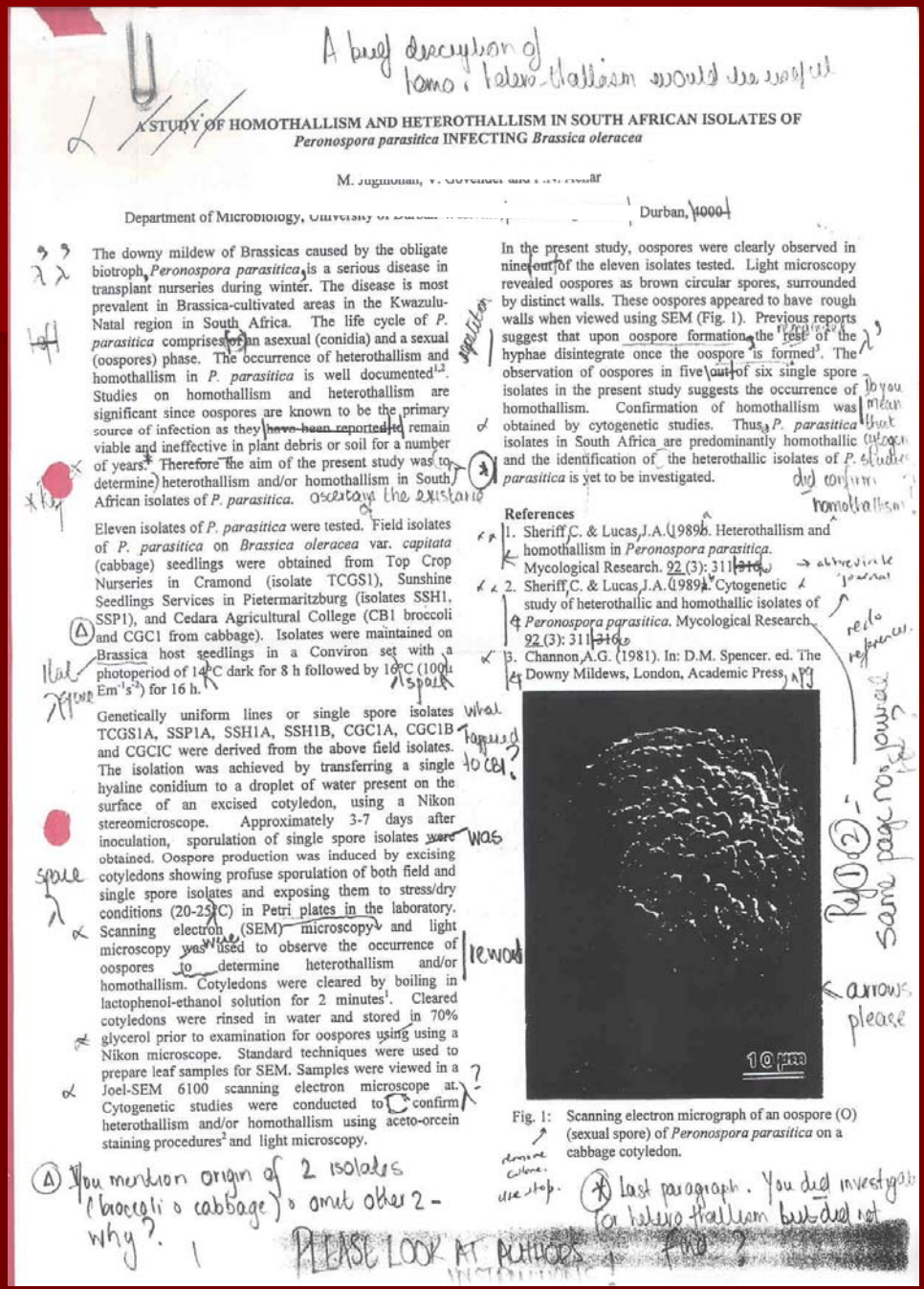

A STUDY OF HOMOTHALLISM AND HETEROTHALLISM IN SOUTH AFRICAN ISOLATES OF *Peronospora parasitica* INFECTING *Brassica oleracea*

M. Jughnohan, V. Govender and P.A. Kumar

Department of Microbiology, University of Durban, Durban, 4000

The downy mildew of Brassicas caused by the obligate biotroph, *Peronospora parasitica*, is a serious disease in transplant nurseries during winter. The disease is most prevalent in Brassica-cultivated areas in the Kwazulu-Natal region in South Africa. The life cycle of *P. parasitica* comprises an asexual (conidia) and a sexual (oospores) phase. The occurrence of heterothallism and homothallism in *P. parasitica* is well documented[1,2]. Studies on homothallism and heterothallism are significant since oospores are known to be the primary source of infection as they have been reported to remain viable and ineffective in plant debris or soil for a number of years. Therefore the aim of the present study was to determine heterothallism and/or homothallism in South African isolates of *P. parasitica*.

