH^- due to either hyperoxygenation or reduced capacity for cells to dispose of oxidants, e.g. reperfusion following ischemia, or by defects in electron transport or O_2^- dismutation (Paraquat). BC converts singlet oxygen to lesser energized forms with release of heat (Krinsky). We therefore think that BC and other intracellular carotenoids may permit oxidative killing of ingested microbes while minimizing damage to surrounding host tissues. Most effective for that purpose would be an injectable or implantable carotenoid, either as adjuvant, liposome, or i.v. lipid. Formulation together with vitamin A would enhance phagocyte-lymphocyte interactions.

IMMUNOLOGY

Production of Monoclonal Antibodies to Connective Tissue Differentiation Antigens

Zev Goldblatt

C. Feit, Y. Hirshaut

Yeshiva University, New York

The primary effort of this laboratory has been to develop monoclonal antibodies directed to connective tissue differentiation antigens. To immunize mice for the production of appropriately sensitized B cells, we have used a variety of human sarcoma cell lines, viewing these tumors as representative of clonal expansions of fibroblasts in variable stages of differentiation. Once produced by fusion, hybridoma supernatants are screened for reactivity by indirect immunofluorescence methods against a limited number of sarcoma and carcinoma cell lines. Those which appear to have promising specificities are further screened against a comprehensive cell line panel including in addition to sarcomas and carcinomas, normal adult and fetal fibroblasts.

By this procedure, we have generated and screened thousands of monoclonal antibodies, of which ten have been found to show specificity for connective tissue markers. When tested against the epithelial cells of human carcinomas in culture, all ten antibodies give negative results. None are reactive with all sarcomas and only two show the same pattern of reactivity against a panel of sarcoma cells. The ten monoclonals also stain normal fibroblasts, both adult and fetal. When test results with the 35 cell lines in the screening panel are considered, sufficient disparities exist to suggest that each of the antibodies has a unique specificity. Of these monoclonal antibodies, four, 6F3, 6E4, 25E2, and 12C9 have, so far,

been studied by immunohistochemical methods against sections of human fetal, adult, and cancer tissues. The remaining six are now being characterized by this approach. None of those monoclonal antibodies tested reacted against sections of carcinomas prepared from 30 patients with six types of malignancy: cancers of the bladder, breast, cervix, kidney, lung, and testes. 6F3 and 6E4 stained most but not all sarcomas, while 25E2 and 12C9 reacted with 9/20 (45%) of tumors studied.

When applied to 27 adult normal tissues, the four tested antibodies reacted only with connective tissue. 6F3, 25E2, and 12C9 stained lung, breast, prostatic, testicular, uterine, and subcutaneous fibroblasts. They did not stain connective tissues in other organs e.g. liver, stomach, or kidney. 25E2 alone stained lung cartilage. 25E2 together with 12C9 reacted with gastrointestinal smooth muscle, uterine myometrium, vascular smooth muscle, and ovarian connective tissue. 12C9 was uniquely reactive with brain and 6F3 with epidermis. The most restricted pattern of reactivity was shown by 6E4 which adult tissue stained only syncytiotrophoblasts.

Finally, against 14 fetal tissues, 6F3, 25E2, and 12C9 were reactive with sections of ten organs. 25E2 and 12C9 continued to show specificity to smooth muscle. 6E4 reacted with none of the fetal sections tested.

A Small Synthetic Peptide Bound via its Hydrophobic Foot to Meningococcal Outer Membrane Proteins Becomes a Highly Immunogenic Vaccine.

Issac Chalom

George H. Lowell, Lynette F. Smith, Robert C. Seid
Wendell D. Zollinger

Dept. of Bacterial Diseases, Walter Reed Army
Institute of Research, Washington DC.

Advances in biotechnology have enabled the identification

and synthesis of large amounts of very pure small peptides. Such peptides, identical to protein epitopes of organisms or toxins that cause militarily important diseases, have great vaccine potential. The major problem impeding the development of small peptide vaccines is their lack of immunogenicity without adjuvants and carrier proteins. Most effective adjuvants are toxic or pyrogenic. Carrier proteins like tetanus toxoid are undesirable because 1) only a small number of peptides can be covalently bonded to a tetanus molecule necessitating immunizing with excessive amounts of protein to deliver enough peptide and 2) epitope suppression of anti-peptide responses occurs in people previously immunized with tetanus.

We now report that a small eleven amino acid peptide can become highly immunogenic by the addition of a small hydrophobic foot to one end of the peptide which is hydrophobically complexed to meningococcal outer membrane proteins. We call these proteins "protosomes" since like liposomes, they are hydrophobic, vesicular and membranous in nature. Without adjuvants, this vaccine induced specific anti-peptide IgG detected by an ELISA in murine sera diluted 1:256,000 after only one booster immunization; titres stayed high over nine months. Results were excellent whether the hydrophobic foot consisted of a short chain fatty acid or six hydrophobic amino acids synthetically added to one end of the peptide. Antibody specificity was confirmed by inhibiting ELISA binding with pure peptide devoid of hydrophobic feet. Alum boosted the 1° response to protosome-peptides but did not enhance the already maximal 2° responses. In contrast, peptides given with Freund's adjuvant, MDP or alum alone were ineffective.

The peptides we used are identical to conserved epitopes of the variable surface glycoprotein and are common to many *Trypanosoma brucei* strains. These trypanosomes cause Sleeping Sickness among millions in Africa and would result in high morbidity among troops deployed in endemic areas. Our protosome-peptide vaccine system is advantageous for the development of protection against many diseases because: 1) Immunization with proteins frequently does not induce antibodies against the common sub-dominant cross-reacting epitopes, 2) Three to four fold more peptide binds hydrophobically to protosomes than can be complexed covalently to carriers like tetanus toxoid, 3) Since protosomes may be derived from many bacterial strains, epitope suppression is unlikely, 4) These outer membrane proteins have been given safely to thousands of persons in meningococcal vaccines, 5) Multi-disease vaccines should be feasible since a variety of peptide foot mixtures may simultaneously complex to protosomes and 6) The protosome-peptide complexes are prepared in a simple one step dialysis procedure which is economically adaptable to large scale production.

