Allegations of a Coverup and Suppression of

Research Related to Cancer Theories.

Each section is a review only, and documentation will be provided to an ethics investigation etc.

1. How material received from the American Freedom of Information Service in 1998 requires PHS to re—investigate.

2. The Cancer Research Myth: the suppression of new ideas/theories to coverup embarrassment to PHS which was scammed by a university advancing private gain (not the PHS mandate).

3. The material sent to Mr. Nicholas Wade, Science Editor for the N. Y. Times.

4. The original early 1990 research proposals and typical replies for direct comparison to published research so to demonstrate that they were both advanced and correct.

1. Freedom of Information (FOI) Material and PHS Regulations

PHS must re-investigate because the FOI material proves that the University of Waterloo intentionally lied to PHS, and violated their guidelines etc. to promote J. C. M. Riley and receive NIH funding not possible by honest means. The PHS money in question is grant # NIH-HD-10718 with Dr. H. R. Behrman of Yale as sponsor/preceptor for J. C. M. Riley. This was an NIH cooperative program administrated through the Medical Research Council of Canada (MRC), and evidence exists to prove the misuse and violation of these guidelines. In short, the University of Waterloo developed a conspiracy to promote J. C. M. Riley beyond honest, ethical academic means.

POINT: if someone misuses their powers and intends to lie—— fabricate a degree, and suppress all contravening evidence, IS THAT NOT FRAUD? That the University of Waterloo fabricated J. C. M. Riley can be proven. Does J. C. M. Riley have any undergraduate degrees in biology or chemistry? Or is he just a C- general BSc. in physics? The NIH—MRC joint guidelines call for an excellent academic background. A C- minus in physics does not qualify for a MSc. program let alone a Ph.D.

Excerpts from F01 material of PHS forms dated 1990 to the University of Waterloo (UW) titled:

" Initial Assurances"

Regarding Procedures for Dealing with and Reporting Possible Misconduct in Science.

Each institution which receives or applies for a research,…,or cooperative agreement "(N.B. J. C. M. Riley and H. R. Behrman,NIH-HD-10718, and the paper work may have begun as early as 1986 or 87), " under PHS Act MUST submit an annual assurance certifying that the institution established administrative policies and that it will comply with those policies and the requirements of the Final Rule as published at 54 FR 32 & 46, Aug. 8, 1989.

Please note that E.A. Greenhalgh through various legal counsel continuously informed UW of scientific misconduct through 1987 to 1993 (plus other avenues).

Please note that when UW conspired to promote J.C.M. Riley irregardless of academic background and contravening experiments, then their ASSURANCES to the Final Rule (and the spirit of PHS' mandate preceding it) etc. were intentionally fraudulent. The evidence to prove intent to commit fraud etc. for the cooperative agreement program can be proven with documentation (at an investigation).

PHS is asked to call the ethics committee investigation because both countries federal police have agreed that a basis for a fraud investigation exists. Therefore, under PHS regulations an investigation must occur.

Further proof that UW lied Concerning Assurances and PHS Regulations

Citing PHS regulations provided by F0l under "An institution (must have):

"1. An IMPARTIAL process for receipt of allegations of scientific misconduct, and for initiating immediate inquiry into each allegation."

For J. C. M. Riley to receive his Ph.D. and NIH monies there could be no blatant flaws in his work exposing his very weak scientific background (i.e., lack of chemistry training so much so he couldn't even recognize oxidation). The Greenhalgh thesis exposed serious flaws in the Riley work , plus the departments' plus work being promoted by H. R. Behrman. The University of Waterloo suppressed these repeatable scientific experiments (contravening work) in the Greenhalgh thesis so the Riley Ph.D. would stand and he would receive (therefore) NIH monies to work with H. R. Behrman at Yale. E. A. Greenhalgh and legal counsel brought the issues before both UW and MRC. Did either report to PHS? The FOI material provides NO EVIDENCE that either did. Therefore, UW (intentionally) violated PHS guidelines with the suppression. Suppression is proof of their intent to commit fraud: i.e., receive PHS monies under false assurances.

Please note PHS—NIH's mandate, pre-dating 1990 - demands truthfulness and adherence to the principles of the NIH mandate. And later under Dr. Bivens, circa 1995 the PHS regulations are considered to this case, though not properly applied-i.e., Dr. Bivens avoids the issue of fabrication and plagiarism as it applied to Riley and Behrman. The spirit of PHS regulations is the issue, not the red herring of dates.

Please note, as requested by UW, E. A. Greenhalgh brought an IMPARTIAL independent report by a Dr. G. L. Nicolson of the M. D. Anderson Cancer Center before UW. However, because Dr. Nicolson's findings supported the Greenhalgh thesis and findings (thereby invalidating the Riley Ph.D.), the University of Waterloo WITHDREW the offer.

POINT: E. A. Greenhalgh was not being funded by PHS. THE PHS MONIES in question are the scholarship monies for J.C.M. Riley to work with H.R. Behrman (circa 1989-90) at Yale. Therefore, any assurances UW made to PHS were deliberately and intentionally false (fraudulent) since they were not truthfully meeting the PHS guidelines. UW did not have an IMPARTIAL process in place as compliance demanded because had they, then PHS monies would have been safeguarded.

Please note the same FOI—PHS material states:

"Notifying the OSI of the final outcome of the investigation with a written report that thoroughly documents the investigation process and findings."

The F01 material contains NO such report. Did UW turn Dr. Nicolson's findings over to PHS? NO! Had they done so, then J.C.M.Riley could not have received PHS monies. This was a deliberate act of concealment leading to fraud. Since UW intended to lie to PHS for monies, would conspirators turn over findings that would expose their conspiracy? Critical of their scholarship student, J.C.M.Riley? NO!!!

Once again from FOI-PHS regulations,

"Selection of impartial experts to conduct inquiries and investigations, Precautions against real or apparent conflicts of interest in an inquiry or investigation."

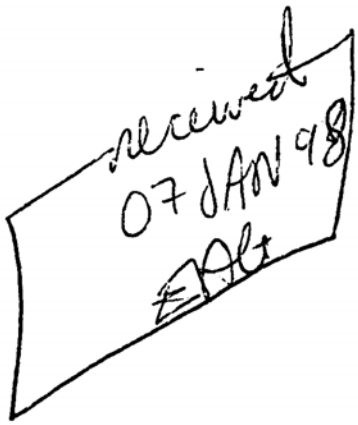
The University of Waterloo with intent deliberately violated this PHS rule. They offered (repeatedly) J.C.M. Riley's Preceptor / sponsor at Yale, Dr. B.R. Behrman, a man whose own work was criticized by the Greenhalgh thesis, as an INDEPENDENT referee. This was a blatant unethical and fraudulent act and must be seen as proof of the allegations. Hence, PHS MUST call for an investigation.

Plagiarism: a Violation of PHS Guidelines

J. C. M. Riley and H. R. Behrman STOLE the intellectual property of the suppressed Greenhalgh thesis and presented the ideas as their own in 1989-90 publications funded by the PHS scholarship-cooperative agreement. Please review the examples found in the section,"The material sent to Mr. Nicholas Wade of the N.Y.Times...

Please note that PBS was made aware of this relationship earlier, and Dr.Lyle W. Bivens in 11 Dec. 95 to Mr. Phil Sharkin falsely writes, after citing 42 CFR 50. 102 "that there is no allegation of fabrication or plagiarism that warrants ORI jurisdiction". Since this is now proven FALSE, PBS must call an investigation.

In summary, enough evidence exists in 1998 to prove misconduct, premeditated misconduct, plagiarism (covered up), and even intentional fraud to warrant PBS to re-investigate. The proper procedure would be to call a proper ethics committee and ask for proper presentations with proper presentation of the documented evidence, but this time be open so to avoid the allegations of cover up. An investigation is warranted.



FOIA Case Number: 98-022D

December 2, 1997

Mr. Edward A. Greenhalgh
265 Regina Street N.
Apartment 7
Waterloo, Ontario
N2J 339 Canada

Dear Mr. Greenhalgh:

This is in response to your September 24 letter addressed to Rosario Cirrincione and your November 10 letter addressed to me.

The Department's policy calls for the fullest responsible disclosure consistent with the requirements of administrative necessity and confidentiality which are recognized by the Freedom of Information Ac (FOIA) (5 U.S.C. 552), and the Department's implementing Public Information Regulations (45 CFR Part 5). Copies of the Act and Regulations are enclosed and referred to below.

In response to the September 24 correspondence, I am enclosing copies of the only records which were located during our search. The Office of Research Integrity (ORI) has no other records responsive to your request. Please note that from the enclosed documents I have removed information the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

My decision to withhold the information is based upon the FOIA, 5 U 552(b)(5), (b)(6) and (b)(7)(C); and the Department's implementing Put Information Regulations, see 45 CFR 5.66, 5.67 and 5.68(c).

Your November 10 letter made a new request for, "..copies of any assurances and dates of association etc., given to NIH by the University of Waterloo, H.R. Behrman, and Yale...". I asked NIH to search its files responsive records. I have been advised that NIH receives assurances from institutions, not individuals. And, a search of its files revealed no assurances received from either the University of Waterloo or Yale. However, we determined that you may have been requesting copies of assurances received by the ORI from these institutions and these documents are enclosed. Again, I have removed information which possibly identify the subject of an ORI investigation. Release would clearly unwarranted invasion of personal privacy.

You, of course, have the right to appeal this decision to deny you full access to records in this agency's possession. You also have a right appeal the adequacy of the NIH search for records responsive to your November 10 letter. Should you wish to do so, send your appeal, within 30 days from the date you receive this letter, to the Assistant Secretary of Public Affairs, U.S. Department of Health and Human Services, Room 13C-24, 5600 Fishers Lane, Rockville, Maryland 20857, following the procedures outlined in Subpart C of the enclosed Regulations. Please mark both your appeal letter and envelope "FOIA Appeal".

Sincerely yours,

Freedom of Information Act Office
Public Health Service

Department of Health and Human Services
Public Health Service (PHS)

Initial Assurance

Regarding Procedures for Dealing with and Reporting Possible Misconduct

PRESIDENT/DIRECTOR
YALE UNIVERSITY
NEW HAVEN, CT 06520
L

Each institution which receives or applies for a research, research-training, or research-related grant under the Public Health Service Act must submit an annual assurance certifying that the institution administrative policies as required by the Final Rule (42 CFR Part 50, Subpart A), and that it will comply and the requirements of the Final Rule as published at 54 FR 32446, August 8, 1989.

1. In accordance with 42 CFR Part 50, the administrative policies provide for the following, and with 42 CFR 50.101-50.105:

- An impartial process for receipt of allegations of scientific misconduct and for initiating immediate allegation.
- Subject to Part 50, completion of each inquiry within 60 calendar days from receipt of allegation, in of a written report.
- Maintenance of detailed documentation of an inquiry for at least three (3) years, which must, upon to authorized HHS personnel.
- Initiation of an investigation within 30 calendar days of the completion of an inquiry, if findings from sufficient basis for conducting an investigation.
- Subject to Part 50, completion of an investigation within 120 calendar days.
- Selection of impartial experts to conduct inquiries and investigations.
- Precautions against real or apparent conflicts of interest in an inquiry or an investigation.
- Affording the affected individual(s) confidential treatment to the maximum extent possible, a prompt investigation, and an opportunity to comment on allegations and findings of the inquiry
- Notification to the PHS's Office of Scientific Integrity (OSI), at the National Institutes of Health that an investigation will be conducted.

- Making efforts to restore the reputations of persons alleged to have engaged in misconduct when confirmed.
- Protecting, to the maximum extent possible, the positions and reputations of those persons who, allegations of scientific misconduct, and those against whom allegations of misconduct are not
- Imposing appropriate sanctions on individuals when the allegation of misconduct has been substantiated
- Notifying the OSI of the final outcome of the investigation with a written report that thoroughly documented investigative process and findings.
- Informing its scientific and administrative staff of the policies and procedures and the importance of those policies and procedures.

2. Name and Title of Official Signing for the Organization

Edward A. Adelberg, Deputy Provost

Address
320 York Street
New Haven, Ct. 06520

I certify that:

(a) this organization has established and will comply with policies and procedures, - the provisions set out in item 1 above, for inquiring into and investigating allegations of scientific misconduct;

(b) this organization will comply with the requirements of the PHS regulations on response of awardee and applicant institutions for dealing with and reporting possible misconduct science (42 CFR Part 50, Subpart A); and

(c) this organization will provide its policies and procedures to the Public Health Service request.

Signature of the person named in Item **2** *(In ink. "Per" signature not acceptable.)*	**Date: 12/**

Department of Health and Human Services (HHS)
Public Health service (PHS)

Initial Assurance
Regarding Procedures for Dealing with and Reporting Possible Misconduct

University of Waterloo
Needles Hall, Room 3015
Water1oo Ontario N2L

Each institution which receives or applies ment under the Public Health Service Act administrative policies as required by the…and the requirements of the Final Rule as

1.In accordance with **42** CFR Pact 5 with 42 CFR 50.101-50.105;

- An impartial process for receipt of all allegation.
- Subject to Part 50. completion of ea of a written report.
- Maintenance of detailed documents to authorized HHS personnel
- Initiation of an investigatjon within 3 sufficient basis for conducting an in
- Subject to Pact 50, completion of a
- Selection of impartial experts to co
- Precautions against real or appare.
- Affording the affected individual(s) Investigation, and an opportunity to comment on allegations and findings of the Inquiry and/or
- Notification to the PHS's Office of Scientific Integrity (OSI). at the National Institutes of Health will be conducted.
- Notification to OS1 within 24 hours of obtaining a reasonable indication of possible criminal violations
- Preparation and maintenance of the documentation to substantiate an investigation's

DEC 11 1995

Mr. Phil Shaikun
Attorney, Civil Division
Commercial Litigation Branch
U. S. Department of Justice
P. 0. Box 261 Ben Franklin Station
Washington, DC 20044

Dear Mr. Shaikun:

The Office of Research Integrity (ORI) has reviewed the material from Mr. Edward Greenhalgh that the Department of Justice (DOJ) submitted to Mr. Robert Lanman and to Mr. Mar on November 13, 1995.

The ORI, Division of Research Investigations (DRI), has received prior allegations from Mr. Greenhalgh concerning the issues he raised with DOJ and closed its file on the matter of September 1994. DRI's review indicated that ORI had no definitional or funding jurisdiction on any of Mr. Greenhalgh's allegations. This determination remains unchanged based on any of the additional material you forwarded.

Our review indicates that the Public Health Service (PHS) funding for---------- Mr. Greenhalgh'----------and the subject of some of his allegations, terminated well before the beginning date of Mr. Greenhalgh' s research which forms the basis of the allegations. --------- received no PHS support at the time he was alleged to have misused or tampered with Mr. Greenhalgh' s experiments. Also, the papers and doctoral thesis involved in the allegations cite no PHS support. Therefore, no funding jurisdiction exists for ORI.

Furthermore, the factual allegations made by Mr. Greenhalgh raise concerns of a different interpretation or judgment of data or a credit dispute between collaborators. These issue outside the PHS definition of scientific misconduct. 42 CFR 50.102. No allegation of falsification, fabrication, or plagiarism is made that would warrant ORI jurisdiction

2. The Cancer Research Myth

Circa 1987, the Medical Research Council of Canada (MRC) was approached by E. A. Greenhalgh with research material from the SUPPRESSED thesis outlining experiments to prove that membrane fluidity was flawedand that free-radical production and oxidation damage were more important .MRC was asked, since many cancer theories are based on membrane fluidity, wouldn't honest cancer researchers want to know?" THIS IS DOCUMENTED IN WRITING!!! In 1990, Weigh et al won the Nobel prize for disproving membrane fluidity. MRC could have acted and promoted research merely by enforcing the existing guidelines and regulations, but because the J. C. M. Riley Ph.D. and NIH scholarship depended on membrane fluidity, they DID NOTHING!!!