Eleven isolates of *P. parasitica* were tested. Field isolates of *P. parasitica* on *Brassica oleracea* var. *capitata* (cabbage) seedlings were obtained from Top Crop Nurseries in Cramond (isolate TCGS1), Sunshine Seedlings Services in Pietermaritzburg (isolates SSH1, SSP1), and Cedara Agricultural College (CB1 broccoli and CGC1 from cabbage). Isolates were maintained on Brassica host seedlings in a Conviron set with a photoperiod of 14°C dark for 8 h followed by 16°C (100 μ Em^{-1}s^{-2}) for 16 h.

Genetically uniform lines or single spore isolates TCGS1A, SSP1A, SSH1A, SSH1B, CGC1A, CGC1B and CGCIC were derived from the above field isolates. The isolation was achieved by transferring a single hyaline conidium to a droplet of water present on the surface of an excised cotyledon, using a Nikon stereomicroscope. Approximately 3-7 days after inoculation, sporulation of single spore isolates were obtained. Oospore production was induced by excising cotyledons showing profuse sporulation of both field and single spore isolates and exposing them to stress/dry conditions (20-25°C) in Petri plates in the laboratory. Scanning electron (SEM) microscopy and light microscopy was used to observe the occurrence of oospores to determine heterothallism and/or homothallism. Cotyledons were cleared by boiling in lactophenol-ethanol solution for 2 minutes[1]. Cleared cotyledons were rinsed in water and stored in 70% glycerol prior to examination for oospores using using a Nikon microscope. Standard techniques were used to prepare leaf samples for SEM. Samples were viewed in a Joel-SEM 6100 scanning electron microscope at. Cytogenetic studies were conducted to confirm heterothallism and/or homothallism using aceto-orcein staining procedures[2] and light microscopy.

In the present study, oospores were clearly observed in nine out of the eleven isolates tested. Light microscopy revealed oospores as brown circular spores, surrounded by distinct walls. These oospores appeared to have rough walls when viewed using SEM (Fig. 1). Previous reports suggest that upon oospore formation, the rest of the hyphae disintegrate once the oospore is formed[3]. The observation of oospores in five out of six single spore isolates in the present study suggests the occurrence of homothallism. Confirmation of homothallism was obtained by cytogenetic studies. Thus, *P. parasitica* isolates in South Africa are predominantly homothallic and the identification of the heterothallic isolates of *P. parasitica* is yet to be investigated.

References

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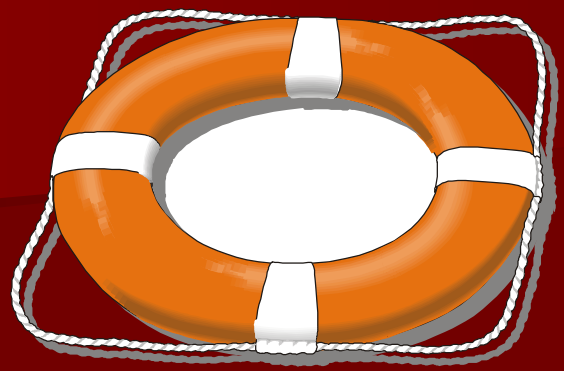
Fig. 1: Scanning electron micrograph of an oospore (O) (sexual spore) of *Peronospora parasitica* on a cabbage cotyledon.

Refereed abstract received in September - October with the return deadline three days away!

How to write a faultless MSSA Conference abstract

Help is at hand!

Prof ES Grossman, Dental Research Institute, MRC/Wits.



MSSA conference abstracts are returned because……

- The content is unacceptable
  - No information, no order, poor spelling, illogical, unscientific, incomplete, incomprehensible
- The format is incorrect
  - Does not follow instructions to authors

"The conference abstracts needs..."

- A clear title
- Some indication of the message
- Whether it is to be believed
- Not to reveal all

...like a good striptease artiste leaving something good and unexpected for the performance

…Whimster, 1997

All scientific writing is ...

- Highly stylised
- Four distinct component parts
  - What was the problem?
    - Introduction
  - How did you study the problem?
    - Methods and materials
  - What did you find?
    - Results
  - What do these findings mean?
    - Discussion

There are two general types of MSSA studies

- Laboratory studies
  - Logic
  - Clarity
  - Precision
- Descriptive studies
  - Don't lend themselves easily to this framework eg
    - Field studies
    - Clinical studies
    - Taxonomic work
    - Materials studies
  - Need a logical progression from problem to solution

Title

- Descriptive sentence stating the exact topic of the report
- It should inform the reader of
  - Groups being studied
  - Effect of one thing on another
- Concise, informative
- Names and affiliations of investigators

Groups?