Designing Peptide Vaccines: The Effects of Hydrophobic Feet, Cysteine Dimerization, and Replicate Epitopes With and Without Carrier Proteosomes

Isaac Chalom

George H. Lowell, Lynette F. Smith and Wendell D. Zollinger

Dept. of Bacterial Diseases, Walter Reed Army Institute of Research, Washington D.C.

Making synthetic peptide vaccines has been complicated by a paucity of efficient carrier proteins and adjuvants acceptable for human use. We have found that without proteins or adjuvants, a small protein can be made immunogenic in mice by the addition of a lauric acid hydrophobic foot to the peptide's amino terminus, and a cysteine residue (to promote dimerization of the hybrid lipopeptide), followed by replication of the peptide epitope three to five times and selective dialysis of the detergent. Hydrophobic complexing (via dialysis) of the cysteine-containing lipopeptides to "proteosomes" (meningococcal outer membrane proteins) made even single-epitope

peptides highly immunogenic and increased the immunogenicity of replicate-epitope peptides. Since proteosomes have been safely administered to people, these methods may serve as models for designing immunogenic synthetic peptide vaccines for human use.

INTERNAL MEDICINE

The Effects of Intravenous Fat Emulsion on Acute Experimental Pancreatitis

Jacob Hakimian

Dr. S. Levenson

Department of Surgery, Albert Einstein College of Medicine, New York

There is controversy in the literature about the effects of intravenous fat emulsions on acute pancreatitis. The present experiment is designed to help clarify this. Acute Pancreatitis was induced in rats by the injection of 4% sodium taurocholate into the common bile duct of anesthetized (i.p. sodium phenobarbital) young adult male sprague-dawley rats which weighed about 375 - 420g. Following this procedure, all rats were fed by continuous intravenous infusion. The rats were divided into two group, groups A and B. The diet of both groups was isocaloric, isonitrogenous, and included equal amounts of vitamins, minerals, and trace elements. Group A received 20% dextrose for its non- amino acid calories and group B received 16% dextrose plus 20% Liposyn (fat emulsion) for its non-amino acid calories. The fat emulsion was given over a period of two hours each day. A comparison of two seemingly healthy rats, sacrificed by phenobarbital overdosage on day 3 after taurocholate injection, showed lower concentration of peri-pancreatic fat necrosis as well as pancreatic malondialdehyde (10% less) in the group B rat. The malondialdehyde concentration is an index of the degree of tissue inflammation. In two very sick (premorbid) rats sacrificed by phenobarbital overdosage one day after taurocholate injection, the pancreas of the animal which received only glucose as the source of non-amino acid calories was more hemorrhagic on gross

examination than the one which received glucose and fat emulsion, but the pancreatic malondialdehyde concentration of the latter rat was slightly (5%) greater. More experiments are needed to provide the basis for a clear conclusion.

Effects of Splenectomy on Wound Healing

Jonathan David Lewin and Chaim Abittan

Dr. Stanley Levenson

Albert Einstein College of Medicine, New York

The general effects of splenic removal upon wound healing is a matter of dubious contention. It was our desire to clarify the matter in a clinical, systematic approach. Twenty rats (weight 500-800 grams) were divided into two groups. Group A rats were splenectomized, and underwent a colon anastomosis, and a 7 cm dorsal incision. Group B rats were operated on in an identical manner but were not splenectomized. Both groups were fed a standard rat chow for a period of 6 postoperative days. After the six postoperative days, the seventeen survivors were overdosed with ether. Studies were made on the breaking strength of the 7 cm dorsal incision and the 5 cm midline laparotomy incision of both groups. Additionally, the colon bursting strength of the anastomotic sites were calculated for the two groups. Although further research is necessary, there appears to be no significant statistical difference in the breaking strength of the 5 cm midline incision, the 7 cm dorsal incision or the colon bursting strength of the splenectomized and unsplenectomized rats.

MICROBIOLOGY

Studies in Leishmania

Sara Weiss

Dr. E. Rowton

Walter Reed Army Institute of Research

Introduction:

Leishmania, a member of the trypanosomatid protozoa, is closely related to the trypanosomes in the evolutionary hierarchy and may have evolved from the lower insect trypanosomatids. Human Leishmaniasis is caused by *Leishmania* and is in most cases a zoonosis disease, transferred from animal to man. Recent investigation, although limited, has revealed that the complexity of the disease is far greater than previously thought. In fact, the World Health Organization (WHO) considers Leishmaniasis to be among the six most important infectious diseases worldwide.

Cultivation of *Leishmania*:

Three types of culture media were tested and compared for their ability to grow three species of *Leishmania*. No single media worked best for all species. *Leishmania mexicana*(λ) grew well in all of the media. *Leishmania major* showed appreciable growth in Schneider's Drosophila media, but RPMI 1640 and fortified MMI were also acceptable. *Leishmania braziliensis panamaensis* grew only in RPMI 1640.

Cloning of *Leishmania* on Agar Plates:

It was hypothesized that *Leishmania major*, which was randomly selected for this experiment, would indeed clone and form colonies on the agar plates. If this were true, then it would have also been possible for the colonies to be transferred onto sheets of nitrocellulose and identified with monoclonal antibodies in a DOT

ELISA test. The purpose of the investigation was to test both of these hypotheses.

It was found that unlike *Leishmania mexicana*, the *Leishmania major* parasites that were spread at three different concentrations did not grow on the blood agar plates. Three possible explanations may account for this. It is likely that *Leishmania major*, which is known to reproduce at a slower rate then *Leishmania mexicana*, merely needs more time to form visible colonies. For this reason, *Leishmania major* will continue to be observed at Walter Reed. It is also likely that *Leishmania major* requires unique nutrients which the blood agar fails to provide. Various nutrients should be experimented with to determine the optimum blood agar mixture for *Leishmania major*. Lastly, it is possible that *Leishmania major* simply will not form colonies on a blood agar surface at all. This hypothesis can be verified only by additional research in the field.