THE QUESTION BECOMES, HOW MANY PEOPLE COULD HAVE BEEN HELPED SOONER? Clearly, a friend's kid and political interests were considered more important than research affecting cancer at MRC (who screened NIH scholarship candidates).

Mr. Nicholas Wade (Sci. Ed., N.Y. Times) has commented that my theories may indeed be the basis for some modern research. This must be put in the context of Dr. Barbara McClintock who won the Nobel prize in 1983 for her PAST work on "moveable" genes: her work had put her in conflict with the established bodies of the past. She was neither an evil person, nor a bad scientist, but actually quite dedicated. She had to become a botanist to get around the establishment obstacle. And so, the proposal must be made that E. A. Greenhalgh is neither an evil person nor a bad scientist, but someone caught in a political trap. Individuals have suggested that Greenhalgh do what McClintock did. However; (1) this has already been tried in the early 90s but evidence exists to prove that positions were lost to mysterious (blacklisting) circumstances. But more importantly, (2) we are talking about theories DIRECTLY relevant to CANCER and AIDS!!! I had cancer, and know that cancer patients want the cure tomorrow, not TWENTY YEARS from now! It is quite realistic, based on the fact that my theories are being proven correct, that real solutions could result in as little as 3—5 years if help were to be received now. N.B. see the discussion concerning R. U. Hausknecht and his use of cancer drugs to induce abortion : this is from my 1986 suppressed work!

QUESTION: What is more important to Sec. Donna Shalala and PHS:

1. Finding new treatments for cancer SOONER than later, or

2. Covering up scientific misconduct and fraud?

Human nature does not take the embarrassment of being scammed well, often going into denial. Unfortunately, in this case, saving face and covering up the fact that the University of Waterloo FABRICATED a Ph.D. and deliberately MANIPULATED PHS regulations also violates PHS/NIH's mandate of "truthfulness in science" and " advancement of science" ,plus the public good. Instead of being angry with Greenhalgh for upholding scientific principles and ethics, you should hold the University of Waterloo ACCOUNTABLE for their actions. No one likes to be taken, but PHS' pride is getting in the way of research that could be beneficial. N.B.: please ask UW to prove that J. C. M. Riley even has an Hons. Biology degree-- the PROPER requirement to be in an MSc. (let alone a Ph.D.) program.

The choice is yours. The work could begin immediately, but because of the conflict—— possible retaliation from PHS since the new theories are based on the suppressed thesis - who will risk their funding to work with me? SPECIFIC EXAMPLE: in the fall of 97 I wrote the Carol M. Baldwin Breast Cancer Center outlining the "Hoechst—Celanese" proposal (re. 1990—93) explaining how induced remission could eliminate surgery and death; and perhaps there would be funding from the pharmaceuticals if their facility was available. Think about it; women do not have to die or have their breasts removed. What happens? NO REPLY!!! A breast cancer center not even discussing the possibility to induce remission and eliminate surgery!!! Quite the conflict!!! PHS was scammed, and now to HELP honest research, PHS must enforce its mandate, the spirit of its mandate, and end the conflict by calling an open and honest ethics committee investigation.

Note the included research proposals (circa 1990—93) and how they relate to research now proven. Please note the proposal on ovarian cancer, and how in 1997 others are only just beginning to do it, seven years later—— delayed. How many could nave been helped sooner? Please consider how the discovery circa 1998 of telomerase fits in with the Cell Death Signal Theory and luteal cells as pre-programmed life span to die; and the ramifications to discover how remission works. Why not help the research as it has a solid basis?

The Cancer Research Myth: government and researchers are working hard to promote new ideas to find a cure.

At least seven to twelve years of suppression has blocked theories that are now being proven by others. All those years to cover up a scam. PHS can help now. Or continue to cover up and let people die.

The Cell Death Signal Theory has grown into the Viroid Thermodynamic Theory on the Origin of Life (VTT) with possible explanations for cancer and AIDS - they are evolutionary mechanisms. If you ask older scientists what cancer is, they will say either a virus or they don't know. If you don't know, then you can't really work out effective mechanisms. In 1986 people treated membrane fluidity as the word of God-- in six months I discovered it to be an artefact; not the real mechanism; however, everyone's GRANT depended upon it. In 1990, Weigh et al won the Nobel prize for saying membrane fluidity was an artefact. Based upon my past theories, I propose a working model for cancer so to help people. Will you help me?

V.T.T. (again) has conflicts with older scientists.

1. VTT doesn't consider evolution to be totally random, but limited by INITIAL STARTING CONDITIONS, and (consequently) the environment.

2. The Central Dogma - genes operate outward ONLY is not correct in the long term of evolution, but can be altered by environmental mechanisms (why is this so fantastic—— genetic engineering is based/on and makes use of these mechanisms). Please recall that Dr. McClintock's "moveable" genes displeased everyone, and the research was held back. Is PHS' mandate to help or hold back cancer research?

The above two points hold great ramifications, especially to the thinking with breast cancer. Just because a woman has the (environmental marker) gene for breast cancer does not mean she should have her breasts cutoff (unless you enjoy mutilating women). No! It is one gene locus, but there are other loci controlling those genes' expression, and whether or not it is allowed to be expressed (i.e., become cancer) depends on whether or not specific signals are received. VTT considers these signals to be (in part) initiated through the environment (very simplistic because this is a letter). Cancer and HIV (both retro viruses) may be evolutionary mechanisms to control both evolution and its RATE. Therefore, evolution may occur much faster than previously considered (or even be held static, dependent on "energy factors" in the environment). And in regard to the Carol M. Baldwin Breast Cancer Center of Foundation, who want to know why a N.Y. area may be more prone to cause women to suffer breast cancer the environment does play a large factor in how areas may be higher risk (the factors are what her foundation are seeking to find). Besides known carcinogens and mutagens etc., there may be organic co-factors (bacterial or secondary viral infections and/or their poducts) necessary and not considered before which act as the environment's messengers or modifiers. Present examples: bacteria have roles in (not previously understood), 1. ulcers, 2. some heart disease, and 3. E. coli and arthritis have a link. Just as with membrane fluidity, other areas must be examined if new answers are to be found.

VTT in its simplest form considers oncogenes to be induced out of the genome (to become viruses again) by one stimulus (simple example, radiation) but whether or not the cancer virus remains active is dependent on the presence or absence of intermediaries, possibly bacterial induced proteins, possibly other; but here is where the work requires help to continue. Please read the early research proposal and how it was proposed circa 1989—90 of breaking up the HIV disease sequence - something later done but others with the AZT regimen. The same concept also applies to cancer and could explain why the simplistic attempts to JUST kill the infected cells, is not effective. The concept may help to induce the sequence to induce remission; and may explain why the chemicals found in certain herbs and other foods (environmental influence) help prevent cancer and indeed may control remission. There are several simple experiments to prove this and would therefore support the claim of inducing remission, and so, eliminate surgery and death. But the work can not continue if it is not helped.

This turning on and off of gene loci is a reflection of environmental factors, and therefore, the environment influences the TREND of evolution (hence questions the Central Dogma). This turning on and off (through diet and INFECTION) depends on what the species needs to reflect of the environment, and to survive most efficiently in the CHANGING environment. Remember it is only in recent history that people have stopped dying of MINOR infections (though our antibiotics are less and less effective). Therefore, if a cancer were induced, the organism would die of an infection long before the cancer could appear--hence, why there are more cases of cancer today and very few reported in history (simplistic : two types of cancer suggested a) integrity of genome correcting and 2) infection inducing both interrelated but operate with two different goals). One point becomes, an individual carrying a specific gene loci would die, allowing the selection of other individuals with a favoured trait to continue (more dominant loci could be repetition of signal or other suggested). Hence, the on and off loci and their location become important.

Simple point emphasized: two types of cancer:

1. if the genome is damaged, then a cancer may be induced to protect the integrity of the genetic signal/message and the individual is eliminated.

2. evolutionary modification by inducing infections (immune system as a reflection of environmental selection) to cause the death of individuals so traits protected by ,i.e., repetition (so promoting desired change) will be promoted and become the "new" subspecies and featured. Fast or slow change as the energy factors of the environment dictate.

All, obviously, over simplified to convey the point that the work needs help to continue, and must be stated here to get around the suppressing politics that have so far trapped it. Please note that nature and evolution are CONSERVATIVE and these oncogenes are also embryonic "growth" sites for embryonic development, and thus explains why THALIDOMIDE IS so successful in stopping brain tumors. Wouldn't finding the actual protein be safer (as asked of the president of Hoechst—Celanese circa 1993)???

Please note that VTT considers viruses to be the origin of hormones; and as such their effect and significance should be seen as very important to understanding and controlling cancer. I have models etc., do I get help, or is a cover up more important?

Therefore, just because a gene for breast cancer is found (or any other cancer) that is not a death sentence. That gene also has controllers, and these may be found, and the factors may be more simple than previously anticipated. There will be mechanisms to keep the so—called cancer gene turned off, or if on, induce remission. Now is that not a worthy goal for PHS to help?

What is more important the potential research or hiding from the embarrassment that HIS was scammed by J. C. M. Riley and the University of Waterloo? It now becomes quite apparent and possible, that potential cancer treatments and research has been blocked simply because P1-IS doesn't want to be embarrassed! Please, it was the University of Waterloo that caused the trouble , let them handle the public embarrassment.

Secretary Donna Shalala, what is more important?

If cancer research, and the public health and safety is to PHS, then there is NO CHOICE but to call for an open ethics committee investigation.

14 April 1988
1400-1

Mr. Christopher W. Besant
Campbell, Godfrey & Lewtas
Barristers & Solicitors
P.O. Box 36
Suite 3600
Toronto-Dominion Centre
Toronto, Ontario M5K 1C5

Dear Mr. Besant:

As I explained to you in our recent conversation the difficulties encountered between Mr. Greenhalgh and the University of Waterloo should be resolved between the two.

The Medical Research Council does not provide research grants to applicants who do not have the assurance of facilities and the ethical conduct of research by an accredited institution. In our case this is a university, hospital or affiliated research institution. Thus, a research grant to Mr. Greenhalgh is out of the question.

However, the possibility of an award to pursue Ph.D training at a Canadian institution is very real. Mr. Greenhalgh would submit an application for an MRC Studentship (MRC 21 enclosed) at the next competition (December 1, 1988). If judged worthy he could receive support. Dr. Pace—Asciak said the same thing to Mr. Greenhalgh on May 26, 1986. With Dr. Pace-Asciak as supervisor, for example, is the only route the MRC could consider supporting his research training.

Canada

From: Edward A. Greenhalgh
265—7 Regina St. N.,
Waterloo, Qntario.
N2J 3B9

To:
Dr. Lewjs Slotin
and the
Medical Research Council of Canada

17 March 1988

re : A Granting Proposal

Dear Dr. Slotin,

This is a direct and open letter to MRC concerning research integrity and funding. Yes, this letter does bypass your normal channels, but because of unfortunate circumstances (manufactured or otherwise) these are not available to me - forcing my actions.

I have been informed by my legal representative, Mr. C. W. Besant of the firm Campbell, Godfrey, and Lewtas that you do not feel that MRC should hold my former supervisor's (Dr. J.C. Carlson of the University of Waterloo(U. of W)) work to judgement even though it may be wrong, and other work (of direct concern to science) proving it inadequate not allowed full public review. The matter must be pointed out that MRC has supported work carried out by an individual (J. C. M. Riley) who only held a General BSc. in physics and whose Ph.D. Thesis is refuted by my original MSc. thesis. You do not wish to compare the two - do your medical research funding referees have general BSc.s in physics? This is a legitimate question because you may not honestly have the ability to perceive the work as flawed.

You may not wish to judge his work but through my own efforts I have had my own theories tested. The material upon which I wish to pursue a Ph.D. have been reviewedwithout biase, by Dr. Pace-Asciak of Toronto's Sick Kids. You should note (see photocopy of the letter) that he considers it may have clinical applications. I do not know (see date) if his offer is still available since I have suffered a terrible delay. Further, review the letter of Prof. G. P. Vinson of the Journal of Endocrinology (England). The work you do not want to judge has already been reviewed, as a paper(but it was actually an abridged version of the thesis that U of W would not accept. Please note that professionals away from U of W consider my thesis to be of interest -- enough so that it may be published if rewritten, which I am actively doing.

You generally fund on the merit of the project and the ability to publish. I have demonstrated merit, but consider the following: as an undergrad at Laurier I produced - without your help or any other funding -- a single author publication (Toxicology (1986) 42, 317 - 330) which has received considerable world attention, along with being requested by the American and Canadian governments, plus the American manufacturer, of chioradane, Vesicol. In this paper I was able to show results to questions that had only been hypothesized before, never conclusively proven.

I have now produced a body of work that seems to be being suppressed since it challenges some established workers. In short, I agree with people like Helmreich and Elson, and Nicolson in criticizing bulk membrane fluidity, but more importantly I prove (if allowed a public audience) that bulk membrane fluidity does not, have a

significant role in signal transmission, and, indeed, that the whole concept may be questionable. This point is of a critical medical importance since a great deal of cancer research is based on membrane fluidity concepts. If it is bogus,wouldn't honest workers want to be so informed in the struggle to save lives? If not, then shouldn't the taxpayer, who trusts you to safeguard his/her life hear this? I believe it is important, so much so, that if you are a scientific body committed to funding legitimate medical research, then I am compelled to ask you directly for funding.

I am presenting you with two possibilities:

1. Out and out funding for a Ph.D. at the centre of my choice;

2. A test, a summer research grant to prove my point, and if I succeed, then point 1 to be granted.

First, let us discuss point 2. My contention is that my MSc. thesis casts very serious doubt on the Ph.D. thesis of J. C. M. Riley and that Dr. Carlson's work in the past has been based on erroneous assumptions. You do not wish to sit in judgement? Fine, let me prove my point.

Edward A. Greenhalgh
265 Regina St. N. Apt. 7
Waterloo, Ontario
N2J 3B9

(519)884-3318

03 December 1997

Nicholas Wade
Science Editor
c/0
N. Y. Times Co.,
229 W. 43rd St.,
N.Y.,N.Y. 10036—3913
USA

tel. (212)—556—7819

Dear Mr. Wade:

Thank you for the telephone conversation on the 3rd of December. In this introductory letter the outline will be very brief as requested, and though later may appear complex, the core will basically consist of suppression, theft of theories and blacklisting along with the cover up of grant fraud.

1. Please note the photocopy of correspondence from the RCMP. We have met twice since then and an investigation is ongoing as of this date.

2. Please read the copy of:

    a. Dr. G. Nicolson's (of M. D. Anderson Cancer Center) 1988 comments concerning the Cell Death Signal Theory.

    b. Dr. Pace—Asciak's (Toronto's Sick Childrens Hospital) comments dated 1986 - of potential clinical value to women.

    c. The reply from Dr. David Kesseler/FDA circa 1994, N.B. before Dr. R. U. Hausknecht published his study on the new abortion technique.

Points a,b & c are meant to PRE-date the Cell Death Signal Theory to 1986 when my thesis work was suppressed. The suppressed work clearly had ramifications to cancer research, and later to the new abortion technique. The point is not to decry Dr. R. U. Hausknecht, who may have seen my theories through Mt. Sinai in LA circa 1993, but to emphasize the work's suppression and how the advancement of science was blocked.

3. Please review the replies from Dr. Shapiro of the U.S. National Bioethics Commission, the U.S. Justice Dept. and the U. S. Supreme Court. Please note that I am awaiting material from the Freedom of Information service, which may prove the allegations that NIH/ORI etc. engaged in a conspiracy and cover up. The former Director may have lied in writing (among others).
Please note the 1997 comments from TAP and GAP, the latter is having difficulty with the academic fraud applications. Please note Dr. Jocelyn Elders", former U. S. Surgeon General, comment.