Effect?

MORPHOLOGICAL CHARACTERIZATION OF MORPHOGENESIS ENTAMOEBA AND RELATIONSHIPS IN THE SYSTEM PARASITE-HOST BY SCANNING, TRANSMISSION ELECTRON MICROSCOPY AND ULTRACYTOCHEMICAL ANALYSIS

K.O.Hovnanyan, K.G.Karageuzyan, M. Hovnanyan, M.K.Karagyozyan

Institute of Molecular Biology of the Ar ian National Academy of Sciences, Yerevan, Re ublic of Armenia

Concise?
Informative?

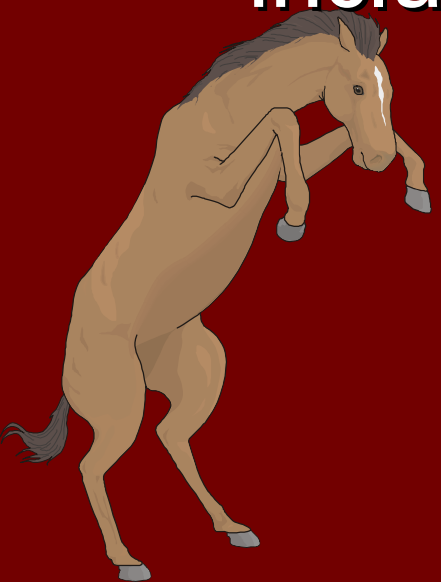
Authors
Affiliations

ULTRASTRUCTURAL, ULTRACYTOCHEMICAL AND LOGICAL CHARACTERIZATION OF ENTAMOEBA PARASITE-HOST RELATIONSHIPS

Authorship

Reasonable rational people become the bitterest of enemies because they could not agree on who was to be included as an author or in which order they should go.

Day, 1983



VOLUME 11, NO. 1 HTTP://ORI.HHS.GOV DECEMBER 2002

Office of Research Integrity

NEWSLETTER

Doctoral Student Sues Over First Authorship

A lawsuit over first authorship ended with a former doctoral student in molecular biology winning over his professor when a German judge ruled that an implicit contract was breached when the professor substituted herself as first author on the final draft according to Nature.

The judge said the original verbal agreement bestowing first authorship on the doctoral candidate constituted an implicit contract because first authorship was not disputed in the 14 months of paper preparation.

The doctoral candidate sued the professor before the paper was submitted for publication, alleging that the professor had substituted herself as first author on the final draft without reasonable cause. The court immediately issued an injunction preventing publication.

The professor said the contribution by the doctoral candidate did not warrant first authorship. The doctoral student replied that he had independently

carried out experiments and helped to write the paper.

The hidden message behind author ranking

- Single author
  - Very bright but not a team person
- First author
  - The one who made the greatest contribution
- Postgraduate research
  - Student first, supervisor second = good manners
- Head of laboratory in last position
  - Last position most prestigious

To counter scientific fraud

- Some journals have a standard letter which all authors sign on submission
  - Agree to the written text
  - Validate the research
  - Accept intellectual responsibility
- All guilty if fraud is uncovered

Introduction

- Supply sufficient background information to allow the reader to:
  - Understand the rationale of the study
  - Evaluate previous results without having to refer to other publications
  - Clearly defined aim
    - Correspond with the conclusions
- Brevity

MORPHOLOGICAL CHARACTERIZATION OF MORPHOGENESIS ENTAMOEBA AND RELATIONSHIPS IN THE SYSTEM PARASITE-HOST BY SCANNING, TRANSMISSION ELECTRON MICROSCOPY AND ULTRACYTOCHEMICAL ANALYSIS

K.O.Hovnanyan, K.G.Karage .Hovnanyan, M.K. ragyozyan

Institute of Molecular Biology of the Ar nian National A demy of Sciences, Yerevan, Republic of Armenia

Amebiasis, despite the reduction of cases in the Armenia and in the other countries, remains as one of the most widespread diseases [1-4]. With the object of establishment of microsructure formation of the entamoeba morphogenesis mechar isms and interactions of Entamoeba histolytica-hosts, we carried out a scanning, tra nsmission electron micr scopic, light-optic microscopic, ultracytochemical, investigation with the application f different methods of modeling the experimental amebiasis.

Previous work?

Rationale of study?