Since *Leishmania major* did not form colonies on the blood agar surface, no identification was made employing the DOT ELISA test. However, this means of identifying the *Leishmania* still appears to be worthy of experimentation.

MOLECULAR BIOLOGY

Studies in Osteogenesis Imperfecta

Marc Weber

Dr. Francesco Ramirez and Dr. Marina D'Alessio

Department of Microbiology and Immunology S.U.N.Y.
Downstate Medical Center

Intercellular connective tissue, such as bone, is primarily composed of a fibrous protein called collagen. At the very heart of collagen diseases, such as Osteogenesis Imperfecta (OI), lies a genetic mutation. OI has been found to cause bone fragility, short stature, and joint laxity, the extent of which depends on the OI type. We were concerned with pinpointing the mutation in one of the non-lethal types in which an altered Type I collagen had been observed.

The human Type I collagen consists of three amino acidic chains: two $\alpha 1$ and one $\alpha 2$. In this case, the mutation has been mapped in the $\alpha 1$ chain. Based on its migration in electrophoresis, one of the fragments (CB8) obtained by chemical reaction, containing amino acids 124-402 was determined to contain the mutation. Cysteine, not normally found in the $\alpha 1$ chains, was found to be present. It was then necessary to discover which amino acid was being replaced by cysteine. Glycine seemed to be an unlikely candidate because the chain follows the general pattern of gly-x-y triplets. Since the mutation is not lethal, and every third amino acid is glycine, it is far too important to be replaced and not to have lethal results.

The mutation found in the protein has a corresponding locus in the gene that codes for the $\alpha 1$ chains between exons 14 and 25. mRNA, extracted from diseased fibroblasts, is used to synthesize the

complementary DNA (cDNA). A primer attached to the mRNA loops around it to produce the cDNA thus removing unnecessary introns.

The cDNA was subcloned in a vector (phage- λgt10) to create an entire library of that specific cDNA. The library was then hybridized to a nitrocellulose filter thereby yielding a "photocopy" of the petri dish. The filter was then screened with a probe to locate the regions which incorporated the cDNA insert. These regions appear positive under autoradiography and their complementary regions can be isolated in the dish. Each positive region was removed and the cDNA was extracted from the phages using chemical techniques and centrifugation. Following this, the cDNA was "cut" using restriction enzymes to free the fragment needed. The cDNA fragment was then subcloned in a plasmid vector for sequencing, and the sequence was compared to the sequence for the normal $\alpha 1$ (I) gene.

After a number of sequencing reactions, results have been inconclusive.

Studies in Plasmid Replication Rate

Gabriel Sosne

Dr. Gordon Churchward

Emory University School of Medicine, Department of Microbiology and Immunology

Bacteriophage of the lambda strain ϕ716 and ϕ716UV5 were utilized to infect a bacterial strain, EQ84. The plasmid's replication rate was measured through the use of hybridization techniques, as well as a ^{32}P probe. This probe was developed using X-rays.

Since ϕ716 contains a DNA sequence quite homologous to another plasmid under observation in the laboratory, it was used for

transfection. It was postulated that a progressive pattern of plasmid replication would be detected after intervals of 5,10, and 20 minutes and one hour after infection, and that their rates could be measured using the aforementioned methods.

Unfortunately, the replication rate proved to be too slow for the purposes of the laboratory. However, this negative result was actually a positive force in encouraging the chief researcher to pursue his experiments relating to this plasmid.

NEUROLOGY

Investigation into the Mechanisms of Opioid Effects on Dopamine Synthesis in Striatum

Miriam Berger

Dr. M. Makman

Albert Einstein College of Medicine, New York,
Dept. of Biochemistry

Opioids have been found to inhibit the synthesis and release of dopamine in neuronal tissue; however, the mechanisms by which this inhibition occurs are still unknown. Two hypothesis are: 1) Opiods inhibit the release of dopamine thereby initiating a buildup of dopamine in the neuron. A feedback mechanism would then inhibit the action of tyrosine hydroxylase, thereby curtailing the production of dopamine. 2) Opioids inhibit adenylate cyclase by interacting with inhibitory G proteins through receptors on the cell membrane. This hypothesis can be tested through the use of Forskolin, a cyclase stimulator which stimulates dopamine production. An opioid's inhibition of forskolin's effect on synthesis, would support the adenylate cyclase hypothesis. This would be corroborated by the opioid having no effect on stimulation of production of dopamine by cyclic-AMP analogues, which is not dependent on adenylate cyclase.

The inhibitory opioid effect has been found using agents which bind to Kappa receptors. To determine if the same effect is found by agents binding to Delta or Mu receptors, D-pen-D-pen enkephalin and 'DAGO' (Tp-D-ala-gly-N-me-phe-gly-op) were used. Striatum was removed from rat brain, and a synaptosomal preparation was made. Different samples of tissue were treated with different agents, and then 3H-tyrosine (8 m) was added. In some samples, synthesis was stimulated by addition of KCl. The medium which included the

released dopamine was separated from the synaptosomes themselves. These samples, in addition to the samples containing the intracellular fluid, were applied to ion exchange columns. This method allowed simultaneous testing of synthesis and release. After eluting the tyrosine remaining in the samples, the dopamine was eluted with 4N HCl. The fractions were then collected and counted. Assays were done with 1 µm D-pen (final) and 3 µm D-pen (final) and the results showed no significant effect on the release or synthesis of dopamine. In a preliminary assay, DAGO also did not show a significant effect on dopamine synthesis or release. These results lead to the conclusion that further research into the method of opioid inhibition should be continued using those opiods that interact with Kappa receptors.

Stroke After Transient Ischemic Attack

Haim Brandspiegel

Milton Alter, Sue Min Lai, Wendy VanBrakle,
Gary Friday, et. al.

Department of Neurology, Philadelphia, PA, and
the Lehigh Valley Stroke Program.