Please note the enclosed copies of publications under H. R. Behrman and J. C. M. Riley circa 1990. Please compare these to the pages from my suppressed thesis——the allegations include that the work was suppressed to allow J. C. M. Riley (who was basically a general physics BSc.) to graduate with a Biology Ph.D., and to protect H. R. Behrman's reputation. They then, Alleged, stole the suppressed theories. Please compare.

The Cell Death Signal Theory has evolved over the years and if you investigate you may be surprised to find in research proposals circa 1990 experiments that pre—date Dolly the cloned sheep: you must read the material to appreciate the comment. These research proposals were sent to a number of universities in the UK. Nonetheless, Cell Death Signal Theory has evolved into the Viroid Thermodynamic Theory on the Origin of Life (V.T.T.) which, again has ramifications to cancer and AIDS research (that present researchers are still catching up on).

As you asked for brevity, I will stop. There is more and everything is documented. I look forward to your reply, but consider this, based on my past work now accepted as correct, would it not benefit society if I was helped to complete the work? I have been stopped because the scientific community is trying to hide and bury their embarrassment for all the dirty tricks used to promote a general BSc. in physics into a Biology Ph.D. while blocking new and original theories. I, myself, have had cancer (probably due the stress from my ordeal, and do not believe that the majority of researchers are working very hard, nor reporting truthfully. It is in society's best interest to expose all the misconduct.

Thank you for your time.

Very truly,

Edward A. Greenhalgh

Mounted Police / du Canada

Protected A

KITCHENER DETACHMENT
150 Frederick Street,
Kitchener, Ontario
N2H6TI

Your File **Votre reference**

Mr. Edward Greenhalgh
265 Regina Street North
Apartment # 7
Waterloo, Ontario
N2J 3B9

Our File **Notre rCférence**

97-441

October 22, 1997

Dear Mr. Greenhalgh

Complaint of Fraud against the University of Waterloo

In response to your letter of October 13, 1997 and telephone message of October 20, 1997, I wish to address your request for a written answer to the following question.

Quote “Is not lying to receive federal monies a felony, fraud? Yes or no?”

The offence of Fraud as set out in the Criminal Code of Canada states the following;

“Every one who, by deceit, falsehood or other fraudulent means, whether or not it is a false pretence, within the meaning of this Act, defrauds the public or any other person whether ascertained or not, of any property, money or valuable security,” is guilty of Fraud.

I have reviewed some of the material which you provided but as yet not all of it Rather than communicate through correspondence it would be more appropriate for both of us to discuss this matter in person and clarify certain concerns. A number of issues which you raise in your various correspondence with other persons are not criminal issues but civil and cannot be dealt with through the criminal process.

In your telephone message to me you mention the RCMP Subpoenaing information relative to the Human Rights Commission. I am unclear as to the reasoning or purpose of this procedure, but that is another matter we will cover when we meet.

Please contact me at your earliest convenience in order that we may set up a meeting at my office.

Sincerely

M.G.C. Lutes, Sgt. i/c
Commercial Crime Section
Tel: 571-6643 (ext. 266)
Fax: 571-6652

Canada

Edward A.Greenhalgh
265 Regina St. N., Apt.7,
Waterloo, Ontario. N2J 3B9

01 December 1997

Sgt. M. C. G. Lutes
RCMP
Commercial Crime Section
Kitchener Detachment
150Predrick St.,
Kitchener , Ontario.
N2H6T1

(519)—571—6640

Dear Sgt. Lutes,

Thank you for receiving this letter, but a comment from our 26th of Nov. 97 meeting must be re-examined. You suggested the possibility that the University of Waterloo may "lose", or by "accident" give the RCMP the wrong academic records: i.e., a "clerical mistake could change a C— into a B-"; for J. C. M. Riley. There are two problems with this "expectation" of low ethical standards and dishonesty:

1. Just how reliable are Centers of Excellence (and the people they produce)?, especially in light of the fact that research impacts the public health and safety (i.e., a new HIV blood tragedy due withheld reports etc.); and

2. the low standards of a Center of Excellence regarding ethics and integrity - the misrepresentation of official documentation is a deceitful and fraudulent act, so how can the public trust that their health and safety is safeguarded by such centers? What good are standards or government objectives and guidelines and regulations if no one has any expectations of their enforcement?

Therefore, a most vigorous investigation is demanded since MRC has placed so much emphasis on these "Centers of Excellence" to its (government appointed) mandate and the public well being. Please cross-reference any requested documents to those that MRC would also have had to have requested. If they do not exist, then the onus (like Jag Bahgdura's (sic) resume qualifications may simply <u>not</u> exist) is on UW et al.. to prove that they did indeed exist. <u>If all</u> files may simply be destroyed (N.B. Somalia Inquiry) why won't another blood tragedy occur. Excellence must mean high standards, or else it is just propaganda, and therefore, MRC Objective (an Act of parliament) were just an Act of Propaganda.

MRC Objectives and Guidelines are quite explicit: candidates MUST be of EXCELLENT academic backgrounds, be of MERIT, HIGH QUALITY, and the research and SIGNED ASSURANCES MUST promote the Objectives of MRC (i.e., advance knowledge and issues' of public health etc; as set out in their booklets) ; and by an Act of Parliament. The apparent suggestion was that if J. C. M. Riley's transcripts would read B+ rather than a C- from a GENERAL BSc. program in physics, then it could be deemed acceptable enough for excellence, high quality and merit. I can not agree that such would meet MRC requirements because:

1. UW has set the PRECEDENT (re., its legal counsel's (R. A. Haney) letters) stating that there IS NO substitute for being PROPERLY enrolled in a Ph.D. program.

Example: In our meeting, I explained that a pharmaceutical representative said that in Europe if you publish a paper then that was considered equivalent to grant a Ph.D. You replied, but this is not Europe. EXACTLY! IN CANADA, to be PROPERLY enrolled in a Ph.D. program you must have an MSc. degree first! Unless you are INCREDIBLY exceptional.

J. C. M. Riley was not:

i. an MSc. graduate (in biology nor other)
ii. nor incredibly exceptional -- so poor that he did not comprehend undergraduate chemistry—— i.e., he claimed NO oxidation occurred in his Ph.D. thesis membranes

IN CANADA, TO BE PROPERLY enrolled in a graduate biology program, the HONEST student is expected to have:

i. an Honours BSc. in Biology
ii. with prerequisite chemistry courses, most notably, Organic chemistry.

Therefore, along with your request for J. C. M. Riley's records please note whether or not that he has the prerequisite chemistry training DEMANDED of honest biology graduates. Hence, it is IRELEVANT if 13W were to make a "clerical" error in changing a C— gen. BSc. in physics to a B+; it is NOT the REQUIRED course program with the REQUIRED chemistry courses DEMANDED to be PROPERLY enrolled in a graduate biology program; let alone a Ph.D. (higher) degree program. This alone must be seen as a fraudulent misrepresentation for MRC monies, with UW giving deliberate

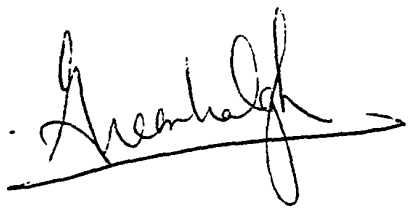
false assurances. Please note that the whole point of the conspiracy was to "create" a Ph.D. and hide Riley's real academic background.

RAMIFICATIONS

MRC Objectives and Guidelines DEMAND EXCELLENCE! UW claims to be a Center of Excellence. Therefore, they MUST meet the most STRINGENT of standards, and excuses for mediocrity are simply UNACCEPTABLE. If degrees can simply be MADE UP, then **80** can safety reports for drugs, and medical products (i.e., HIV blood products) by a Center of Excellence. Alan Rock, former Justice Minister, now responsible for Health, has publicly apologized for the blood tragedy, and vowed that such will never happen again. Why not? If 13W believes that it can get away with lying about assurances and standards and not be held accountable, why not about safety reports etc.? What message does it send? If the RCMP will stop an investigation due a "clerical" error with out cross-referencing transcripts sent to MRC etc.? Then why shouldn't another blood scandal, DES etc. occur? Pharmaceutical companies and research centers will now have the precedence that they are not accountable, and that FALSIFIED records are all that is necessary to get them off the hook. The public health and safety is compromised if the RCMP does not vigorously use all avenues available; especially following Mm. Rock's vow.

Thank you, Sgt. Lutes, for your time in reading my concerns. I sincerely hope you will examine all possible sources.

Very truly,



Edward A. Greenhalgh.

555 University Avenue
Toronto, Ontario
Canada M5G 1X8
PHONE (416) 597-1500

THE HOSPITAL FOR SICK CHILDREN

RESEARCH INSTITUTE

May 26, 1986

Edward A. Greenhalgh
Apt.7
265 Regina St., N.
Waterloo, Ontario
N2J 3B9

Dear Mr. Greenhalgh:

This is a reply to your letter of the 20th of May. I found your project well worthy of pursuing for a Ph.D. program. It is both interesting and has good prospect of proliferating into clinically relevant problems.

Unfortunately funds for salary support are not immediately available to consider your acceptance into the Ph.D. program. If, however, you are capable of obtaining salary support from some agency e.g. NSERC or MRC, I would gladly consider your application for Ph.D. studies in the Department of Pharmacology.

Sincerely,

C.R. Pace—Asciak, Ph.D.
Professor
Departments of Pediatrics
and Pharmacology

THE UNIVERSITY OF TEXAS
MDANDERSON
CANCER CENTER

DEPARTMENT OF TUMOR BIOLOGY. 108
(713) 792.7477

August 30, 1988

Mr. Edward A. Greenhalgh
265—7 Regina St. N.,
Waterloo, Ontario N2J 3B9
Canada

Dear Mr. Greenhalgh:

I apologize for the time involved in reviewing your two manuscripts, but you must appreciate that my own academic and journal responsibilities come first. Also, I was out of the country, and during that time I had a young colleague (Instructor) examine your manuscripts. Since this colleague had experienced similar problems to your own, I felt it appropriate to have this person take a look at your two papers. I then examined the manuscripts and made some minor corrections and added a few additional comments into the reviews.

As you will see from the enclosed reviews, we felt that your research is interesting and appropriate for a thesis, although it is somewhat preliminary for scientific publication. The journal reviews that you received on these manuscripts were, in my opinion, fair and balanced and the criticisms are potentially answerable (in revised manuscripts). Thus, I believe that your papers were not blocked for publication. They received the type of reviews that many manuscripts receive upon initial submission.

I cannot really comment on your hypothesis that PCF_2a is a signal for cell death, because it isn't in my area of expertise. Certain cells actually secrete PCF_2a and must grow in high extra—cellular PGF_{28} concentrations, so such a mechanism cannot be universal, if indeed it does exist. The whole area of PGs is rapidly moving, and simple hypotheses relating the action of these compounds to intracellular events involving cyclic nucleotides and cell division and death will take time, I believe, to sort out. However, your hypothesis is potentially interesting, and I am sure that you might find interested laboratories working in this area.

I wish you luck in your academic dispute, and I hope that you are successful.

Sincerely,

Garth L. Nicolson
David Bruton Jr. Chair in Tumor Biology
Professor and Chairman, Department of Tumor Biology

CLN/pb

1515 HOLCOMBE BOULEVARD
HOUSTON, TEXAS 77030
(713) 792-2121

Food & Drug Administration
Rockville, MD 20857

July 11, 1994

Mr. Edward A. Greenhalgh
265-7 Regina Street, North
Waterloo, Ontario, N2J 3E9
Canada

Dear Mr. Greenhalgh:

Thank you for your letter of June 10, 1994, and accompanying materials referring, in part, to RU486 and arsenic. I have shared these materials with our Division of Metabolism and Endocrine Drug Products in the Food and Drug Administration's (FDA) Center for Drug Evaluation and Research.

FDA is committed to approving safe and effective products, and we work with sponsors -to ensure that the necessary steps to secure approval are taken. Approval of a drug is not a quick process, due to the need for a drug's sponsor to conduct clinical studies demonstrating that a product is safe and effective in humans. These requirements are specified in the Food, Drug, and Cosmetic Act and the implementing regulations. In general, clinical studies are sponsored by drug manufacturers, conducted by clinical investigators, and monitored by FDA.

FDA approves a drug for use in the United States after it has reviewed the results of the manufacturer's/sponsor's New Drug Application, containing data (results of human, animal, and laboratory testing, and manufacturing information) which demonstrate the product's safety and efficacy. Investigational drugs may not be distributed or imported for trial on humans unless the sponsor has filed an Investigational New Drug (IND) application as specified in FDA'S regulations.

I hope this information has been helpful, and once again thank you for taking the time to write.

Sincerely yours,

Mary K. Pendergast
Deputy Commissioner
Senior Advisor to the Commissioner

A PROPOSED FUNCTION STUDY OF R1J486 vs. ARSENIC POISONING vs. NEMBUTAL TREATMENT (re.: CELL DEATH SIGNAL THEORY)

An 0utline by E. A. Greenhalgh to Coincide with a Request to the National Institute of Health (NIH) for an Investigation into Scientific Misconduct in Reproductive Endocrinology.

10 June 1994

FROM:

E. A. Greenhalgh

265-7 Regina St. N.,

Waterloo, Ontario, N2J *3P9*

Canada

ph. (519)—884-3318

TO:

Dr. Samuel Narrow, the National Institute of Health (NIH)

Ms. Cindy Peirson, Program Director, National Women's Hea1th Network

Mr. David Kesseler, Commissioner, the Food & Drug Administration

Rep. Christopher Smith (D-NJ), RU486 Review,

Rep. John D. Dingell (D), the Oversight Committee.

Enclosed is a scientific argument with support material. The request to Dr. Samuel Marrow of NIH will follow at a later date due to the time consuming requirements of accuracy and cross-referencing. And human testing of RU486 will begin in the fall. The material can be examined as you desire. A longstanding academic dispute (threatening to some major researchers) based on ethics and safety has been ongoing since 1987. The major surprise has been the appalling lack of safety standards and agencies to investigate problems in Canada. To highlight this point, Canada has had to invite the FDA to provide safety standards concerning blood products for the Canadian Red Cross.

Note my publications: Toxicology (1986)V.42; a histological study/comparison of pesticides, and the two Journal of Endocrinology (UK) papers v.425 (1990) regression studies of luteal cells, mentioning the Cell Death Signal Theory. Please note the 1986 letter from a Dr. Pace-Asciak of Toronto's Sick Children's Hospital: "work could be of clinical value". Similarly, Dr. G. L. Nicolson of the M. C. Anderson Cancer Center and his incredibly kind support noting that the theory should be followed up on. Also, Dr. Peddie of Princess Anne Hospital (UK) original kind reply about my work being in accord with their own research. The concerns about blacklisting and suppression are in the copy of the letter sent to the Ontario Human Rights Commission and government agencies. Note replies.

The above stresses that my work is credible being published in divergent disciplines and the theory has a basis in reality. The work had been started but blocked. Why? I do not know, but theories must be tested and blacklisting prevents same. Suppression should be a concern to the reader suggesting something important. That concept/theory follows (in very simplified form).

RU486 vs. Arsenic/Nembutal or Other Poisoning

In spontaneous and/or induced abortion there is:

1. A prostaglandin surge affecting the pituitary initiating the resetting of the menstrual cycle. This overrides the positive hormonal signa1 from the fetus. In induced abortions this is the prostag1andin injection.

2. The fetus must be harmed/killed to over-ride the positive signa1 of the feta1 hormones that are maintaining the pregnancy. RU486 harms/kills the fetus.

Point : Harmful side—effects (mutagenic/carcinogenic/other) by RU486 to the adult female are unknown.