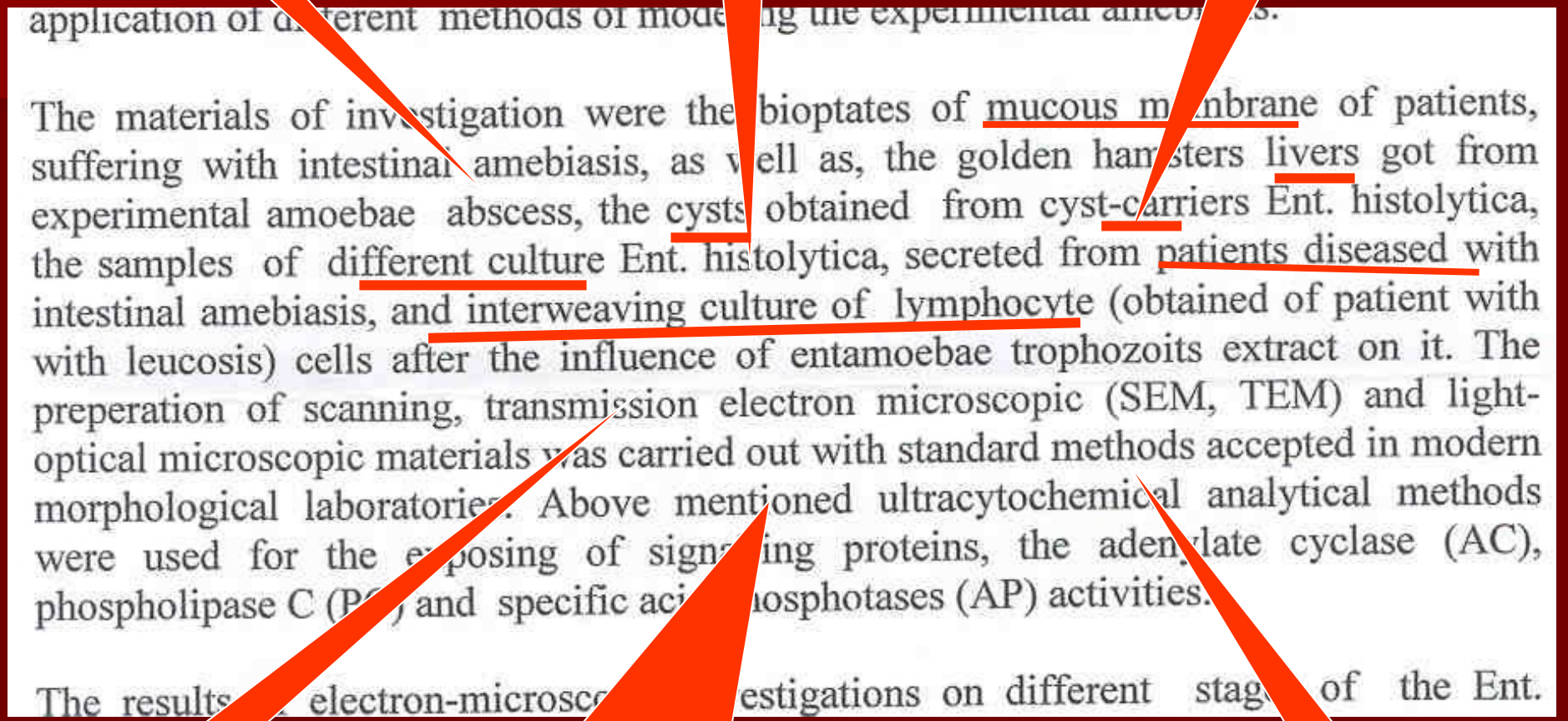
Brevity ?

5 x PhDs

Aim

Methods

- How the study was carried out
- Three main points of interest
  - Subjects
    - Who were they
    - How many
    - How were they selected
  - Apparatus
    - Equipment
    - Reagents
  - Procedure
    - Specimen preparation
    - Measurement and/or assessment
    - Data collection
    - Statistical manipulation



application of different methods of modeling the experimental amebiasis.

The materials of investigation were the bioptates of mucous membrane of patients, suffering with intestinal amebiasis, as well as, the golden hamsters livers got from experimental amoebae abscess, the cysts obtained from cyst-carriers Ent. histolytica, the samples of different culture Ent. histolytica, secreted from patients diseased with intestinal amebiasis, and interweaving culture of lymphocyte (obtained of patient with with leucosis) cells after the influence of entamoebae trophozoits extract on it. The preperation of scanning, transmission electron microscopic (SEM, TEM) and light-optical microscopic materials was carried out with standard methods accepted in modern morphological laboratories. Above mentioned ultracytochemical analytical methods were used for the exposing of signaling proteins, the adenylate cyclase (AC), phospholipase C (PC) and specific acid phosphotases (AP) activities.