A register of TIA and stroke was established in the Lehigh Valley in 1982. Patients admitted to any hospital in the region are registered and their diagnosis is verified. By June 1986, 889 admissions for confirmed TIA were registered giving an average annual incidence of 38.3 / 10^5 population. This incidence is higher than reported from the population-based Mayo Clinic study, providing assurance of relatively complete case ascertainment. Among the 889, 41 patients were subsequently hospitalized for stroke. Of these, 34% occurred within the first month, 17% in 1-6 months, 22% in 7-12 months and 12% in 13-18 months. The longest

interval between TIA and stroke in the 41 cases was 43 months. Neither gender nor age affected the risk of stroke after TIA. Clearly, the first month after TIA is associated with increased risk of stroke. Study of those who develop a stroke early after TIA may yield clues which will aid prevention.

ONCOLOGY

Isolation and Mechanism of Action of a Serum Inhibitor to a Tumorolytic Factor, ATF.

A.J. Burdowski, E.Bartfeld, M.Scanlan, H.S. Grob, F.Friedman, D. Jaffe

Stern College for Women

Murine Autotumorolytic factor (ATF), a serum derived protein, induces the breakdown of mammary adenocarcinoma both *in vivo* and *in vitro*. A serum fraction has been identified which inhibits the tumorolytic activity of ATF, when incubated with ATF prior to injection into tumor bearing mice.

This inhibitor was prepared from serum of tumor bearing C3H (t/f) mice. The serum was diluted with 0.05 Na-K phosphate buffer (pH 7.5) and centrifuged at 6500 x g (4 min), then at 20,000 x g (5 min). The precipitate was resuspended and applied onto a DEAE-Sephacel column. Material was eluted with a phosphate buffer gradient. Activity was determined by incubation of the peaks with ATF (30 min at 37°C), and s.q. injection into tumor bearing mice. Active samples displayed the ability to inhibit tumorolysis by ATF. The active peak was applied onto a Sephacryl-SF-200 column, with the resultant peak applied onto a chromatofocusing column (PBE 94) and eluted with a Polybuffer 74. The active fraction eluted at pH 5.56. Electrophoresis was carried out with a Phast system utilizing a gradient gel of 10-15%, and silver staining revealed one band with a molecular weight of 15,000-20,000 d.

Enriched populations of splenic lymphocytes derived from tumor bearing mice were obtained, cultured in RPMI 1640, and treated with ATF for 4 hrs. Cells were harvested, the media was collected, then added into isolated mammary carcinoma cell cultures and incubated for 24 hrs. ATF treated lymphocyte media lysed the

mammary carcinoma cells, while the isolated inhibitor prevented this lysis. The Cytotoxicity assay was based on crystal violet incorporation.

Mechanisms and Effects of Protein Induction by TNF and Interferon

Yochanan Burnstein

Dr. B. Y. Rubin

Blood Derivatives Program of the New York Blood Center

Interferon, one of the body's defenses against foreign microorganisms, stimulates cells to halt the replication of viruses. Studies have also shown that interferon (IFN) may be effective against certain forms of cancer. While IFN is currently being administered in clinical trials, the mechanisms by which it mediates its antitumoral effect is not yet fully understood.

Fibroblasts cells grown in vitro and treated with the IFNs have been found to produce several new protiens with molecular weights of 42, 56, 67, and 80 kd. Certain forms of IFN have been found to be more efficient than others in the induction of the synthesis of these proteins; IFN-α induces the 56, 67, and 80 kd proteins, while IFN-γ induces 42, 56, and 67 kd proteins. These same proteins have also been found in cells treated with Tumor Necrosis Factor (TNF), a product of activated macrophages that has anti-tumor activity. These proteins and their role in the observed effects of IFN and TNF are currently being studied by our laboratory.

A primary method of studying these proteins is SDS-PAGE (Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophoresis), whereby they are separated based on differences in their molecular weights. To obtain the protein extract of IFN or TNF treated cells,

cells were labeled with ^{35}S-methionine in the presence of either IFN or TNF and then lysed. Immunoprecipitations (in which the extracts were incubated with antibodies specific for desired proteins) were then carried out to precipitate the proteins to be studied. These samples (containing the various precipitated proteins induced) were fractionated by SDS-PAGE and compared.

Several experiments of this type were carried out with various modifications in the original cell treatment. The following results were obtained:

(A) In experiments utilizing actinomycin D (an inhibitor of transcription) and cycloheximide (an inhibitor of translation) it was determined that:

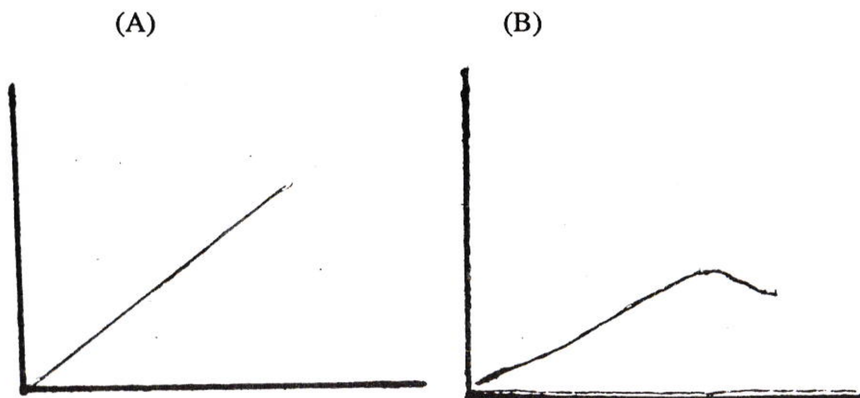
(1) The induction of the 56 and 67 kd protein in IFN-γ linked cells is a one step process.

(2) The induction of the 42 kd protein by INF-γ is a two step process requiring the synthesis of an intermediary protein which in turn induces the synthesis of the 42 kd protein.

(B) IFN-α treated FS_4 cells which were washed free of the IFN were found to continue to contain high levels of the 56 and 67 kd proteins even 72 hours after the removal of the IFN. However the amount of the 80 kd protein present in the cells decreased over time. The rate of decay of the 80 kd protein parallels the rate of decay of the anti-viral in this cell line, indicating that the 80 kd protein may play an important role in the IFN mediated anti-viral effect.