Let us examine concepts suppressed by the University of Waterloo, Canada. Why is not known, but a directly comparable model is available. In examination of luteal regression, Greenhalgh called regression a form of induced cell death (hence, Ce11 Death Signal theory:) Experiments were begun to compare the effects of sodium pentabarbitol (aká Nembutal) on the ovary/luteal cells (see enclosed photocopied pages of suppressed thesis). Here is the point, sodium pentabarbitol caused the same effects of regression (decreased progesterone) as had the prostaglandin injections. Consider the effect as induced cell death, then using my toxicology experience I considered, "what kills cells?". Combining toxicology and endocrinology, I looked for similar experiments. Two papers I reviewed before my work was stopped were:

Edward A. Greenhalgh
265-7 Regina St. N.
Waterloo, Ontario
N2J 3B9
(519)884-3318

25 August 1992

President A.W Schuele
Hoechst Canada Inc.
P.0.B. 6160, Station A
Montreal, Quebec
H3C 3K8

Dear President Schuele:

I am writing an update to my 31 July 1992 letter. Your response can be considered no worse than other pharmaceutical firms. Those whose main goals are not exactly as my proposal wrote back saying so and wishing me well. The few firms where my proposal was exactly what their Industry is based upon have simply avoided the issue. Although you are no worse than the industry standard; however, according to Quality Assurance and Road Map to Problem Solving, shouldn't you want to be better?

Please note the kind reply to my request for scientific papers from Dr. Ohno (21 July 1992 - The Ben Horowitz Chair of Distinguished Scientist...). On a strictly scientific basis I receive considerable worldwide courtesy still. On a strictly scientific basis I wish to up date my proposal and its benefit. Please contact Dr Kott as I have explained the theoretical details to him, and if my theory (of evolution) is correct the benefits are immense. If I am correct, I may be able (within a year) produce a protein responsible for remission. The protein could then be mass-produced by genetic engineering. Is Hoechts going to turn such a project down? Again we can "brainstorm" the possibilities.

On a sadder note, a poor individual (24 Aug. 1992) has settled a foolish dispute with Concordia by murdering people. A tragedy. I asked you to read a Time magazine article concerning academic problems; further many people in the USA have settled dispute similarly. I, too, have been involved in an academic dispute; however, like Ms. O'Toole (end any proper pharmaceutical firm) I have retained legal counsel. McMillan and Binch are proceeding with my plagiarism charge: such a responsible firm would not do so unless they were very convinced of the validity of the case. I have watched positions in England and the US disappear while driving a forklift for Hoechst. Nevertheless, I kept a good work record, a positive attitude and paid my bills (the Province has announced it is going after students who have defaulted their loans as far back as 1965). Do I not fit your Quality Values as the type of individual your QA program states you should support.

Why not meet with me and discuss the project? Taxol will soon be on the market, so why not have an equally valid alternative? I honestly do not see you risking very much capital on the project, while the returns are potentially incredible.

Saint-Laurent
Montreal, Québec H4R 1R6
Tel (514)333-3500
Fax~(514)331-1526

800 Rene-Levesque Blvd West 0
P0 Box 6170 Station A
Montreal, Quebec H3C 3K8
Tel (514)871-5511
Fax (514) 871-5635

September 11 1992

Edward A. Greenhalgh
265-7 Regina Street North
WATERLOO, Ontario
N2J 389

Dear Mr. Greenhalgh:

Upon reading the documentation you forwarded to me on July 31st, which illustrates your impressive educational background, I can understand your sentiments on receiving notice of a job opening at our Resco Plant for general labourer positions. I would, however, like to clarify that it is the responsibility of our Human Resources representatives to advise a11 of our employees affected by the recent business changes of any job openings available within our organization. This gesture on our part is in no way meant to lessen the importance of our employee's qualifications, and was forwarded to all the Cambridge employees concerned.

It is a fact that our North American business orients itself towards the marketing of our product line and not in the domain of scientific research. Therefore, we cannot sponsor the type of research project you have presented.

I have asked Mr. Jean-Pierre Kolo to contact you in the near future to assess with you if there are any other avenues that you could explore.

I am confident that your experience and perseverance will lead you to a successful career and I wish you the best of luck in your future endeavors.

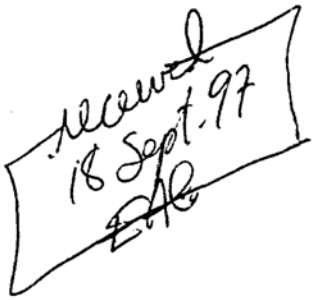
Yours truly

Alban W. Schuele
President

TAF AX PAYERS AGAINST

THE FALSE CLAIMS ACT LEGAL CENTER

FRAUD



September 17, 1997

Edward A. Greenhalgh
265 Regina St. N., Apt. 7
Waterloo, Ontario
N2J 3B9 Canada

Dear Mr. Greenhalgh:

Thank you for contacting Taxpayers Against Fraud, The False Claims Act Legal Center (TAP). We have reviewed the information that you sent to us. Unfortunately, TAF is not in a position to join you in filing a False Claims Act lawsuit based on the information you have provided. Please be assured that our decision is not intended to reflect on the merits of your allegations.

TAF is a private, nonprofit organization that promotes awareness of the False Claims Act and provides litigation assistance to private plaintiffs under the Act. Due to our limited resources, TAF can assist in only a small number of cases each year.

You should be aware that, under the False Claims Act, an action must be filed within the later of the following two time periods: (1) six years from the date of the violation of the Act; or (2) three years after the Government knows or should have known about the violation, but in no event longer than ten years after the violation of the Act. (However, at least one district court has interpreted the Act to require that private actions be filed within the six-year, rather than the ten-year, period.) Further, if before you file someone else files a False Claims Act lawsuit or helps to publicize allegations similar to yours, you may lose your right to bring a suit under the Act.

I am returning all of the materials that you sent to us. I am sorry that we are unable to offer you any assistance. Everything you have been through sounds awful. I hope that somehow, someday you will reach a satisfactory resolution. Good luck.

Sincerely,

Alan Shusterman, Esq.
Associate Director

Enclosures

1220 19th Street, NW Suite 501 Washington, DC 20036 phone (202) 296-4826 fax (202) 296-4838
internet: *http://www.taf.org* or taf-info@taf.org

National Bioethics Advisory Commission
6100 Executive Boulevard • Suite 3C01
Rockville, MD 20892-7508
Telephone: (301) 402-4242
Facsimile: (301) 480-6900

May 15, 1997

Mr. Ed Greenhalgh
265 Regina St., N., Apt. 7
Waterloo, Ontario
N2J 3B9

Dear Mr. Greenhalgh,

Dr. Shapiro forwarded your letter to me to implement your request that it be entered into the official record. To that end, I will include your letter in the briefing book for the May 17, 1997 meeting of the National Bioethics Advisory Commission which will put it into the public domain.

On the issue of the protection of human subjects, you may want to contact Dr. Gary Ellis, Director, Office for Protection from Research Risks, National Institutes of Health, 6100 Executive Boulevard, Suite 3B01, Rockville, Maryland, 20892-7507. He can be reached by telephone on (301) *496-7005*.

Sincerely yours,

Henrietta Hyatt-Knorr
Deputy Executive Director (Acting)

cc: Dr. Shapiro
Dr. Ellis

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Office of the

Surgeon General

Rockville MD 20857

OCT I7 I994

Mr. Edward A. Greenhalgh
265-7 Regina Street, North
Waterloo, Ontario N2J 3B9
Canada

Dear Mr. Greenhalgh:

Thank you for your recent letter to Surgeon General Joycelyn Elders in which you allege that certain officials of the University of Waterloo have engaged in improper conduct with respect to your research activities.. Dr. Elders asked that I respond to your letter.

The Office of the Surgeon General of the U.S. Public Health Service has no authority to conduct investigations of the type you request. Within the Public Health Service, authority to review and investigate allegations of scientific misconduct is under the purview of the Office of Research Integrity Carl). Although you have previously corresponded with ORI personnel, we are forwarding your letter and accompanying materials to that office for review and appropriate response.

We regret the difficulties that you have encountered at the University of Waterloo, and we sincerely hope that these matters can be resolved satisfactorily.

Sincerely,

Winston J. Dean, J.D., M.P.H.
Senior Advisor
Office of the Surgeon General

Government Accountability Project
1612 K Street, NW Suite 400
Washington, DC 20006
202-408-0034 fax: 202-408-9855
Email: gap 1@erols.com Website: www.whistleblower.orglgap

11 September 1997

Edward A. Greenhalgh
265 Regina St. N., Apt. 7
Waterloo, ON
N2J 3B9 CANADA

Dear Mr. Greenhalgh:

Thank you for contacting the Government Accountability Project (GAP). As you may know, GAP is a nonprofit, public interest organization committed to promoting government and corporate accountability by providing assistance to employees who "blow the whistle" on fraud, waste, abuse of authority, and threats to the environment, public health, and safety. Assistance and services offered by GAP include legal representation, advice and/or advocacy, media outreach, political outreach, and other support for whistleblowers. If you have access to the Internet, you may wish to survey some of GAP's past and current work by visiting our website at www.whistleblower.org/gap.

This correspondence is to acknowledge receipt of your recent letter and documentation. Please allow a couple weeks for GAP attorneys to give your information proper attention and review. GAP is, unfortunately, working with a small budget and staff thus slowing down the expedience of our intake process. We apologize for this inconvenience.

You should also be aware that there are statues of limitations on most legal claims, and failure to observe these deadlines could result in possible legal claims being time-barred. Please note that if you are in need of immediate legal assistance, you should seek representation outside of GAP to ensure that any time limitations are met.

For the past twenty years, GAP has dedicated its effort to supporting whistleblowers across the country. Unfortunately, we do not have the staff capacity and resources to become involved in every worthwhile claim, legally speaking. We will, however, make every attempt to review your request and concerns in a timely manner and offer our assistance however possible, be it through legal advice, media outreach or other means of support. We thank you for your patience, and will be in contact with you in the near future.

Sincerely,

Rod Frey
Intake Director

P.s. Enclosed is our recently published newsletter 'Survival Tips for Whistleblowers', if you have not received one. I hope you find it helpful.

U. S. Department of Justice
Office of the Inspector General

April 9, 1997

Edward A. Greenhalgh
265 Regina Street N., Apt. 7
Waterloo, Ontario
N2J 3B9 Canada

Dear Mr. Greenhalgh:

The purpose of this letter is to acknowledge receipt of your recent correspondence. The matters that you raised have been reviewed by the staff of the Investigations Division, Office of the Inspector General.

The primary investigative responsibilities of this office are:

- Theft, fraud and bribery committed by U.S. Department of Justice employees and contractors; and

- Waste and abuse by high ranking Department officials, or that affects major programs and operations.

This Office does not have jurisdiction in the matter you described. We suggest that you consult with private counsel or a legal aid organization to determine what remedies, if any, are available to you.

Sincerely,

Roger M. Williams

Special Agent in Charge
of Operations
Investigations Division

SUPREME COURT OF THE UNITED STATES
OFFICE OF THE CLERK
WASHINGTON, DC 20543

WILLIAM K. SUTER

CLERK OF THE COURT

December 16, 1996

Edward A. Greenhalgh
265 Regina St. N.
Apt. 7
Waterloo, Ontario
N2J 3B9 Canada,

RE: Edward A. Greenhalgh

Dear Mr. Greenhalgh:

In reply to your letter or submission, received December 13, 1996, I regret to inform you that the Court is unable to assist you in the matter you present.

Under Article III of the Constitution, the jurisdiction of this Court extends only to the consideration of cases or controversies properly brought before it from lower courts in accordance with federal law and filed pursuant to the Rules of this Court. The Court does not give advice or assistance or answer legal questions on the basis of correspondence.

Your papers are herewith returned.

Sincerely,
William K. Suter, Clerk
By:

Clayton R. Higgins, Jr.
(202) 479—3019

Enclosures

U.S. Department of Justice
Civil Division
Office of the Assistant Attorney General *Washington, D.C. 20530*

November 9, 1994

Mr. Edward A. Greenhalgh
265-7 Regina St. N.
Waterloo, Ontario
N2J 3B9 Canada

Dear Mr. Greenhalgh:

Thank you for your letter of October 3, 1994, to Attorney General Janet Reno, requesting an investigation into allegations of scientific misconduct by the University of Waterloo (Canada) and Collaborating Extramural Researchers to suppress research contrary to their shared and associated research grants.

We have carefully reviewed the materials presented in your correspondence, and based upon your description of the allegations, it would not be appropriate for the Department of Justice to take any action with respect to these matters since we are not a scientific investigatory agency.

According to your letter, you have provided copies of your correspondence to the National Institute of Health and the Inspector General's office of the Department of Health and Human Services, which are the appropriate bodies for review of such matters.

Therefore, we regret that the Department of Justice cannot be of further assistance to you in this matter. Thank you for your inquiry.

Cordially yours,

Frank W. Hunger

MFH:AK:MOConnell
46—92—2

P.O. Box 261 Ben Franklin Sta.
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November 13, 1995

Mr. Edward A. Greenhalgh
265-7 Regina Street, North
Waterloo, Ontario
N2J 3B9 Canada

Dear Mr. Greenhalgh:

Your correspondence to the Attorney General and to Douglas E. Crow, Deputy Chief, Organized Crime and Racketeering Section, Department of Justice, requesting an investigation into allegations of fraud by the University of Waterloo, Yale University, and certain individuals regarding a training grant made by the National Institutes of Health ("NIH"), has been copied to this office. We have forwarded copies of both sets of correspondence to the Legal Advisor, National Institutes of Health, and the Acting Legal Advisor, Office of Research Integrity, United States Department of Health and Human Services, for proper consideration.

Thank you for your letters.

Sincerely,

MICHAEL F. HERTZ
Director
Commercial Litigation Branch
Civil Division

MINIREVIEW

Oxygen radicals and Reactive Oxygen Species in Reproduction (43321C)

JOHN C. M. RILEY AND HAROLD R. BEHRMAN

Reproductive Biology Section, Departments of Obstetrics/Gynecology and Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

Abstract. Free radicals and reactive oxygen species play a number of significant and diverse roles in reproductive biology. In common with other biological systems, mechanisms have evolved to minimize the damaging effects that these highly reactive molecules can have on reproductive integrity. Conversely, however, recent findings illustrate the constructive roles that oxygen radicals and reactive oxygen species play in a number of important junctures in the development of germ cells and the obligate endocrine support they receive for the successful propagation of the species. Specifically addressed in this review are some aspects of sperm development and action, the uterine environment, oocyte maturation and ovulation and corpus luteum function and regression. (P.S.E.B.M. 1991, Vol.198)

In this review a relatively new group of cell and tissue .regulators in the reproductive system is addressed, namely, oxygen radicals and other reactive oxygen species such as hydrogen peroxide. Beyond their role in the thyroid and the immune system, much of what is known about free radical chemistry in biology concerns damaging or pathological processes, including aging, cancer, radiation damage various diseases and toxicity of xenobiotics. Here, however, a different perspective will be brought to bear, where potentially important functional roles of oxygen radicals and hydrogen per-oxide are discussed with respect to cells and tissues of the reproductive system. Most tissues of the reproductive system have an intrinsic plasticity with a host of developmental and regressive states. It may not seem unnatural, therefore, that oxygen radicals and associated agents may serve as important mediators in tissue remodeling, hormone signaling and steroidogenesis, and germ cell function. While this field of investigation is just emerging, an overview of the early information in this area is presented, as well as a brief review of the general nature and actions of oxygen radicals and reactive oxygen species.

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0037-9727/91/1983-0781$3.00/0

Free Radicals and Reactive Oxygen Species

Free radicals have been described as molecular entities that contain at least one unpaired electron in a given atomic or molecular orbital (I). The generally enhanced reactivity of free radicals over more stable molecules results from the fact that more energy is required, for example, to maintain two separate species each with an unpaired electron than to allow them to come together and share electrons such that a filled molecular orbital is formed, with the attendant formation of a covalent bond. The reactivity of a free radical is inversely related to its stability.