The results of electron-microscopic investigations on different stages of the Ent.

Subjects

No numbers

Selection

Apparatus
Reagents?

What about?
Assessment; Measurement;
Data collection; Statistics
Data manipulation

Procedure

Begin writing the abstract while the work is in progress. This makes the writing easier while everything is still fresh in your mind - especially for methodology.

Day, 1983

Results

- Presents findings
- Draws attention to points of interest
- Displays summarised and analysed data
  - micrographs, tables, graphs, stats
    - be sure these are correctly labeled and identified

Findings?

phospholipas[illegible] (PC) and specific acid phosphotases (AP) activities.

The results of el[illegible]ctron-microscopic investigations on different stages of the Ent. histolytica life-cir[illegible]le's (trophozoits in culture, hematophages, tissue-forms, mature/inmature cysts) showed that in the entamoebae's cells have been established some reorganizations both in surface and cytoplasmatic structures. The SEM study of the cell surfaces (vegetative forms entamoeba) can be seen in pinocytic and phagocytic invagination stages of the plasma membranes. There was revealed the new particular aspects of entamoebae's morphogenesis. The fission of entamoebae take place by means of closed mitosis and accompanied by formation of innuclear center of the microtubes' organization. The amount of virus-like structures, which have been propounded for the taxonomy of entamoebae as an additional ultrastuctural sign, was increases in hematophages and decreases in cysts.

Using the method of ultracytochemical determination of the AC activity localization it was able to reveal the enzym on the inner layer of the plasmatic membrane.This phenomenon gives the evidence concerning of the functional similarities of the plasmatic membrane both entamoebae and eukaryotic cells of multicellular

Data?
Micrographs?

Points of interest?

Discussion

- Discuss results in context of aims
  - Did you find what you expected?
  - Compare results with previous studies?
  - Why were your results un/expected?
- Avoid unimportant, unconstructive and negative argument
- Speculation ??
- End positively!

the data obtained the acidic phosphatases are localized in inner phagosomal membranes.At the same time they are placed also as the surface-active lysosomes on the plasmatic membranes.According to the results of the biochemical analyses, it became pissible to establishe the high level of phospholipase activity in cultures of Ent.histolytica.The data obtained have shown, that PC and AP play an important role in the cytopathogenic action of Ent.histolytica.The ultracytochemical investigation demonstrated the presence of signalling proteins (AC, PC) in the cells of entamoebae. The results complex the functional morphological investigations have shown that cytopathic action o ifactor processes of enzymes, as well and highly active phagolysosomal sy nerous organic and unorganic microen rties of entamoebae are reveald as a nat f intestine's mucous membrane of patie and the liver of golden hamsters with the experimental amoebae abs changes are conditioning be development of the hosts cells edema in the s acualization ,rarefaction of cytoplasm and by the lytic deformation of cells in nnal period.It is well know that the increasing of the phospholipase activity s to the hydrolytic degradation of the membrane –bound phospholipids.This p ess is characterized with the simultaneous formation on significant pool of nisterified fatty acids,prodominantle polyenic , lysoforms of phospholipids, mainly lysophosphatidylcholines , and toxic products of free radical peroxidation of polyenic fatty acids.All these substances have a pronounced membranetoxic-membranelytic properties and they lead to the degradation of cell membranes, occuring the breaches in ion balance, which is very tipical for cell edema.These data obtained prove d also in the presence of the Ent.histolytica extract trophozoits actions on the cells injterweaving culture lymphocytes.

Too much of everything!

Thus plasmatic and phagosomal membrane's cells of Ent. histolytica cells are the

The preparation of a good abstract has almost nothing to do with literary skill but everything to do with organisation -
of both content and construction

Conclusion

- Main findings summarised
- Suggestions made for further research
- Taking findings and generalising them to phenomena not directly tested in the present research

remains as one of the most widespread diseases [1-4]. With the object of establishment of microsructure formation of the entamoeba morphogenesis mechanisms and interactions of Entamoeba histolytica-hosts, we carried out a scanning, transmission electron microscopic, light-optic microscopic, ultracytochemical, investigations with the application of different methods of modeling the experimental amebiasis.

trophozoits actions on the cells injterweaving culture lymphocytes.

Thus plasmatic and phagosomal membrane's cells of Ent. histolytica cells are the carriers of multifunctional enzymatic structure, which can play a definite determiming in entamoebae's life-cicle mechanisms, as well as , in the different tipes of interactions of parasite-host at amebiasis.

References:

Do you remember the Aim?

Main findings

trophozoits actions on the cells injterweaving culture lymphocytes.