(C) Induction of the 67 kd protein following IFN treatment was found to continue over time in one cell line (DAUDI) (Panel A), while in another cell line (GM2767) it was found that the synthesis of this protein occurs for several hours after which production slows down (Panel B). This observation indicates that the mechanism of induction of the 67 kd protein differs in different cell lines.

(Diagrams on next page)



A comparison of the 67 kd proteins synthesized (Y axis) over time (X axis) for IFN treated DAUDI (A) and GM2767 (B) cell lines.

Induction of Lymphocyte Humoral Cytotoxicity by an Autotumorolytic Factor (ATF)

M. Scanlan, F. Friedman, A.Burdowski, and H. S. Grob

Adelphi University and Stern College for Women

An autotumorolytic factor (ATF) derived from the sera of spontaneous mammary tumor bearing C3H (T/F) mice has been shown to induce quantitative lysis of primary mammary carcinoma cells, *in vitro*, in crystal violet uptake assays. Lymphocyte enriched cultures obtained from tumor bearing C3H mice were exposed to ATF for 1,3,6,12,18,24, or 48 hours. ATF, ATF-primed lymphocytes or ATF-primed lymphocyte culture supernatants were added to the primary mammary tumor cell cultures maintained in 96-well trays. Following a 24 hour incubation, tumor cells were stained with 0.1% crystal violet and then lysed with 0.5% SDS. Tumor cell lysis was indicated by A_{590} measurements of SDS cell lysates that were 50%

of those found in corresponding control cultures. Tumor cells exposed to ATF directly showed little lysis. Cytotoxic activity was evident in tumor cell cultures exposed to 1,3,6,12, and 18 hour ATF-primed lymphocyte culture supernatants. Furthermore, *in vitro* data was substantiated *in vivo* as indicated by lysis of spontaneous mammary carcinoma following subcutaneous injection of ATF-primed lymphocyte culture supernatants into tumor bearing hosts.

Induction of Differentiation of Colon Cancer Cells

Alexander Losef

Chief Researcher: Dr. Augenlicht

Department of Oncology, Montefiore Medical Center, Bronx, New York.

Mucinous colon tumors which exhibit a principle hallmark of colonic epithelial cell differentiation, as well as the production of mucin, and which in a biological sense (but not pathological) can be considered very well differentiated, have a poorer prognosis than the majority of colon tumors. It is one of Dr. Augenlicht's goals to determine whether objective criteria, such as changes in expression or structure of genes associated with mucin production / secretion and goblet cell differentiation are more useful in distinguishing mucinous tumors with a poor prognosis from those which do not differ from non-mucinous colon carcinoma.

The overall experimental approach is as follows: to use nucleic acid probes associated with colon cell differentiation, specifically mucin secretion; to investigate the expression and structure of genes in mucinous tumors of defined phenotype; and further to correlate the results with prognosis for the patient. The probes are from three sources:

(1) The human c-k_1-ras gene, whose expression is linked to

the lineage of mucin secretion in the colon and mutations which characterize many colon tumors.

(2) Cloned sequences whose relative levels of expression characterize the lineage of goblet cell differentiation and mucin secretion, including probes, if isolated, for the mucin peptide backbone.

(3) A panel of 8 cloned sequences whose expression is linked in a complementary way to butyrate induced colon cell differentiation *in vitro* and transformation in human colonic biopsy material.

Plans will be laid for the further analysis of the results in light of the heterogeneity likely to be present in all human material, and the nature of the sequences involved and mechanisms by which they are in tumor development or progression.

PHYSIOLOGY

Microcomputer Data Acquisition and Data Analysis in an Undergraduate Physiology Lab.

A.J. Burdowski, M. Berger, Z. Farkas, L. Bartfeld, L. Brandwein

Stern College for Women

The goal of this project involved the updating and the modernization of our undergraduate physiology laboratory. Our students now acquire data in real time, see the data on the screen and then analyze the data with various analysis programs.

IBM PS/2 model 30 microcomputers were utilized along with Keithly system 570 data acquisition systems. The software for the data acquisition and analysis was LabTech notebook and Lotus 123. Various physiological transducers were employed (Narco Biosystems). Students were divided into groups (2 students) and 2-3 student groups utilized one computer. Each group had its own transducers, amplifiers and input couplers. The computer screen was divided into regions, one region per group. Acquired data was analyzed and printed utilizing Lotus 123. Students were able to record data in muscle physiology experiments (isotonic, isometric, and smooth muscle contractions), EEG, ECG, respiration, Galvanic skin resistance, as well as the determination of the speed of conduction in the sciatic nerve. We also use various simulation and modeling programs in the course to enhance the visualization of the biological process. This approach to the physiological labs has reduced the frustration of the students in the labs. We are expanding the use of these systems to enhance the teaching of our general biology, animal behavior and psychobiology courses.

Molecular Studies of Alcoholic Liver Disease

Robert Wind

Mark Zern, Francis Weiner, Mark Czaja,
Marie-Adele Giambrone

Albert Einstein College of Medicine,
Deptartment of Medicine

The liver, probably the most multi-functional and complex organ of the body, is composed of a variety of cells organized in a network of extracellular matrix. Several factors play a role in maintaining the proper equilibrium of matrix deposition and remodeling.

Fibroblasts are ubiquitous mesenchymal cells; they synthesize collagen and other matrix molecules for the scaffolding of connective tissue, are important in wound repair, and contribute abundant connective tissue proteins to areas of chronic inflammation. In certain diseases, such as hepatic cirrhosis, they oversynthesize the extracellular matrix components, thus becoming detrimental to the host. One of the objectives of our work was to examine hepatic cirrhosis and the molecular mechanisms responsible for increased collagen content in the fibrotic liver.