Oxygen radicals are intermediate, short-lived species produced by the reduction of oxygen (addition of electrons), ultimately forming water. The addition of a single electron to oxygen leads to the formation of the superoxide anion radical, gaining another electron produces hydrogen peroxide, and trivalent reduction generates the hydroxyl radical. Some enzymes catalyze either single (NADPH oxidase) or double (glucose oxidase) electron additions that form superoxide or hydro

0013-7227/91/1284-1749$03.00/0
Endocrinolgy

Vol. 128, No. 4
Printed in U.S A.

In Vivo Generation of Hydrogen Peroxide in the Rate Corpus Luteum during Luteolysis*

JOHN C. M. RILEY AND HAROLD R. BEHRMAN

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Abstract. The hypotehesis that bydrogen peroxied generation occurs in the corpora lutea of superovulated rats during luteolysis was tested using a peroxide-dependent inhibitor of catalase, 3-amino-1,2,4-triazole (AT). Luteal regression was induced during midpseudopregnancy by injection of 500 ug prostaglandin F2a (PGF2a) 1 h before administration of AT (0.1 g/kg ,ip) and was confirmed by progesterone analysis of peripheral blood serum. Within groups of both PGF2A-treated and untreated control rats, other rats also received ethanol (0.2 g/kg, ip), which prevents hydrogen peroxide-mediated inhibition of catalase by AT> Diluted homogenates of ovaries removed 1 h after AT administration were assayed for catalase activity by measuring the decrease in absorbance at 240 nm for 30 sec after the addition of bydrogen peroxide (10 mM). Ethanol-sensitive catalase inhibition by AT was significantly higher (47.9 + 3.38%) in samples from PGF2a-treated groups than in controls (23.1+4.82%;P<0.01;n+9). Similar increases in catalase inhibition by ATt were found inluteal tissue of rats treated with PGF2A 24 h earlier and in rats in which luteolysis was allowed to occur spontaneously in late pseudopregnancy. Hemoglobin and AT assays revealed that the changes in catalase activity were not the result of altered blood contamination or AT concentration in the luteal homogenates. Since catalase inhibition by AT is only seen in the presence of hydrogen peroxide, these results support the conclusion that an early and sustained component of corpus luteum regression is the generation of hydrogen peroxide in luteal tissue.
(*Endocrinology* 128: 1749-1753, 1991) *see thesis page 200-201*

That reactive oxygen species (including hydrogen peroxide and free radicals, such as superoxide and hydroxyl radical) are generally deleterious to tissue function is underscored by the ubiquitous presence in both prokaryotic and eukaryotic cells of enzymes that serve as detoxifying agents. Some of these include superoxide dismutase, glutathione peroxidase, and catalase. Reactive oxygen species have been implicated in immune responses (1,2) and inflammatory processes (3) and have been suggested to be a component of the aging process (4,5).

Regression of the corpus luteum is an example of degeneration of cellular function, which is a normal and necessary part of the reproductive cycle. Perhaps the first indication that oxidative processes are involved in ovarian function was the observation that LH causes depletion of ascorbic acid in the corpus luteum (6). The luteolysin prostaglandin F_2a (PGF_2a) also depletes ascorbic acid, and a similar effect is seen in natural regression (7,8). It has been reported that along with LH-induced ascorbic acid depletion in the ovary are increases insuperoxide dismutase and peroxidase activities (9). Recent studies have shown that superoxide generation occurs during luteolysis (10) and that as little as 50 uM hydrogen peroxide has luteolytic effects on ovarian cells, including depletion of aTP- and gonadotropin-dependant cAMP and progesterone production (11,12)

A difficulty in examining the role of reactive oxygen species in cellular events is their short lifespan; this problem is exacerbated with *in vivo* studies. One approach for observing *in vivo* production of hydrogen peroxide is measurement of catalase inhibition by 3-amino-1,2,4-triazole (AT) (5, 13, 14). Catalase is a cytosolic enzyme that converts hydrogen peroxide to water and oxygen via an intermediate catalase-peroxide complex termed compound I. Compound I combines with AT to form a permanently inactive compound. Consequently, AT irreversibly inhibits catalase activity only in the presence of hydrogen peroxide, and the resultant degree of catalase inhibition can be used to gauge the relative quantity of hydrogen peroxide present. Compound I can oxidize ethanol and other alcohols to aide-hydes and in the process be regenerated to catalase. Inhibition of catalase by AT via compound I inactivation is prevented in the presence of excess ethanol, which effectively competes with AT for compound I. Thus, intact animals can be treated with AT in the presence and absence of ethanol for periods of time, and the degree

Received November 2, 1990.
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2514 Hydrogfen Peroxide Generator in Regressing Rat Luteal Cells, JCM Riley. C. Leranth and H.R,.Behrman, Reprod. Biol. Sect., Depts. Ob/Gyn and Pharmacology, Yale University School of Medicine, New Haven CT. (Spons. by J.L. Luborsky.)

Hydrogen peroxide (H_20_2 ……abrupt alytic response in rat luteal cells characterized by desensitization of gonadotropin receptors and abrogation of progesterona synthesis. The objective of the present study was to assess whether the production of H_20_2 is a component of luteolysis using biochemical and ultrastructural methods. Experimental: Catalase is inhibited by amiriotriazole (AT), but only in the presence of H_20_2 which provides an in *vivo* method which provide *a* specific assy of H_20_2 (Biochem J74:339,1969). Rats were treated with a luteolytic dose of PGF_{2a} (0.5 mg/rat) for 1 hr followed by AT (O.1 g/kg) ± ethanol (0.2 g/kg). Luteal tissue was removed 1 hr later and assayed for catalase activity. In the morphological studies, luteal slices were incubated with $CeC1_3$ which forms an electron-dense precipitate in the presence of H_20_2 (J. Cell Biol. 67:566, 3975). Results: Peroxide-dependent inhibition of catalase activity by AT In PGF_2 -treated rats was 45.9 ±3.5%, signiflcantly greater than that found with control animals (21.0 ± 4.3%, 5—10, pcO.01). This effect is unlikely to be pharmacological, since rats whose corpora lutea were allowed to regress naturally showed levels of catalase inhibition similar to $PGF_{2\ a}$-treated animals (38.1±2.1%, *n-7).* Luteal tissue treated with $CeCI_3$ revealed dense staining around lipid droplets that was more Intense in samples $PGF_{2\ a}$ from treated rats. Conclusions: An early and sustained component of luteal regression Is the production of H_20_2, which appears to be generated in the lipid droplets of luteal Cells.

2516 Localization of Glvcogen Svnthase Activity in Liver of Fasted Adrenalectomized Rats Prior to and After Injection of Desamethasone. LL Michaels. T. Shepard and R.R. Cardell Jr.. Dept. of Anatomy and Cell Biology. Univ. of Cincinnati College of Medicine. Cincinnati. OH 45267-0521.

Hepatic glycogen synthase (OS) activity was localized in normal and adrenalectonsized (ADX) overnight fasted rats and in ADX rats oversight fasted and injected with 2 mg of dexamethasone (DEX) to stimulate glycogen synthesis, 2-6 hr prior to sacrifice.' Overnight fasting reduces hepatic glycogen content more substantially in ADX rats than in normal rats. Liver from anesthetized rats *was* immediately frozen in isopentane cooled by liquid nitrogen. Frozen sections were incubated in medium containing substrate lot total OS activity. Sections front normal fasted rats revealed limited dispersed enzyme activity in a few periporsal and centrilobular hepatocytes. In contrast, in hepatocytes of ADX rats GS activity appeared *as* large aggregates of glycogen (the reaction product) in specific cells within the lobules. The aggregates were stained with both iodine and FAS and were removed by treatment with amylase confirming that she aggregates were glycogen. Also. aggregates increased in size during incubation in she medium for GS activity. Two hours after injecting rats with DEX, hepatocytes showed dispersed activity as well as some aggregates of reaction product. GS activity *was* evident with iodine staining in more cells after 4 hr and after 8 hr of DEX treatment virtually all hepatocytes contained a large amount of reaction product. Controls were negative except at the eight hr interval some hepatocytes exhibited pale—staining, pre-existing glycogen that could be distinguished from reaction product. The results suggest that GS that is responsive to incubation medium becomes concentrated in limited regions in hepatocytes of ADX rats after fasting. Stimulation of glycogen synthesis with DEX results in reaction product becoming more dispersed within cells and more hepatocytes displaying GS activity. (Supported by NIH **DK* 27097)

2518 Cell number and Morphometrv of Corpora Allata from Larval and Ovarlectomized and Normal Adult Panale Dinlontera punctata.

GD. Johnson and B. Stay. Department of Biology. University of Iowa, Iowa City. IA 52242.

Corpora allata (CA) of the cockroach Diploptera punctata respond to hormonal signals by changes in the rate of juvenile hormone (311) production. To further undarstand this response. we save measured morphological parameters of CA cells from: 1) small and large active glands (mid-stadium penultimate larva and mated adult females at the peak of the first vitellogenic cycle) and 2) small and large inactive glands (last instar larva, and virgin adults and ovarectomised. mated adults). Gland volume; volume of neucoplasm cytoplas, and neurosecretory endings; cell membrane; number of cells: and JH synthesis were measured. In most physiological states hormone synthesis correlated positively with cell number, gland volume, cytoplasmic volume and cell membrane area and negatively with neurosecretory ending volume and nuclear/cytoplasmic ratio. However glands of penultimate larva and ovarlectomized adult females showed an increase in cell number , CA volume, and cytoplasmic volume while they maintained a low level of hormone synthesis.

2515 Heterotypic Celll Contacts with Chromaffin Cells Induce Vasculogenesis in the Developing Neonatal Rat Adrenal Medulla. P.I.Lelkse and BR. Unsworth', (1) Dept. Medicine, Univ. Wisc. Med. School, and (1,2) Dept. Biology. Marquette Univ., Milwaukee, WI 53201

The long term goal of our research is to investigate the novel concept that. heterotypic cell interactions between vascular endothelial cells (ECs) and parenchymal cells modulate organ specifIc differentiation. In the adrenal medulla neural crest-derived cells of the sympathoadrenal lineage locally differentiate into neuroendocrine chromaffin cells (CCs) which, in the mature gland, are found in close proximity of fenestrated capilliary ……cells. To test our hypothesis, we are studying the ultrastructure of the rat adrenal medulla during neonatal development front the vantage point of CC-EC interactions. In medullae harvested immediately after birth, *we* observed close apposition, without an intervening basement membrane, between CCs, erythrocytes and Indistinct mesenchymal cells (MCs) within apparently avascular clusters of CCs. Heterotypic interactions between CCs and MCs include junctional contacts. Immunofluorescence using frozen sections suggests that MCs' might be endothelial cell precursors. After three days, MC5 in contact with CCs are found so develop EC-like extensions. including fenestrae, which begin to line newly formed true

vascular spaces. Our findings raise the possibility that heterotypic contacts with CCs might be required for vasculogenesis, yjz the in situ transdifferentiation of endothelial cell precursors Into fenestrated endothelial cells. Taken together with our previous report of EC-induced in vitro transdifferentiatjon, of PCI2 cells toward the CC phenotype, (Mizrachi et al.. PNAS, 1990, in *press),* these ultrastructural observations support our working hypothesis that bidirectional interactions (via heterotypic cell contacts and humoral factors) between ECs and parechymal cells provide pivotal cues for organ specific differentiation.

2517 Growth and Differentiation in Normal Human Bronchial Epithelial Cells: Effects ot Formaldehyde. C.D. Albright, R.T. Jones, P.M. Grimley, B.F Trump, and J.H. Resau, Department of Pathology, University of Maryland School of Medicine, Baltimore, MD 21201 and USUHS*, Bethesda, PD 20892.

Formaldehyde at a non-lethal concentration (<30 m.M) has previously been shown to cause an 8—fold increase in cytosolic calcium. Since changes in cytosolic calcium, influences growth and differentiation, we have studied the in vitro effects of formaldehyde on growth, differentiation and the cell-to-cell communication of normal human bronchial epithelial cells (NBE). These cells were isolated using protease from a piece of mainstem bronchus obtained at autopsy and cultured in low calcium, serum free medium. Following exposure to varying concentrations of formaldehyde (3, 30, 300 opt) the cells/colony (C/C), population doublings/day (PD/D) and the mitotic index (MI) as well as the morphological characteristics were determined arid compared to non-treated controls. The number of C/C decreased fram 43 to 17, the PD/D fram 0.75 to 0.57 and the MI 2.3 to 0. Cell-to-cell communication was quantified by fluorescence recovery after photobleaching of 5 (arid 6) carboxyfluorescein labeled NBE cells using an ACAS 470 laser microscope. Formaldehyde inhibited cell-to-cell communication between bronchial epithelial cells (27 to 83 % of control) . These data indicate that NBE cells respond to formaldehyde by growth inhibition arid terminal differentiation. Supported in part by the Medical Free Electron Laser Program of the Solo at USUNS (MAALT Project GM74AQ].

2519 Effects of Secretin and Caurelein on Pancreatic Digestive enzymes in Cultured Rat Acinar Cell,. KK Hirschi and P.M. Brannon. Dept. of Nutrition & Food Science, University *of* Arizona, Tucson, AZ 85721.

Caerulein is proposed to regulate the synthesis of pancreatic proteases and amylase. Similarly, secretin is implicated in the regulation of pancreatic lipase synthesis. Supporting both proposed regulations is evidence predominately from in vivo studies. In this study, we examined the effects of caeruletn and secretin directly on cultured primary acinar cells to eliminate possible interactions of these gastrointestinal hormones with other hormones or metabolites in these regulations. Cellular *and* media enzyme activities, and relative synthesis were measured after 24 h of hormonal treatment. Cells were incubated with ^{14}*C-amino* a acids and then subjected to two-dimensional gel electrophoresis to separate individual acinar proteins for subsequent determination of incorporated radioactivity and relative synthesis. In general, enzyme activities decreased 33% over time in culture ($p<O.02$). while media enzyme activities increased (370% $p<0.00001$) in all treatment groups. Over *24* h in culture, the relative synthesis of chymotrypsin increased, while that of amylase decreased. Caerulein further decreased cellular content of all enzymes ($p<0.002$) and increased media amylase and ….activities (p,0.02) Caerulein. however, significantly increased the synthesis of trypsin (28%) and tended to increase that of chymotrypsin (25% $p<0.06$), which supports its proposed role *in* protease regulation. Secretin on the other hand, did not significantly affect the cellular or media activities of the relative synthesis of any pancreatic enzyme evaluated; therefore, this study does *not* support the proposed direct role of secretin in lipase regulations. Further the cellular content of digestive enzymes in vitro, unlike in vivo, are not their apparent synthetic rates, possibly because of altered secretion. (Supported in part by NIH DK32690).

[redacted] All other comments are Carlson's criticisms EAG-98

Pages from Discussion.....of suppressed thesis EAG

Please note enclosed comments are those of J.C. Carlson

24h after PGF_{2a} injection. Thus the results in Figure 1A appear confirmed by the literature demonstrating that a major effect of Regression in luteal cells is a loss to form steroidogenic responses to stimulation.

Although Figure 1A would appear to substantiate the method as a good model mirroring physiological responses in vitro, other issues needed to be addressed before making a concrete conclusion.

Figure 2 represents experiments towards this end. Figure 2A was a series of experiments testing the use of 0.1% FBS as a medium supplement, plus methodology of specimen sacrifice (there was an instability in the colony, and it was considered that perhaps CO_2 asphyxiation caused trauma): CO_2 asphyxiation vs. sodium pentabarbitol (NaPB) peritoneal lethal injection for the Saline-Control.

In the serum—free medium of Figure 2A the same hCG treatment range as in Figure 1A was used and the Saline—Control CO_2 expired luteal cell suspension exhibited the same biphasic pattern (Tukey and Dunnett's test, $p < 0.05$). The same 4—5:1 ratio Saline—Control:P24—Regressed was also evident (Tukey

Test, $p < 0.05$). Similarly the P24—Regressed failed to demonstrate any steroidogenic response other than basally by hormonal treatment as before. Therefore, the cells were functioning as previously noted, and therefore could be used to contrast the other methodologies.