Thus plasmatic and phagosomal membrane's cells of Ent. histolytica cells are the carriers of multifunctional enzymatic structure, which can play a definite determiming in entamoebae's life-cicle mechanisms, as well as , in the different types of interactions of parasite-host at amebiasis.

References:

Do you remember the Aim?

Generalising

References

- List only significant published references
- No secondary sources
  - Unpublished data; in press; abstracts; theses
- Check all parts of reference for accuracy against original publication
- Ensure the number in the text corresponds with the correct reference

Space

Caps

References:

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The extended abstract can supply virtually as much detail as a full paper. What it lacks is experimental detail. Precisely because it lacks experimental detail it cannot qualify as a scientific paper.

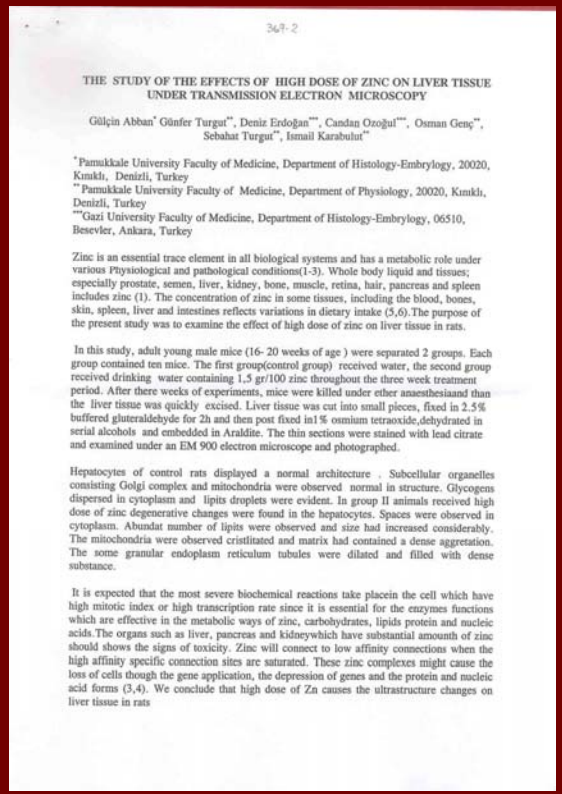
Day, 1983

Why is it necessary to follow the instructions to authors?

- Camera-ready copy reduces printing costs
- Eliminates typesetting
- Consistent font, style and size brings uniformity to the publication
  - Appearances are everything!
- Distributed to:
  - 9 local and overseas libraries; 4 electronic abstracting services; 5 subscription services

If you were a referee ...

Why three pages when two are all that is permitted?



367-2

THE STUDY OF THE EFFECTS OF HIGH DOSE OF ZINC ON LIVER TISSUE UNDER TRANSMISSION ELECTRON MICROSCOPY

Gülçin Abban* Günfer Turgut**, Deniz Erdoğan***, Candan Ozoğul***, Osman Genç**, Sebahat Turgut**, Ismail Karabulut**

*Pamukkale University Faculty of Medicine, Department of Histology-Embrylogy, 20020, Kınıklı, Denizli, Turkey
**Pamukkale University Faculty of Medicine, Department of Physiology, 20020, Kınıklı, Denizli, Turkey
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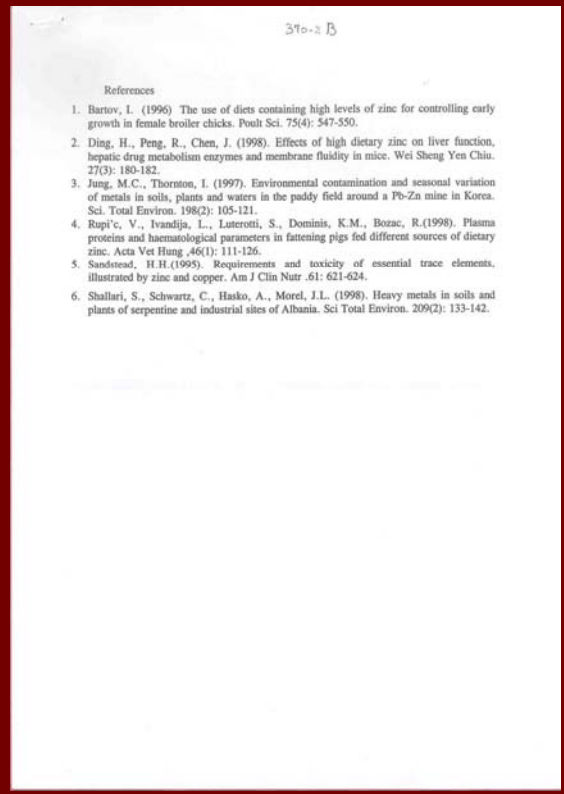
Zinc is an essential trace element in all biological systems and has a metabolic role under various Physiological and pathological conditions(1-3). Whole body liquid and tissues; especially prostate, semen, liver, kidney, bone, muscle, retina, hair, pancreas and spleen includes zinc (1). The concentration of zinc in some tissues, including the blood, bones, skin, spleen, liver and intestines reflects variations in dietary intake (5,6).The purpose of the present study was to examine the effect of high dose of zinc on liver tissue in rats.