We hypothesized that there are several endogenous effector molecules that normally enhance the deposition of the liver matrix. For example the cytokines (molecules secreted by phagocytic mononuclear cells): TNF- Tumor Necrosis Factor, TGF-β- Transforming Growth Factor-β, and IL-1- Interleukin-1. During hepatic injury from any number of causes, including alcohol ingestion, there is an overabundance of the fibrogenic factors which result in both fibrosis and cirrhosis.

To describe the role of the cytokines in hepatic fibrogenesis, we performed Northern and dot blot hybridization analyses of hepatic RNA. By these techniques we probed denatured RNA with various protein DNA sequences to determine

whether TNF, TGF-β, and/or IL-1 mRNA is increased in the livers of carbon tetrachloride-treated rats (and occasionally ethanol and acetaldehyde) or in the livers of cirrhotic and non-cirrhotic patients. We additionally sought to determine if there was any significant change in the steady state mRNA levels of β-actin, albumin, or types I or IV procollagen mRNA levels in these hepatocytes.

Various animal and human sample hepatocytes pre-treated with various fibrogenic factors were subjected to Northern and dot blot analysis. These samples were probed with a wide range of cytokines and cellular proteins that both inhibit and enhance the deposition of liver matrix in different liver types. The levels of different types of procollagen mRNA were then evaluated to determine the effect of these cytokines and other stimulatory factors on the proliferation of fibrosis and cirrhosis. Hepatocytes treated with TGF-β showed an increase of greater than thirteen-fold in type I procolagen mRNA levels. No significant change in the steady state mRNA levels of β-actin or albumin in these hepatocytes was observed, but more work is necessary. Both TNF and IL-1 were found to have similar effects *in vivo* and *in vitro*.

Using this working model for the pathogenesis of hepatic fibrosis, we will be able to explore potential therapeutic agents.

PSYCHOLOGY

The Influence of Self-Selected Tempo Upon The Performance of Eye-Hand Coordination Tasks Among Down's Syndrome Children

Rena Hartstein

Various physiological functions serve as time keepers for the body, finding expression in a person's performance of daily routines. Recent research has established that an individual's internal functions manifest themselves in visible rhythmic patterns. These patterns are especially present among Down's Syndrome children, a group thought by many scholars to possess an unusual music ability. Absent from literature is the study of the effect of adjusting tempo to create a mood which is conducive to a group mastering new tasks. This study, developed in conjunction with professionals from two universities and fifteen institutions, examines the relationship between self-selected tempo and performance of eye-hand coordination tasks among Down's Syndrome children.

Subjects from one group listened to their self-selected tempo of the song, "Row Your Boat" while performing nine gradated tasks. The second group performed the same tasks while listening to a standard variation of the song. No group performed the tasks without music since research has already established that music serves as a reinforcement during the performance of work tasks.

Results indicated a significant difference in the rate, degree, and quality of task performance. The individualized music not only compensated for the subject's intellectual disadvantage but enabled the child to have a higher degree of performance than the members of the higher I.Q. control group. This study as a whole, indicates the tremendous impact that a self-selected tempo can have on the performance of eye-hand coordination tasks among Down's Syndrome children.

RHEUMATOLOGY

The Role of a Histadine, Cystine, and Copper Complex in the Pathogenesis of Rheumatoid Arthritis

Murray Fuksbrumer

Dr.D.A. Gerber, Assoc. Prof. Med.

State University of New York Health Science Center at Brooklyn, New York

It is widely believed that the inflammation of joints associated with rheumatoid arthritis is a response to IgG which has denatured as a result of some antigenic challenge, such as a virus. However, an alternative biochemical hypothesis proposes that the manifestation of denatured IgG in a pseudoimmune complex is a response to a sulfhydryl-disulfide interchange reaction. Such a reaction would result from the presence of certain enhancers or the absence of certain inhibitors or both. One possible endogenous inhibitor is a complex of histidine, cystine, and copper.

The role of a mixture of histidine, cystine, and copper in the sulfhydryl-dependent aggregation of human IgG was tested as follows: A solution of IgG (3.3 mg IgG, 9.9 ml NaCl, 0.1 ml pH 7.4 0.1 M phosphate buffer) and of histidine, cystine, and copper (30 ml H_2O, 10.2mg cystine, 18.6 mg histidine, .075 ml 40 mM $CuSO_4$) were prepared. The IgG solution (1.2 ml) and the histidine, cystine, and copper solution (.025 ml) were then mixed together to form a solution which was placed in a shaking apparatus (rate=174 complete cycles / minute, amplitude=7.6 cm, temp=6.6°C). Concurrently, a solution of IgG and saline, in the same proportions as the aforementioned solution, was placed in the shaking device. After shaking for approximately 21 hours, the solutions were

removed from the apparatus and a blind test was applied to the counting of particles in each solution on a hemacytometer counter chamber.

Particles were classified based on size. Since each particle was an aggregate of IgG, the greater the size of a particular particle, the greater the aggregation of IgG. In order for this experiment to suggest that a lack of an inhibitor comprised of histidine, cystine, and copper leads to IgG denaturation as manifested by protein aggregation, it would have to be found that significantly greater aggregation occurs in the complex-deficient solution than in the complex-containing solution.

As shown in the accompanying tables (tables 1-2), significantly greater amounts of medium, large, and extra-large particles were present in the complex deficient solution than in the complex-containing solution. Such results support the contention that the absence of an inhibitor comprised of histidine, cystine, and copper permits the pseudoimmune denaturation of IgG and the subsequent inflammation of joints characteristic of rheumatoid arthritis.