More interesting were the NaPB treated Saline-Control luteal cells. They exhibited the same response as the P24—Regressed luteal cells. Such action would denote these as dying cells; or atretic as reported by Uilenbroek et al. (214) where the degeneration of large pre—ovulatory follicles was induced by Nembutal injection. Recall from the Introduction that atresia and regression have been considered similar (if not the same) processes. They (214) also found decreased estradiol production and increased 20a—OHP concentration, which has been implicated in the introduction as a possible 1ute~rlytic mechanism. Then the response of the NaPB luteal cells supports the view that the regressing CL is a dying cell complex (39) since these and the P24—regressed cells gave identical steroidogenic responses. The contention is further supported by the observation that the degenerating

cells of regressed CLS have cytoplasmic structures gragment and then the cells lyze and phagocytes invade the region to dispose of the debris (39. 169,200,207).

Figure 2A was one study, each cell suspension prepared from 4 rats, the tests all run in triplicate, and because the Saline—Control serum—free tests matched Figure lA so closely, then the NaPB group's response should be considered a typical result of such treated luteal cells. Statistically, it was the experimental result from the body of a larger work and as such should be acceptable: especially since a paper proposing a luteolytic theory (256] required only one ewe as an experimental model. If the aforementioned is acceptable, then a result supported by literature, in a large body of work should also be acceptable.

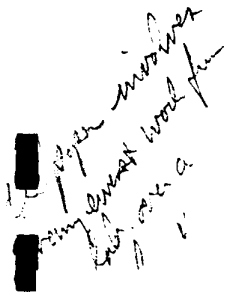


Figure 2A also compared serum—free medium vs. the same medium augmented by 0.1% FBS. This was to discern if FBS would provide beneficial or inhibitory properties to the cell suspension. The first striking result seen in the Saline—Control suspensions was the decrease in progesterone concentration of the FCt1 compared to Ctl by approximately 50% (Tukey test

Nevertheless, this thesis inherited the experimental design verbatim from Riley and Carlson (48), and as such it was this methodology that was compared to the luteal cell suspension for the comparison; and contrasted for physiological function, or the lack therein.

A variation from (48) was the use of the modified membrane preparation (76) with a further modification of using EPPS/Saline buffer. This was done because this buffer has been reported to interfere in calcium studies (61), phosphate buffers interact with the phosphate quantification of Na+-K+-ATPase enzyme assay, and to match the physiological plasma concentration seen in vivo. It has been reported in humans and other species that the electrolyte concentration in the follicular fluid is very similar to that of blood plasma, sometimes slightly exceeding the serum concentration. Since the majority of biological studies generally accept physiological saline as a standard, it was included in the dextranglycol biphase membrane preparation, polarization membrane preparation medium and the enzyme study buffers.

however, there are no reports of this. Since their method must be taken on trust, then similarly this thesis preparation based on their methodology assumes the same latitude. Further, it should be noted that Bangham et al. (258) used a salt solution as their aqueous phase, and therefore such use in this thesis has precedence.

There is one other major deviation from accepted practise in the technique employed by Riley and Carlson (48), and therefore also incorporated into the experimental design of this thesis, and that is the preparation and study of a lipid suspension under normal atmosphere. Indeed part of the mixing of the probe and suspension is via pasteur pipette, and frothing the mixture. The accepted practise is to, at least prepare, and work with lipids under an inert atmosphere and possibly incorporate an anti—oxidant (124,125,126) and/or EDTA (208) into the aqueous phase since unsaturated lipids are extremely labile and undergo oxidation/peroxidation damage readily (42,152,153,208). Of the reference papers many authors used in some stage of preparation a nitrogen atomosphere (6,9,13,14,17,18, 22,25,26,33,35,40,44,62,64,71, 72,73,78,81,

84,122,123,126,130,134,135,136) and argon (101,124,125, while only a limited number used normal atmosphere (47,48,97,103,131). The aforegoing emphasizes that there is a large understanding in the majority of workers that possible damages exist via oxidation processes to lipids and some redress to the problem made. Oxidation damage was found to be critical in the explanation of the results seen and will be-pivotal to further discussion.

Figures 4 to 6A were the earliest study on the membrane suspension design adapted from Riley and Carlson (48) and was therefore performed at 40°C. These figures clearly show a calcium dependency on the polarization results. The study can be enhanced by comparing the slopes (m) of the preparations allowing insight into the forces acting on them. In Figure 4A, at 1.0 mM calcium concentration, for the first 35 mm. the P24—Regressed suspension was changing its polarization values twice as fast as the Saline—Control suspension but for the next 30 mm. interval the rate was reversed with the former slope approaching 0, until near the end both preparations slopes approach 0 indicating that the rate of change was completed. Both

interaction. As noted in all thesis figures where calcium was excluded from the preparations rigidification does not occur and the line remains horizontal. The author concludes rigidification was due to ionic interactions because to make the statements about Mg^{2}+ ion requires background information that the authors (48) apparently lack. Harris (81) working with PS micelles in 100 mM NaC1, 5 mM Tris—HC1, 0.2 M sucrose medium (pH 7.4) found that divalent ions have pronounced effects on polarization (10 mM concentrations) and the changes caused by Mg^{2}+ were <u>smaller</u> but similar to those caused by Ca^{2}+. Jacobson and Papahadjopoulos (71) have shown that 1 x l0-^{3} M Ca^{2+} abolishes phase transition of both phosphoglyceride and PS in a range of 0—70°C whereas 5 x i0'^{3} M Mg^{2}+ only broadens the transition, but does not abolish it. The point here is that even 5 X the level of calcium's, Mg^{2}+ is not a good substitute. Further, it is known that in biosystems that $Mg^{2\sim}$ is <u>only effective</u> at lOX higher concentrations than that of Ca^{2}+ and appears to form <u>less solid</u> membranes (195). Therefore, for Riley and Carlson (48) to form such a statement they would have had to had used lO X their calcium concentration levels with Mg^{2}+: they did not.

thesis the penetration of t—PNA and $Ca^{2}+$ are part of the polarization response: how many bilayers are involved? It has been shown that t—PNA uptake by MLV dispersions involves at least two processes after binding to the outer leaflet. 1. flip—flop (these are no longer biomembranes in <u>situ</u> but are bilayers lacking microtubules and microfilaments) with the subsequent transfer across an aqueous compartment to inner lamallae (125]. Kornberg and McConnell (125) suggest that the fatty acid flip—flop is fast while the transfer steps are slower. They (125) also note that fluorescence changes are generally highly reversible in the <u>absence</u> of free <u>radicals</u> and <u>oxygen.</u> Calcium has been reported to increase the cation permeability of liposomes made from PS such that $Ca^{2}+$ added to one side of a membrane destabilizes same so creating an increased permeability, whereas if added to both sides produces a highly stable membrane (195). This would suggest the added calcium and t—PNA should establish some sort of equilibrium mixture in MLVs. Although vesicles formed from biornembranes would probably favour a natural orientation the aforegoing establishes some parameters so the responses reported should be accepted

The Saline—Control has increased its rate of change while the BW755c treated (P24—Regressed suspensions) have decreased or stopped, virtually remaining in one polarization state. Why? The answer lies in the true mechanism of action for BW755c.

Generally, BW755c is accepted as a lipoxygenase/cyclooxygenase enzyme inhibitor preventing PG formation in vitro and in vivo (165). This is relevant because arachidonic acid metabolism and PG biosynthesis via the cyclooxygenase pathway is involved in the luteolysis of a number of non—primate species, including sheep, guinea pig, cow, pig and rat (198). Now it is important to review some regression theories. The counter—current mechanism operating between the uterine vein and ovarian artery allows uterine PGF2a to reach the ipsilateral ovary and so avoiding metabolism in the lungs (198). Pulsatile PGF2a discharged into the uterine venous blood increases toward the end of the luteal cycle, possibly as a consequence of estradiol and progesterone secretions by the ovary (261). In ewes, changes in the uterine PGF_2 secretion have been correlated to increased uterine cyclo—oxygenase activity (198,262). A slight luteal PG

increase has been shown in naturally regressing porcine CL coincident with decreased progesterone secretion (30).

The mechanism by which PGF2a induces regression is a controversy. It may cause vaso constriction, loss of LH receptors and the decreased production of CAMP and progesterone (198). Recently the hypothesis has been suggested that $PGF_{2\sim}$ can antagonize the stimulating action of LH on luteal adenylate cyclase and this may be the basis for its luteolytic action. Support for the hypothesis has been shown with experiments in vitro and in vivo on the rat (198,260). To this Wallach (87) reports PA2's role in the arachidonic cascade: the enzymatically catalyzed oxidative transformations of arachidonic acid leading to the formation of PGs and other eicosanoids, to provide substrate for PG formation. To this, Patek and Watson (88) reported that the ability of the rat CL and bovine ovary to synthesize $PGF_{2\sim}$ has been shown in vivo, converting aracidonic acid to PGF2a. Riley and Carlson (48) report on increased arachidonic acid in regressing luteal tissue which may have two implications: 1. arachidonic acid is the precursor of

PG5 via the cyclooxygenase system in luteal cells; 2. superoxide anion ($0T_2$) is also formed by lipoxygenases which utilize arachidonic acid as substrate. Indeed, Hemler et al. (170) have found that a variety of peroxides can accelerate cyclooxygenase to produce PGs. Lipid hydroperoxides formed by lipoxygenase were the most potent activators (of cyclooxygenase) with the next potent being the endoperoxides and then peroxides non—specifically formed during arachidonic acid autooxidation. They (170) stated that a peroxide tone was required to maintain optimum cyclooxygenase action, and actions, including agents which non—enzymatically reduce peroxides (i.e., certain sulfhydryl compounds) or prevents the occurrence of peroxidation (anti oxidants) can lower this tone.

Therefore, at first glance, the responses shown in Figure 7 appears to be a breakthrough; a chemical agent known to interfere in the PG cascade at the enzymatic—cyclooxygenase level (BW755c (164,165,167)) causes a fluidity change (decreases rigidity). However, having established that changes noted thus far were $Ca^{2}+$—anionic head group interactions, another

answer must exist. It is a very intricate one an requires a chemistry background to appreciate.

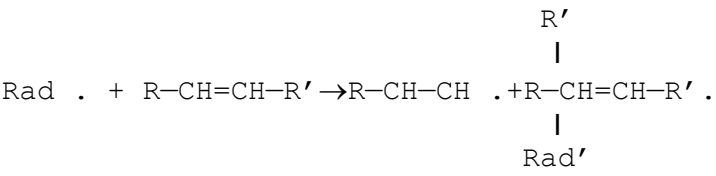
The first clue is provided by Lengfelder (who asked if anti—inflammatory drugs (a class in which BW755c belongs) act as oxygen radical scavengers Lengfelder notes that oxygen radicals and other activated oxygen species are common products of cell metabolism of which superoxide radicals are pivotal being derivative for H_20_2, hydroxyl radical (OH) and singlet oxygen (102). He continued to state that superoxide radicals are produced by neutrophils and macrophages and during enzymatic activity of cyclooxygenase/lipoxygenase; and one way to follow superoxide radical formation is by lipid peroxidation and membrane damage. He notes that the copper complexes of non—steroidal anti—inflammatory drugs (NSAID5) such as salicylate and various derivatives, pencillamine, indomethacin, and prioxicam can eliminate superoxide radicals (160). And along these lines of testing, 755c has been shown to be an antioxidant/free radical scavenger (163,168). Therefore, the actions shown in Figure 7, and by extension all the

polarization studies shown involve oxygen radicals, radical damage and scavenging.

The question now becomes, what is generating the oxygen radicals, and consequent lipid damage? Please note the earlier discussion concerning other researchers with lipids and the use of inert atmosphere (N_2, Ar), and antioxidants in their lipid preparations, plus the fact that lipids are extremely labile to oxidation/peroxidation damages (152,153). Then note that this membrane preparation was not prepared under inert atmosphere, and in fact part of the probe mixing process required frothing with a pasteur pipette: source of lipid oxidation/peroxidation and oxygen radical formation.

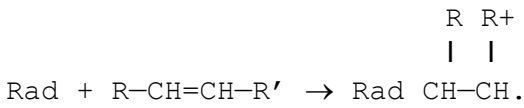
A very important organic chemical reaction is Chain Reaction (free—radical initiated) Polymerization (235). Polymerization is the joining together of many small molecules to form larger molecules. Chain Reaction Polymerization involves a series of reactions each consuming a reactive particle and producing another similar particle; each individual reaction is dependent on the previous one. The reactive particles

can be free radicals (Rad.), cations, or anions (235). General Reaction:



(new radical and reaction can repeat) The reaction continues, and therefore the chain— carrying particles are free radicals, each adding a new monomer unit, forming a bigger radical. Generally an initiator to start the reaction is a free radical, typically a peroxide (235).

An important variation is Free Radical Vinyl Polymerization: the reaction occurring at doubly bonded carbons (the vinyl groups), hence the name. The reaction involves the addition of free radicals to the double bond of the monomer:



(Chain Initiating Steps)

The reaction may be terminated by an inhibitor: many amines, phenols and quinones act as inhibitors (235).

It is then proposed that the unsaturated PL5 of the membrane vesicles were acting as the monomer units and via free radical, oxygen induced, chain reacting, interacting and forming a reticulum over the vesicle/ liposome. This network's significance becomes important shortly. Precedence for this comes from the fact that a method for increasing the viscosity of vegetable oils is by blowing them with hot air and the oils undergo chemical and physical property changes. The percentage of combined oxygen in the oil increases in the form of peroxides, carboxylic acids and other hydroxylic compounds. Oxypolymerization was noted, actually as two steps: 1. an induction period, and 2. an oxypolymerization period which produces changes at greater rates (222). Although this is an extreme case, it should be noted for what it i5: a chemical example. Further, systems using soap micelles, calcium and polarization have been employed to follow polymerization reactions (252). It is known that unsaturated lipids do undergo free radical reactions easily and polymerize to form C—C linkages rather than peptide formation (153).

Much closer to the actual work presented in this thesis is that of Hochstein and Jam (156) where they have related the association of lipid peroxidation and the polymerization of membrane proteins. They state that lipid peroxidation has been implicated in free radical reactions and membrane alterations associated with aging cells and tissues. They also feel that polymers may form in the absence of lipid peroxidation/hydroperoxide decomposition by direct radical attack. They express the opinion that this polymerization could increase the rigidity of erythrocyte membranes (156).

This is an extremely interesting theory, but what is its direct relevance to the experimental evidence shown (Fig. 7). BW755c did abolish the rigidity. Then what is suggested is that the polymers are formed either by lipid—lipid linking, or lipid— protein-linking forming a reticulum over the vesicle/ liposome. This is equivalent to introducing a restricting and segregating force creating domains of lipids inside the "holes" of the net. This restriction then allows electronic interactions between $Ca^{2}+-$ anionic PLs not normally seen; or is <u>additive</u> to the

rigidification force. Note that BW755c completely abolished the polarization change, and in fact when the slopes were compared at the later time period,

Ctl m vs. P24+BW755c m vs. P12+BW755c m

were shown to be: 7°, 2° and 0° respectively, indicative of oxidation occurring in Ctl.

What other precedents are there for this? One example comes from Riley and Carlson (48) where they add calmidazolium to their suspension claiming that calmodulin may be a second messenger or necessary co— agent for PA2. As such calmidazolium could abolish PA2's action. The literature accepts that PA2 is calcium dependent (though Irvine (172) states that it does not mean PA2 is calcium controlled), only Stocker and Richter claim indirectly calmodulin sensitivity. However, Riley and Carlson (48) claim its use as an inhibitor to a regression causing agent, but the calcium—calinodulin system is an important regulating mechanism to steroidogenesis independent of the stage of follicular maturation and cellular differentiation (90). It would seem suicidal to a cell to interlink a steroidogenic mechanism with a regression system. Riley and Carlson (48) did note that calmidazolium

caused a decline in polarization values. Then it is of considerable interest to note that Degenhart et al. (251) found that calmidazoliuin had a profound effect on O~2 and HO~ in their studies. In this light the effect of Riley and Carlson (48) can be seriously reviewed to as to what mechanism is actually operating.