In this study, adult young male mice (16- 20 weeks of age) were separated 2 groups. Each group contained ten mice. The first group(control group) received water, the second group received drinking water containing 1,5 gr/100 zinc throughout the three week treatment period. After there weeks of experiments, mice were killed under ether anaesthesiaand than the liver tissue was quickly excised. Liver tissue was cut into small pieces, fixed in 2.5% buffered gluteraldehyde for 2h and then post fixed in1% osmium tetraoxide,dehydrated in serial alcohols and embedded in Araldite. The thin sections were stained with lead citrate and examined under an EM 900 electron microscope and photographed.

Hepatocytes of control rats displayed a normal architecture . Subcellular organelles consisting Golgi complex and mitochondria were observed normal in structure. Glycogens dispersed in cytoplasm and lipits droplets were evident. In group II animals received high dose of zinc degenerative changes were found in the hepatocytes. Spaces were observed in cytoplasm. Abundat number of lipits were observed and size had increased considerably. The mitochondria were observed cristlitated and matrix had contained a dense aggretation. The some granular endoplasm reticulum tubules were dilated and filled with dense substance.

It is expected that the most severe biochemical reactions take placein the cell which have high mitotic index or high transcription rate since it is essential for the enzymes functions which are effective in the metabolic ways of zinc, carbohydrates, lipids protein and nucleic acids.The organs such as liver, pancreas and kidneywhich have substantial amounth of zinc should shows the signs of toxicity. Zinc will connect to low affinity connections when the high affinity specific connection sites are saturated. These zinc complexes might cause the loss of cells though the gene application, the depression of genes and the protein and nucleic acid forms (3,4). We conclude that high dose of Zn causes the ultrastructure changes on liver tissue in rats



370-2 B

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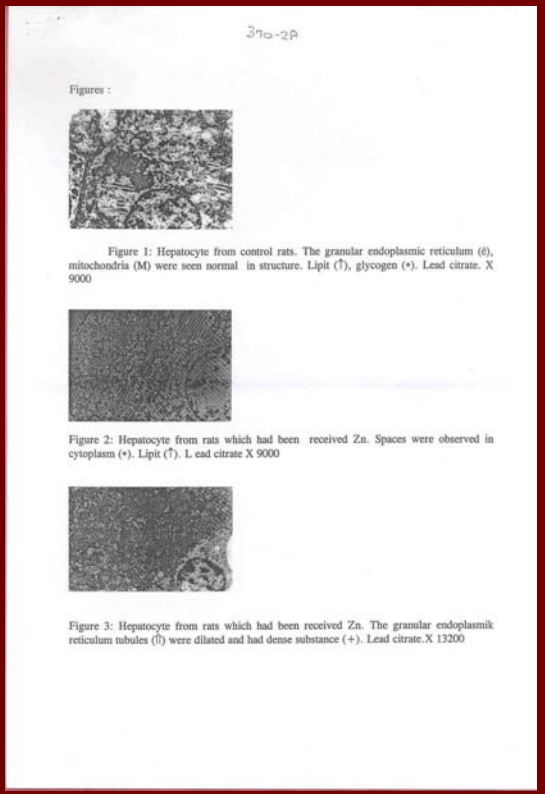

370-2A

Figures :

Figure 1: Hepatocyte from control rats. The granular endoplasmic reticulum (ê), mitochondria (M) were seen normal in structure. Lipit (↑), glycogen (•). Lead citrate. X 9000

Figure 2: Hepatocyte from rats which had been received Zn. Spaces were observed in cytoplasm (•). Lipit (↑). L ead citrate X 9000

Figure 3: Hepatocyte from rats which had been received Zn. The granular endoplasmik reticulum tubules (⇑) were dilated and had dense substance (+). Lead citrate.X 13200

The heading

- The title
  - Bold capitals, centred, 14> words

  SPACE
- Authors names
  - Mixed case, centred, one line, no end punctuation

  SPACE
- Affiliations
  - Mixed case, centred, one line, no end punctuation

  SPACE

1 line?

17 words?