Currently, histidine, cystine, and copper are being tested separately in experiments similar to the one described above in order to establish that the complex, and not an individual component of the complex, expresses inhibitory activity. As of yet, no significant differences in particle sizes have been found between a copper-deficient solution and a copper-containing solution (tables A-B)

(see tables on next page)

Results :

Table 1

Number of Particles in Each Size Classification

Rx	Date	XS	S	M	L	XL	Tot	1x1	mlx1	lel/T	mlel/Te(%)
TC	23-Jul	378	0	0	0	0	378	0	0	0.00	0.00
NaCl		806	4	1	3	0	814	3	4	0.37	0.49
Diff		-428	-4	-1	-3	0	-436	-3	-4	-0.37	-0.49
TC	28-Jul	60	7	0	0	0	67	0	0	0.00	0.00
NaCl		210	21	2	0	0	233	0	2	0.00	0.86
Diff		-150	-14	-2	0	0	-166	0	-2	0.00	-0.86
TC	29-Jul	65	4	2	0	0	71	0	2	0.00	2.82
NaCl		46	4	3	0	0	53	0	3	0.00	5.66
Diff		19	0	-1	0	0	18	0	-1	0.00	-2.84
TC	30-Jul	275	12	2	0	0	289	0	2	0.00	0.69
NaCl		98	13	4	0	1	116	1	5	0.86	4.31
Diff		177	-1	-2	0	-1	173	-1	-3	-0.86	-3.62
TC	04-Aug	400	5	2	0	0	407	0	2	0.00	0.49
NaCl		213	5	2	1	0	221	1	3	0.45	1.36
Diff		187	0	0	-1	0	186	-1	-1	-0.45	-0.87
TC	05-Aug	123	11	5	0	2	141	2	7	1.42	4.96
NaCl		267	6	2	4	0	279	4	6	1.43	2.15
Diff		-144	5	3	-4	2	-138	-2	1	-0.02	2.81
TC	06-Aug	105	4	0	0	0	109	0	0	0.00	0.00
NaCl		340	6	3	1	0	350	1	4	0.29	1.14
Diff		-235	-2	-3	-1	0	-241	-1	-4	-0.29	-1.14
TC	11-Aug	448	24	2	0	0	474	0	2	0.00	0.42
NaCl		1470	9	4	2	1	1486	3	7	0.20	0.47
Diff		-1022	15	-2	-2	-1	-1012	-3	-5	-0.20	-0.05
TC	25-Aug	266	10	2	0	0	278	0	2	0.00	0.72
NaCl		70	10	8	1	0	89	1	9	1.12	10.11
Diff		196	0	-6	-1	0	189	-1	-7	-1.12	-9.39

Date= date of counting
TC= solution containing a complex of histidine, cystine, and copper
NaCl= complex-deficient solution (control)
XS= extra-small, S= small,
M= medium, L= large,
XL= extra-large, Tot= total

Table B

Statistical Analysis of Results Shown in Table A

30-Aug L8 Fuksbrumer   Copper

	n	Avg	SE	%inhib / t
Extra-small				
Cu	6	257.67	45.09	12.46
NaCl	6	294.33	85.64	0.38
Diff	6	-36.67	76.38	-0.48
Small				
Cu		18.83	3.66	-8.65
NaCl		17.33	6.72	-0.20
Diff		1.50	5.21	0.29
Medium				
Cu		2.00	0.63	-20.00
NaCl		1.67	0.49	-0.42
Diff		0.33	0.67	0.50
Large				
Cu		0.00	0.00	100.00
NaCl		0.33	0.21	1.58
Diff		-0.33	0.21	-1.58
Extra-large				
Cu		0.00	0.00	ERR
NaCl		0.00	0.00	ERR
Diff		0.00	0.00	ERR
Total				
Cu		278.50	44.36	11.21
NaCl		313.67	85.00	0.37
Diff		-35.17	78.80	-0.45
l+el				
Cu		0.00	0.00	100.00
NaCl		0.33	0.21	1.58
Diff		-0.33	0.21	-1.58
m+l+el				
Cu		2.00	0.63	0.00
NaCl		2.00	0.52	0.00
Diff		0.00	0.58	0.00
l+el/total				
Cu		0.00	0.00	100.00
NaCl		0.12	0.09	1.29
Diff		-0.12	0.09	-1.29
m+l+el/total				
Cu		0.79	0.29	22.65
NaCl		1.03	0.36	0.50
Diff		-0.23	0.39	-0.59

n= number of trials
Avg= average number of particles found for that particular size classification and solution
SE= standard error
t= measure of statistical significance

Table A

Number of Particles in Each Size Classification

Rx	Date	XS	S	M	L	XL	Tot	1x1	mlx1	lel/T	mlel/Te(%)
Cu	12-Aug	422	13	3	0	0	438	0	3	0.00	0.68
NaCl		672	1	0	1	0	674	1	1	0.15	0.15
Diff		-250	12	3	-1	0	-236	-1	2	-0.15	0.54
Cu	13-Aug	321	15	3	0	0	339	0	3	0.00	0.88
NaCl		168	5	3	1	0	177	1	4	0.56	2.26
Diff		153	10	0	-1	0	162	-1	-1	-0.56	-1.37
Cu	18-Aug	155	6	1	0	0	162	0	1	0.00	0.62
NaCl		257	17	1	0	0	275	0	1	0.00	0.36
Diff		-102	-11	0	0	0	-113	0	0	0.00	0.25
Cu	19-Aug	160	27	4	0	0	191	0	4	0.00	2.09
NaCl		159	25	3	0	0	187	0	3	0.00	1.60
Diff		1	2	1	0	0	4	0	1	0.00	0.49
Cu	20-Aug	175	29	1	0	0	205	0	1	0.00	0.49
NaCl		394	46	1	0	0	441	0	1	0.00	0.23
Diff		-219	-17	0	0	0	-236	0	0	0.00	0.26
Cu	28-Aug	313	23	0	0	0	336	0	0	0.00	0.00
NaCl		116	10	2	0	0	128	0	2	0.00	1.56
Diff		197	13	-2	0	0	208	0	-2	0.00	-1.56

Date= date of counting
Cu= copper-containing solution
NaCl= copper-deficient solution (control)
XS= extra-small, S= small,
M= medium, L= large,
XL= extra-large, Tot= total

Table 2

Statistical Significance Between Particles Found in TC and NaCl (based on size and number of particles)