Further, Stocker and Richter (173) to support their claims also used trifluoperazine (a potent inhibitor of calmodulin—sensitive enzymes) and saw the prevention of anisotropic (rigidification) increase. It must be noted that their isolated PM5 were not prepared under an inert atmosphere but free to atomspheric oxygen (173,174). Kornberg and McConnell (101) even with their precautions conceded that they could not exclude a contribution from traces of molecular oxygen and other oxidizing agents. Trifluoperazine contains heterocyclic rings with conjugated bonds (265) that are susceptible to free radical attack and is termed a phenothiazine derivative (266) which as a group have antioxidant properties (267). The hydroperoxide—induced $Ca^{2}+$ release and oxidation of mitochrondrial pyridine nucleotides are decreased in a concentration dependent manner by both

the calinodulin—inhibitor, trifluoperazine and the phospholipase inhibitor, p—bromophenacyl bromide with complete inhibition achieved (263). Richter (263) then states that any conclusions drawn from these results must take into account possible hitherto unknown effects of these inhibitors. Therefore, oxidation of their (48) preparation and concommitantly antioxidant questions can be posed.

Further evidence for this free radical—lipid peroxidation polymer was also found from Riley and Carlson (48) who used heat denaturation on PGF_{2a}—treated membrane samples in boiling water for 20 mm. followed by cooling on ice. They reported that any precipitated material was resuspended by mild vortexing for 5 mm. From this they found this completely inhibited any polarization increase. They concluded that the heat treatment had had a non—specific fluidizing effect on the membranes probably by eliminating any organization of different bilayer lipid population into domains and in association with proteins (48). This seems reasonable in the light that such treatment would hydrolyze conjugated bonds (see appropriate section (235)) in the lipids and generally

destroy the phospholipids and triglycerides such that extensive polymerization could not occur. Hence there would not be the needed polymerization to be additive to the $Ca^{2\sim}$ electronic interaction for rigidification/ increased polarization to be seen.

Other evidence? The 90 min. pre—incubation experiment of Riley and Car1son (unpubl. results). They claim a maximal response after waiting 90 minutes (under normal atmosphere) and then running the experiment. From all the aforegoing (especially the industrial example and its induction period (222)) would suggest that this 90 minutes allowed the polymer to form and hence a maximal polarization expression. Nevertheless, Figure 1B, the similar luteal cell suspension did not show any enhancement of steroidogenesis. The earlier comments should be reviewed.

Other researchers have also performed pre—incubation studies, all under normal atmosphere and without anti—oxidant protection. One example is from Danforth et al. (131) who claim membrane fluidity increases (less rigid) could be correlated to changes

in gonadotropin binding. Their pre—incubation times consisted of 3h storage in the dark with either ethanol or neuramidase (a chemical which removes cell surface sialic acid and therefore unmasks binding sites). Ethanol is supposed to make membranes more fluid and therefore receptors more mobile. They found that neuramidase gave no polarization effects while ethanol caused a polarization decrease. Therefore they concluded that ethanol was an important tool to unmasking receptor sites. However, given the proposed theory (oxidation damage—polymerization), and the fact that ethanol can serve as an anti oxidant, being an •OH scavenger (223,264) would suggest an alternative explanation.

The work of Dave et al. (151,154,157) presents an extremely interesting twist on the oxidation/peroxidation theory. These are workers not using inert atmosphere, and extended pre—incubation times (30 mm. to lh) and finding decreased microviscosity associated with PG synthesis. However, it is noted (151) that the changes in membrane fluidity may relate to changes in the PL:cholesterol ratio since they had noted such changes in other studies. Regarding this aspect,

Figure 5B should be reviewed wherein P24 was more fluid than P12h and P6h. Cholesterol is interesting because it oxidizes easily (75)). Auto—oxidized cholesterol products exert expansion effects on surface area—surface pressure curves (chpt. VIII (75)) opposite to unoxidized cholesterol that causes condensation (rigidification) of membranes. As such this offers a logical, though not (and not meant to be) a complete explanation. It must be noted that polarization values (151) were 0.191 to 0.165 to 0.152 without significance testing clearly stated. This much fluctuation (though it is generally lower than my own values) was seen in the Saline—Control suspensions. Their studies were at 24—25°C and not physiological Comments were made (154,157) on the artificialness of temperatures, and questions can be raised as to if the PRL injections were physiological examples and how when the binding of PRL was lost at C that the incubation times were cavalierly shortened. Their experiments appear to be short of recreating' physiological conditions and, therefore, can they reflect physiological roles?

Perhaps more reasonable (174) was their implication that a chemical agent via oxidations (O-.2)

can elicit cellular damage resulting in membrane ordering. This too is implied in the experiments of this thesis, especially that a dying/degenerating cell was suffering such damage. Strauss et al. (40) note that in luteal demise (rat) that the increased store of triglycerides containing polyunsaturated fatty acids may leave the luteal cells vulnerable to lipid peroxide damage. Especially in light of the theory that PG5 cause chemotaxis in phagocytes, and phagocytes invade the regressing CL to clean up the debris.

Therefore, it becomes quite reasonable to accept the polarization responses shown were the results of $Ca^{2}+$ anionic head group interaction made possible due to oxidation damage polymerization. Since the regressed membranes were more susceptible, a physiological function will later be suggested. First, though, a brief mention must be made (Fig. 8) that no dilution effects were found from dilution by vehicle volume used to introduce hormone (or other treatments) to the membrane suspensions. So the effect of hormone treatment can be covered without such concerns.

manner. Luteal cells could be compared to a machine that responds to a loci of commands as each command is met independent of order (the receptors formed to be available and the combined hormones of the pituitary, placenta and uterus set the order) and to isolate the CL as a completely independent entity is not a true presentation of its reality. Rather it is a smaller unit contained within a larger cycling unit under intricate, complex, but organized control. Hence the biphasic pattern shown strongly suggests an in vivo response reproduced in vitro.

An interesting aside is that this response would not have been noted had serum or BSA been included in the medium. This supports the need and importance of the use (and development) of good serum-free media for experimentation.

The luteal cell suspension of the NaPB killed Saline—Control specimens represented the reaction of dying cells. Since the P24—Regressed cell suspensions' response appeared the same as the NaPB's this emphasized that the CL is a structure in demise, part of which is cell death. More importantly, many

steroidogenic lesions occur well beyond the PM, before changes in the same are noted. Niswender et al. (133) suggests that the internalization of the LB receptor complex is associated with the termination of its acute effect on steroidogenesis. This becomes important in regard to the plasma membrane suspension experiments.

There have been suggestions concerning regression that alterations in the PM fluidity does not allow ligand to receptor, or ligand—receptor to adenylate cyclase interactions. Given that the membrane suspension experiments were calcium cation—PL anionic head group and free—radical damage dependent, plus reports of 4.2 Bragg spacing, suggest that only physiological changes or physical damages occurred. This is emphasized by the fact that none of the preparations without calcium exhibited any polarization differences. As noted earlier, the cytoplasmic side of the PM bilayer would be more fluid and as such adenylate cyclase would not be greatly inhibited. Strulovici et al. (116) reports that changes in membrane fluidity are unlikely to be the sole cause of desensitization since: 1. pretreatment of cells with fatty acids that increase viscosity did not induce

desensitization to PSH, and, 2. the desensitization of granulosa cells to lutropin and PGE_2 by exposure to homologous hormone did not increase membrane viscosity. Ueda et al. (140) note in the rat that changes in the fluidity of the luteal cells' mitochondrial membranes always accompany rapid and permanent luteolysis; and that PGs and PRL affect the fluidity; as well as other physical characteristics in opposite directions (60, 140,151). These are physical characteristics, not, as shown by others, definitive receptor changes such as microtubule up—, and down—regulation (65,69,90,94,133, 178), phosphorylated receptor changes (100,114,175), or changes to the adenylate cyclase complex (114,159), but membrane phase changes. Phase or fluidity changes are not necessarily controlled by a cell's DNA but by responses to earlier (PM biogenesis) signals or blockage of said signals. Dying cells do not necessarily continue to replace membrane components.

It is of great interest then, if these are dying cells (regressing' cells) as to what is happening. As extensively explained throughout the Introduction that the granulosa—luteal cell has all the steroidogenic mechanisms required, but they respond

differently during different stages of maturation. One mechanism, however, that may be assumed to remain constant throughout is the adenylate cyclase complex, and it responds differently during each luteo—follicular complex stage by a changing preference to which ever receptor is available and intercommunication is possible. It is accepted that during regression that adenylate cyclase loses its ability to respond to ligand, such as LB, but may be directly activated by an antagonistic agent as NaF or epinephrine (100). This is directly contradictorial to the theorem that reduced microviscosity is inhibiting the interaction of the components of adenylate cyclase.

The above is more interesting in that adenylate cyclase (231) is now reported to have two pathways:
1. a stimulatory, and 2. an inhibitory one. Each pathway responds to its own complement of agonistsligands. Tying this to what has been observed in the rat, PGF_2 has its own receptor and the ability to antagonize the stimulation of LH on luteal adenylate cyclase which may be the biochemical basis for luteolysis in the rat (260). Accepting that regressed cells are dying cells, and PG metabolism has been noted

in these cells such formed PG5 have been implicated in the chemotoxis of phagocytes to areas of inflammation (l65) events seen at the end of the CL luteolysis and corpus albicanticans formation. This has important physiological implications to the physical actions of regression.

In agreement with the PM suspension experiments which indicated membrane disruption due to the combined actions of $Ca^{2}+$, PA2 and free radical actions, it is suggested that PGF2a receptor occupancy of the alternate adenylate cyclase pathway would allow their actions by somehow limiting LAT action, increasing cyclooxygenase production and depleting energy stores. It is further noted that under physiological conditions the divalent ion, $Ca^{2}+$ concentration (=3 mM) extra cellularly is always lower than monovalent anions (i.e., Na+ 140 mM) and phospholipids exposed to such a medium exhibit approximately 25 percent binding capacity (141,220). In luteal tissue intracellular $ca^{2\sim}$ levels are in the 0.1—10 MM range. In healthy cells, a calcium pump maintains safe levels and whose surface membrane structures (topography) is not so simple or "naked" as the vesicles in this thesis and

of the erythrocytes. Particularly important is the dual pathway for adenylate cyclase; positive for LB (and analogs) and negative for PGF_2 (and others). Therefore, at the end of the CL'S functioning, the luteal PM is not only susceptible to damages, but an apparent mechanism exists to enhance the destruction and provide chemotoxic signals to attract phagocytes to clean up the debris. All of which is one part of the continuing luteo—follicular complex cycle of the ovary.

As for the contention that membrane fluidity is important to receptor—binding (or lack of) or adenylate cyclase functioning (or hindrance thereof) no support could be provided for such from the work presented in this thesis. What the thesis does conclude, is that physical changes do occur in regressing luteal cell membranes, but they are just that: physical responses after the fact. They may be part of the mechanism providing arachidonic acid for PG biosynthesis; however, they are not the signally mechanism. The literature still places the origin of that signal deeper in the cell and an investigation of genetic expression would provide greater insight than more polarization studies could hope to.

HEALTH MONITOR

Genes and cancer

American researchers may have discovered a genetic problem that could play a role in many breast cancer cases. Scientists at the Oregan Health Sciences University and the University of California at Santa Cruz are focusing on a gene called WT1. It normally produces a protein that functions as a tumor suppressor—it controls the growth of cells so that they do not begin proliferating wildly and form tumors. Reporting in the *Proceedings of the National Academy of Sciences,* the researchers said that in two-thirds of the 21 breast tumors examined by the team, the WT1 gene was present, but the protein it normally produces was either absent or significantly reinduced. Stressing that their finding is preliminary, the researchers suggested that if a faulty WT1 gene is a cause of breast cancer, a therapy to overcome that problem might eventually be developed.

Birth Defect breatkthrough

In a breakthrough that may be applied to babies, scientists in Boston say they have repaired a birth defect in lambs by growing fetal tissue in a laboratory and implanting it in the newborn animal. A team under pediatric surgeon Dario Fauza used surgical tools less that $1/12^{th}$ of an inch wide to make tiny incision in the mothers' uteruses and remove tissue from defective bladders in unborn lambs. With cells from the extracted material, researchers grew new tissue and repaired the defective organs shortly after the lamb's birth. Medical experts said that applying the technique to human babies could solve many of the difficulties facing doctors in trying to correct infant organ defects, including cases in which the developing bladder fuses with the body wall. One problem has been that the tissue taken from other parts of the body to make repairs can cause functionoal problems later and, in some cases, even cancer. Fauza, a post doctoral fellow at the Harvard University medical school, announced his feat at a meeting of British pediatricians in Istanbul. He said he hoped to begin testing engineered tissue grafts in human babies within five years.

Earlier Diagnosis

An international team that included Canadian researchers has developed a test for ovarian cancer that could eventually save lives by identifying the disease in its early stages. Douglas Gaudette, a University of Guelph biochemist developed the test with Dr. Gordon Mills, a Canadian oncologist who works in Houston and a Japanese scientist. The method, described in the International Journal of Cancer, measures a fatty molecule called lysoPC that reaches high concentrations in the blood of women with ovarian cancer. Because ovarian cancer has no obvious early symptoms, about 70 percent of women have advanced cases by the time the disease is diagnosed. Ovarian cancer-the fifth most deadly form of the disease among Canadian women- is expected to claim 1350 victims this year.

HELLO POLLY

The British scientists who in March brought the world Dolly-the first clone of an adult animal-achieved another breakthrough by creating Polly, a cloned lamb that carries human genetic material. Unlike Dolly, who was produced from the cells of an adult sheep, Polly-a Poll Dorset lamb-and her four nearly identical sisters, were created by fusing a fetal lamb cell altered by the inclusion of human genetic material to the nucleus of a cell from a sheep's ovary. Officials of the Edinburgh based firm PPL Therapeutics said they hope eventually to establish herds of sheep carrying human genes to produce proteins and blood products for treating such diseases as hemophilia and osteoporosis.

How sweet it is for saccharin users

Saccharin- the artificial sweetener partially banned in 1979 in Canada and subsequently overshadowed by aspartame, may make a comeback. Saccharin's problems began after studies by Health Canada scientists during the late 1970's showed that exposure to the sweetener appeared to cause bladder tumors in some make rats. As a result, saccharin was banned as a food additive. Canadians can still buy it over the counter for personal consumption but package labels warn that pregnant women should not take it and that continued use may be a health hazard. For anyone in the United States, saccharin remains in general use-but with a label declaring that it can cause cancer in laboratory animals. Now Health Canada and the U.S. Food and Drug Administration are looking at saccharin again in the light of new studies suggesting that its effect on rats is specific to the rodent-and considering whether to recommend removal of saccharin's current warnings and restrictions.

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MJP14/JB

9 July 1990

Mr E A Greenhalgh
Apt 7
265 Regina Street North
Waterloo
Ontario N2J 3B9
CANADA

Dear Mr Greenhalgh

Thank you for your enquiry about research possibilities in the School of Biological Sciences at Southampton. Your application was handed to me by Professor Walker.

I read your CV with much interest, and your recent research interests seem to have much contingency upon ours. I work in collaboration with Dr Richardson at the Princess Anne Hospital; we are primarily interested in the luteinization and luteolysis of the human corpus luteum. We might be able to help you obtain a research position if you were interested in this area. Perhaps you would contact me (by Fax if possible) if you wish to find out more about our activities.

Yours sincerely

Dr M J Peddie
Lecturer in Physiology

INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH

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Edward A. Greenhalgh
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Waterloo, Ontario
Canada N2J 3B9

27th March 1990

Dear Mr. Greenhalgh

Linda Sheldrick has passed on to me your letter concerning PhD research studentships.

I regret that as a non-UK/EEC national that you would be ineligible for the UK Research Council Studentship Scheme we run at this Institute.

Yours sincerely,

Dr. R.J. Bicknell
Tutor for Research Students

King's College London

UNIVERSITY OF LONDON

DIVISION OF BIOMOLECULIAR SCIENCES

BIOPHYSICS SECTION

HEAD OF DIVISION
Professor R. M. Simmons, PhD

11 April 1990

Mr. E A Greenhalgh
265—7 Regina
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N2J 3B9
Canada

Dear Mr. Greenhalgh,

Thank you for your enquiry about entering a Ph.D programme here. I am passing on your papers to those of my colleagues who are active in your field of interest.

Yours sincerely

R M Simmons

From 6 May 1990
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1. Luteal Cell Studies. Studies to examine the possible cell death signal. There is some evidence that regressed cells are already programmed to die. First, what is a healthy functioning luteal cell must be established. Then the two types, control vs. regressed, can be compared and contrasted to what adenylate cyclase function is in each, which protein kinases and related nucleotides are activated or not and if the dual (hCG/PGF_{2a}) pathway does exist.
The progesterone secretion may be measured as a hormone response while the regions of the genome are examined for each stage. Further, the adenylate cyclase enzyme complex, nucleotides, G proteins and other components could be removed from each cell type (control vs. regressed) and tested as to their ability to function independent of their local environment (so placing hormonal control either at the membrane or other location, i.e. the genes).

2. To measure the progressive changes in dispersed luteal cells cultures to differing periods induced and the related progesterone secretion (and related adenylate cyclase, nucleotide, protein kinase responses). The plasma membrane can be examined for changes in the lipid population; is there a difference in the "(electro)static" charge of the plasma membrane (due different headgroups) and is such a static charge also seen in tumour cells? How does the static charge relate to lymphocyte chemotaxis/attraction or repulsion? Does the cholesterol content increase, and how are the enzymes, and nucleotides affected (or not)? What parts of the genome are activated? What phospholipids are present or lost with regression and are the eicosanoid precursors? Are these part of the negative adenylate cyclase pathway, cyclooxygenase enzyme and the cell death signal? Is this genome missing (or masked) in transformed cells? Could the genome be reactivated to terminate "immortal" cancer cells? Do cells have the phospholipid precursors for eicosanoid synthesis? Hormones and eicosanoids seem to be in a balance and eicosanoids may be part of intercellular communication.

Part of the phospholipid population change can be examined by s-adenosylmethionine (Aldomet) augmentation. Control and regressed luteal cells would be studied to see if the hormone response is due to a fluidity change, a phospholipid change due carbon chain methylation or whether the genome was methylated. Related questions would concern the effect of adenylate cyclase, G-proteins, etc. The Aldomet experiments could then be applied to transformed cells to determine a response (phospholipid? gene change? or none?).

3. Plasma membrane/Phospholipid Population Change

To design monoclonal antibodies to the regressed luteal plasma membranes. A ligand or tracer could be attached to the antibody so that the regressed cells could be tagged and identified from control cells. The tag could be a number of methods, immunofluorescent, a metallic (NMR) or radio isotope (not preferred). The method could be applied to an ovary in vitro and then ultimately in situ. The method then could be developed for tumours and the unintrusive detection in the whole body (specifically for applications in human medicine).

4. Lymphocyte/cyclooxygenase/Regression Taxis Experiments

Essentially a “barrier” culture experiment where control cells are separated by a barrier from lymphocytes by another barrier from regressed cells:

i.e. control I lymphocytes I regressed cells

↑porous barrier↑

Variations would include control/lymphocytes/control; control/lymphocytes/medium plus PGF_{2a}, and/or other agents. The theory is based on arthritis and infection studies wherein lymphocytes are guided to inflammation sites.

The agents to be tested may be:

1. To inhibit the lymphocytes: leukotrienes, interferon, NSAIDS, antioxidants and other drugs which may be under pharmaceutical consideration.

2. To promote or direct the lymphocytes: such as oxidants, essential fatty acids (components of blood, serum/plasma from cancer/AIDS victims could be considered). The study would examine various media for secreted products, and measure these products (and/or the media) on various sections of the brain.

Alternately, or inclusive, the experiment could also consist of: cells tested, barrier, brain section, to study the concept of the feedback loop (and remission). Since the hippocampus involves the emotions, remission is a valid question to study. In the brain sections, prostaglandin receptors could be studied along with related biochemical reactions and other changes.

Future consideration would involve in vivo studies on whole specimens, but these would be further along at a later date.

5. The Zygote-Ova Fusion Experiment

This model, as noted in my earlier letter, interests me and has implications for genetic engineering, cancer research, plus other areas. However, I only mention the work now as a future consideration dependent on how all the other research goes. Perhaps, these studies would be the springboard to a later research period.

RESEARCH PROPOSAL TO EXPLORE THE OVERLAP OF THE ENDOCRINE, LYMPHATIC AND NEURAL SYSTEMS TO DEVELOP DIRECT CLINICAL METHODS FROM THESE STUDIES USING THE RAT MODEL AND THE OVARY, DISPERSED LUTEAL CELLS AND UTERUS.

1. The luteal cell dispersion would study the responses of various toxins, chemicals and drugs:

I chemicals and drugs:

a. In vivo injection of pseudopregnant females. A preliminary study with selenium gave measurable results. Perhaps new drugs could be compared to established and known safe products to extrapolate levels for clinical testing, plus potential problems and pitfalls. Effects on the neural system (hypothalamus) could be measured. An early suggestion involves the treatment with NSAIDS/antioxidants to help pregnant women, with difficulty, carry to term.

b. In vitro treatment of the dispersion to study drugs/toxins and their effects on the cell's: membranes, cytoplasmic functioning and DNA.

To note how (a) and (b) may (or not) invoke the proposed cell death signal, the plasma membrane's "static charge/energy barrier" theories and the relationship to eicosanoids, intercellular communication/homeostasis (health). To examine how these studies may show an interrelationship of eicosanoids, interferons (others) and the lymphatic system to the hypothalamus/pituitary. The above may indicate how cancer/AIDS circumvent the immune system, so that intercellular communication may have important consequences to stress and remission.

2.To study tumours in the ovary and uterus and examine feedback to the 3 systems. Tumour and luteal cell membrane changes could constitute the basis for a non-intrusive early detection technique. The uterus is especially interesting because prostaglandins (PGs) formed post partum may guide lymphocytes to clean up debris (and reduce tissue mass: part of the cell death signal theory). Cancer/AIDS may circumvent such, hence a broad area of study and implication.

3.A fusion/cloning experiment using ova from the same female and via centrifugation/PEG techniques produce a zygote. The zygote could be bi-, tri- and tetra-nucleate. The result would further explain DNA and other cellular functioning. The direct clinical application would use the binucleate zygote implanted in a primed surrogate uterus. By studying the difficulties to achieve implantation of this "trophoblast", the clinical applications could lead to:

a. "sterile" fathers may be missing a chemical (sufficient quantity) to facilitate sperm penetration of the ova membrane. By forcing the cloning experiment, the treatment could uncover the required substance to augment the semen so a zygote (child) would result under more natural conditions (i.e. artificial insemination of the mother and not more complicated procedures). A direct clinical application.

b. Humans have a low success rate (plus the risk of uterine cancer) with present techniques, so solving the cloning-implantation experiment could lead to definitive fertilization treatments with less risk and higher success. Also gained would be increased knowledge on the interrelationship of the three (neural, endocrine, lymphatic) systems.

4. Using the fusion experiment, there is the possibility of fusing cancer/HIV virus within the zygote to study oncogenes and the incorporation of viral DNA (viral RNA? function) into the genome (a new species: a theory states that viruses control our evolution). The zygote through morula-gastrula-germinal layer stages could be examined for (new) chemical signals, and the effects of drugs and chemicals (Dr. Duesberg's. lifestyle theory).

HCCI

HCCI Management Services Inc.

September 11, 1992

Edward A. Greenhalgh
265-7 Regina Street North
WATERLOO, Ontario
N2J 3B9

Dear Mr. Greenhalgh:

Upon reading the documentation you forwarded to me Aug. 31st, which illustrates your impressive educational background, I can understand your sentiments on receiving notice of a job at our Resco Plant for general labourer positions. I would however, like to clarify that it is the responsibility of Resources representatives to advise all our employees after the recent business changes of any job openings available in our organization. This gesture on our part is in no way to lessen the importance of our employees' qualifications, but forwarded to all the Cambridge employees concerned.

It is a fact that our North American business oriented towards the marketing of our product line and not in the scientific research. Therefore, we cannot sponsor the research project you have presented.

I have asked Mr. Jean—Pierre Kolo to contact you in future to assess with you if there are any other avenues you could explore.

I am confident that your experience and perseverance you to a successful career and I wish you the best of luck in future endeavours.

Yours truly,

Alban W. Schuele

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25 August 1992

President A. W. Schuele
Hoechst Canada Inc.
P.O.B. 6160, Station A
Montreal, Quebec
H3C 3K8

Dear President Schuele:

I am writing an update to my 31 July 1992 letter. Your response can be considered no worse than other pharmaceutical firms. Those whose main goals are not exactly as my proposal wrote back saying so and wishing me well. The few firms where my proposal was exactly what their industry is based upon have simply avoided the issue. Although you are no worse than the industry standard; however, according to Quality Assurance, and Road Map to Problem Solving, shouldn't you want to be better?

Please note the kind reply to my request for scientific papers from Dr. Ohno (21 July 1992 - The Ben Horowitz Chair of Distinguished Scientist…). On a strictly scientific basis I receive considerable worldwide courtesy still. On a strictly scientific basis I wish to update my proposal and its benefit. Please contact Dr. Kott as I have explained the theoretical details to him; and if my theory (of evolution) is correct the benefits are immense. If I am correct, I may be able (within a year) to produce a protein responsible for remission. The protein could then be mass produced by genetic engineering. Is Hoechst going to turn such a project down? Again we can "brainstorm" the possibilities.

On a sadder note, a poor individual (24 Aug. 1992) has settled a foolish dispute with Concordia by murdering people. A tragedy. I asked you to read a Time magazine article concerning academic problems; further many people in the USA have settled dispute similarly. I, too, have been involved in an academic dispute; however, like Ms. O'Toole (and any proper pharmaceutical firm) I have retained legal counsel. McMillan and Binch is proceeding with my plagiarism charge: such a responsible firm would not do so unless they were very convinced of the validity of the case. I have watched positions in England and the US disappear while driving a forklift for Hoechst. Nevertheless, I kept a good work record, a positive attitude and paid my bills (the Province has announced it is going after students who have defaulted their loans as far back as 1965). Do I not fit your Quality Values as the type of individual your QA program states you should support.

Why not meet with me and discuss the project? Taxol will soon be on the market, so why not have an equally valid alternative? I honestly do not see you risking very much capital on the project, while the returns are potentially incredible.

…2

RESEARCH PROPOSAL

This research proposal has two parts:

One: A practical short term (one year) project to establish a work base.

Two: A longer term (to run concurrent to One) basic research project.

Both projects are eligible for government assistance and cost sharing with a private firm under existing NSERC, NRC and MRC programs. This should meet the spirit of Bill C-22.

Part One: Development of a New AIDS Test

The Federal government has set aside funding for AIDS research and should be interested in this project. Only a preliminary outline follows. On conditional approval actual cost estimate and logistics will be provided.

Broad Spectrum Analysis

Blood samples would be acquired from the Red Cross, hospitals and other clinics who would associate themselves with the project. Such involvement represents a positive and practical image to the public through a working relationship between a pharmaceutical firm, government and medical centres for medical research. The blood samples would be collected as required by law for safety and anonymously (ethical and privacy concerns) with a code designation, i.e., for collection source, disease condition, sex and age.

The first expense will be the fee applied to the participating agencies for collection and labeling of the blood samples. The samples will be transported to the testing facility. The second expense will be the fee applied to the use of the facility, equipment and any required personnel for necessary technical skills.

Blood Testing would be:

A Healthy control (uninfected)
B. AIDS
C. HERPES
D. SYPHILIS
E. CANCER – i.e. leukemia,
i.e. lupus
F. Influenza
G. Meningitis

(Concept of a spectrum because HIV may "piggyback" with other diseases.)

The chosen diseases could cover the following categories:

i. Attacks the immune system
ii. Not affect the immune system
iii. Viral vs. bacterial
iv. Affect or not the nervous system

Testing to Develop Profiles (a cataloguing)

1. Microscopic examination (L.M. & E.M.) associated with video recording and computer scanning and counting to produce a rapid comparison and contrast.

2. Blood Segmentation

   a. Fractional centrifugation methods to give:
   -plasma
   -solid segments – r.b.c.
   - other cells
   - viral, viroid, etc.
   - other (proteins, hormones, ions)

   b. Examination of Segments

i. spectrophotometric profiles (i.e., light, flame)
ii. chromatographic profiles
iii. the solid segments can be examined for their physical components, i.e., membrane lipids can be compared
iv. viroid, viral, etc., segments can be studied for known and unknown particles using accepted culture methods.

The results of (2) may be used in a dagnostic computerized spectrophotometer scanner that could use a very small blood sample (not centrifuged) to clearly and quickly diagnose a patient. The result would denote the total state - - i.e., HIV present, helper virus present, associated protein present, etc. Normal vs. abnormal health states and how advanced any disease present would also be determined.

Further, once "catalogued" and all the components (HIV, satellite virus, etc.) detected then:

i. A simple, i.e., anti-body test could be developed for a reliable "over -the-counter" AIDS test. There may be several levels of the disease and each could be identified. The potential is significant.

ii. If each state can be recognized, then different drug regimen may be used to "break the chain" and interrupt the disease with a less drastic therapy. Similarly, cancer treatments could be examined on this experimental theme.

Part Two: The Basic Research

To explore the theoretical work demonstrating the possibility that viruses are the basis of life representing a "living crystal" concept controlled by the laws of thermodynamics. One experiment would make energy measurements based on the theoretical paper's mathematical predictions (work presently in progress). Then, an experimental model would be designed: i.e., the original prototype cell (a protocyte, to coin a phrase), from a virus, a protein and a micelle/vesicle. Another area would examine viral induced lysis in cells — the actual genes activated plus the formed products from a variety of infected bacteria. These would then be compared to an evolved cell model. One such model is the luteal cell and regression lysis. By

comparing the gene sequences, lysis as evolutionary conservation may be explored. Part of the evolutionary study is the central theorem of the conservation of genes (a vivid example is the use of coral in bone surgery). Coral is quickly accepted by the body. Similar genes from two dissimilar organisms: the genes were conserved to be utilized by higher organisms.

The lysis mechanism (see my papers *v125(3)* J. Endo 1990 and the mention of a possible cell death signal), related parts and functions, should prove, on a wide scale, to be of medical importance.

Lvsis and cell death (for a variety of cells). There may be a common (conserved) gene sequence with related (i.e., enzyme) components that are activated.

The Important Occurrences: red blood cells and aging; muscle atrophy which may be healthy (i.e., the decrease in uterium size post partum) or dangerous (i.e., heart damage as in ischemia and heart disease).

Why cancer cells do not lvze. Either because the gene sequence is absent or blocked. Therefore, can the proper gene sequence be specified and the cancer cells then be given a specific signal and told to "die", i.e., with a cell specific signal drug.

Basic Research Goals

a. To discover how viruses were developed to seek and attach to the cell, etc.: all of which have significant consequences to viral control and drug delivery.

b. How self and identity of self (of the cell and the environment) were developed.

c. How control of lysis was developed with consequences to reproduction, arthritis and feedback to the brain.

d. How the colony (higher organism) was developed: healthy coexistence and its implications.

e. How the nucleus and genome evolved: how energy was stored in the nucleus and passed along.

f. Development of membranes and how the proteins (enzymes) came to be placed in same. This has implications for disease control and drug delivery.