25-Aug L8 Fuksbrumer   H-Cy-Cu

	n	Avg	SE	%inhib / t
Extra-small				
TC	9	235.56	50.63	39.77
NaCl	9	391.11	154.87	0.95
Diff	9	-155.56	129.82	-1.20
Small				
TC		8.56	2.32	1.28
NaCl		8.67	1.84	0.04
Diff		-0.11	2.55	-0.04
Medium				
TC		1.67	0.53	48.28
NaCl		3.22	0.68	1.80
Diff		-1.56	0.80	-1.94
Large				
TC		0.00	0.00	100.00
NaCl		1.33	0.47	2.83
Diff		-1.33	0.47	-2.83
Extra-large				
TC		0.22	0.22	0.00
NaCl		0.22	0.15	0.00
Diff		0.00	0.29	0.00
Total				
TC		246.00	51.46	39.19
NaCl		404.56	154.81	0.97
Diff		-158.56	128.73	-1.23
l+el				
TC		0.22	0.22	85.71
NaCl		1.56	0.47	2.54
Diff		-1.33	0.37	-3.58
m+l+el				
TC		1.89	0.72	60.47
NaCl		4.78	0.74	2.80
Diff		-2.89	0.81	-3.58
l+el/total				
TC		0.16	0.16	70.00
NaCl		0.53	0.17	1.59
Diff		-0.37	0.13	-2.79
m+l+el/total				
TC		1.12	0.56	61.94
NaCl		2.95	1.08	1.51
Diff		-1.83	1.12	-1.63

n= number of trials
Avg= average number of particles found for that particular size classification and solution
SE= standard error
t= measure of statistical significance

SURGERY

The Effects of Choline and DMEA (Dimethylethanolamine) on the Toxicity of Lithium

Tamara Carmel, Renee Friedman, Jonah Wieder

Eli Seifter, Ph.D.

Albert Einstein College of Medicine, Bronx, New York
Department of Surgery

The use of lithium compounds in psychotherapy for ailments such as bipolar manic depression in human patients has brought about undesirable side-effects of unknown etiology.

The use of lithium in psychotherapy for acute bipolar disorders such as manic depression often causes harmful side-effects such as renal disease. In severe cases, diabetes insipidus results, a condition in which the patient is easily dehydrated. Lithium causes this by blocking ADH (antidiuretic hormone) receptors in the distal convoluted tubule of the kidney. The resulting unresponsiveness to ADH causes overproduction of dilute urine which in turn leads to dehydration.

The risks involved in lithium treatment are enhanced by the fact that the drug's effective dosage is very close to toxicity levels (0.41 - 0.89 mmole/liter). Thus, in some individuals, toxicity is approached.

The purpose of our experiment is twofold: It is firstly to study what effects, if any, choline and/or DMEA have on the toxicity of lithium, and secondly to determine whether or not choline and DMEA can be used as a substitute therapy for lithium.

Methods

Groups of white healthy female Fischer rats were maintained on nutritionally different diets. The animals weighing approximately 338 grams were kept individually in mesh steel cages at room temperature and were fed every other day. The fifty-six three month old rats were divided into eight groups of seven. All of the rats in a given group were fed the same diet so eight diets were required for the eight groups. Each diet had a base of nutritionally complete commercial rat chow and contained 10 g/Kg dextrin with a designated supplemental ingredient. The control group received chow and 10 g/Kg Dextrin, group 2 received control and lithium carbonate (2g/Kg), group 3 received control and choline chloride (3g/Kg), group 4 received control, lithium carbonate (2g/Kg) and choline chloride (3g/Kg), group 5 received control and DMEA (.5g/Kg), group 6 received control, DMEA (.5g/Kg) and lithium carbonate, group 7 received control, DMEA (.5g/Kg), lithium carbonate (2g/Kg) and choline chloride (3g/Kg) and group 8 received control, DMEA (.5g/Kg) and choline chloride (3g/Kg).

The toxicity was measured primarily by loss of body weight. The rats were weighed every other day. The results of these experiments as of the time of printing are unavailable.

Although the method of lithium administration may be important (Plenge et al showed that fluctuations in levels caused less damage than the relatively constant serum levels produced by oral feeding), the specific mechanism that accounts for the lithium response is not yet known. The reason that lithium therapy has been effective requires more carefully controlled clinical and biochemical studies.

The Effect of Dietary Supplements of Nucleotides on the Survival of Irradiated Mice

Tamara Carmel, Renee Friedman, Jonah Wieder

Eli Seifter Ph.D.

Albert Einstein College of Medicine, Bronx, New York.
Department of Surgery and Anesthesia

Radiation harms living organisms by interacting with nitrogenous bases within the chromosomes thus causing deformed production or sequence of proteins.

There is reason to believe that a dietary supplement of nucleotides of nitrogenous bases will prevent damage induced by radiation in any of three possible ways. By incorporating free nitrogenous bases into the animal's daily food they will act firstly as decoys so that the radiation would combine to the radicals of the free bases rather than to the bases in the DNA of the chromosomes and thus prevent genetic alterations. Secondly, the bases could combine with the free radicals that might be produced by the radiation to prevent them from causing cell damage. Thirdly, the bases will help supplement the body with bases which might be lost during cell deaths as a result of radiation. (These building blocks can then be used to build nucleic acids -- DNA and RNA. This is more efficient than having the cells manufacture the nucleic acids from scratch.)

The study was performed on 242 mice (male, CBA mice). Each cage contained 6 mice, and two cages constituted a group. Each mouse was individually labeperfomesled by magic marker color notations on its tail. Twenty-one groups were made and then every seven groups were divided into three sections. The first section was irradiated with 600 rads, the second with 700 rads and third with 800 rads, (^{137}Cs - γ). One week before radiation seven diets were prepared. Each diet was given to one of the three

groups respectively.

Diets consisted of the following: Control received chow and dextrin (10g/Kg), group 2 received control and D.L. methionine (2g/Kg), group 3 received control and uridine (1g/Kg), group 4 received control and adenosine (1g/Kg), group 5 received control and thymidine (1g/Kg), group 6 received control and guanosine (1g/Kg) and group 7 received control and cytidine (.1g/Kg).

As the study involves a 30 day experiment that is still in progress, results as of yet are inconclusive.

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