THE STUDY OF THE EFFECTS OF HIGH DOSE OF ZINC ON LIVER TISSUE UNDER TRANSMISSION ELECTRON MICROSCOPY

Gülçin Abban*, Günfer Turgut**, Deniz Erdoğan***, Candan Ozoğul***, Osman Genç**, Sebahat Turgut**, Ismail Karabulut**

*Pamukkale University Faculty of Medicine, Department of Histology-Embrylogy, 20020, Kınıklı, Denizli, Turkey

**Pamukkale University Faculty of Medicine, Department of Physiology, 20020, Kınıklı, Denizli, Turkey

***Gazi University Faculty of Medicine, Department of Histology-Embrylogy, 06510, Besevler, Ankara, Turkey

Zinc is an essential trace element in al ological systems and has a metabolic role under

1 line, centred?

complete evaluation of $Al_xGa_{1-x}As$ and $In_xGa_{1-x}As$ it is necessary to determine the alloy compositions x accurately.

In this paper the factors influencing the accuracy of energy dispersive X-ray spectroscopy (EDS) analysis of thin foil semiconductors are discussed. The thin foil EDS analysis was performed on a 120kV Philips transmission electron microscope (TEM) with a EDAX EDS system. The compositions of the films were determined using a modified approach of the Cliff-Lorimer[3] ratio method for binary alloys. The Al mole fraction x in the $Al_xGa_{1-x}As$ epilayer was calculated by substituting the k_{AsGa} factor, determined from the GaAs substrate, in

$$x = 1 - \frac{A_{As}}{A_{Ga}}\left[k_{AsGa} \cdot \left(\frac{I_{As}}{I_{Ga}}\right)\right]^{-1}$$

where A_{As}, A_{Ga} are the atomic masses of As and Ga, and I_{As}, I_{Ga} are the characteristic X-ray intensities of the $Al_xGa_{1-x}As$ epilayer. The indium mole fraction x in $In_xGa_{1-x}As$ was determined in exactly the same manner.

The mole fractions x, determined using the above approach, are dependent on the accurate determination of

Poor print quality

Some have too much to say..... but that's OK

LOW EDGE DISLOCATION DENSITIES IN STEP-GRADED STRUCTURES

G MacPherson and P J Goodhew

Department of Materials Science and Engineering, University of Liverpool, United Kingdom

For single layers of $In_xGa_{1-x}As/GaAs$ the growth of the epitaxial layers remains in the 2D (Frank-van der Merwe) mode until the indium composition exceeds x~0.25[1]. Above this level the growth mode becomes strain-induced 3D (Stranski-Krastanow) growth. Growth of epitaxial layers with final indium compositions up to x~0.50 that remain in the 2D growth regime can be achieved by using graded structures[2] or low temperature growth techniques such as ALMBE. Associated with 3D growth is a rapid increase in the density of threading dislocations which can seriously degrade the quality of any device. Although there is a residual strain at the surface of graded structures which provides a driving force for threading dislocations to leave the structure, some threading dislocations may remain because their path has been blocked. Freund[3] suggested that the path could be blocked by the strain fields of orthogonal 60° dislocations. An extension of this idea is blocking by orthogonal edge dislocations. Edge dislocations are potentially a greater problem since they are sessile and lie within the layer.

For two 60° dislocations to form an edge dislocation in a single layer they must glide along their respective {111} glide planes as shown in fig.1(a). This will only remain possible provided the 60° dislocations do not have a spacing greater than $\sqrt{2}h$, where h is the epilayer thickness. Once this spacing is exceeded the {111} planes intersect outside the layer as shown in fig. 1(b). Extending this idea to the "layers" of thickness h_i comprising a step-graded structure, if the spacing of the 60° dislocations exceeds $\sqrt{2}h_i$ then to form an edge dislocation another interface within the structure would have to be crossed (fig1(c)). When this is the situation edge dislocation formation can be suppressed in two ways. Firstly, the two gliding segments will interact with 60° dislocations already present at the interface, and secondly there is a change in elastic modulus. For a continuous interface, where the lattice constants are similar but the elastic modulus is different, a dislocation in a softer material will be repelled from an interface with a harder material[4]. For single layers, Krishnamoorthy et al[5] provide empirical equations for the residual strain. MacPherson et al[6] have used these equations, and the fact that subsequent growth further relaxes layers within the structure, to predict the mean dislocation density at the interfaces.

$In_xGa_{1-x}As/GaAs$ step-graded structures up to a nominal indium composition of x=0.30 were grown by chemical beam epitaxy (CBE) at a temperature of 550°C on semi-insulating GaAs substrates.

Cross-sectional TEM showed the majority of dislocations to be at the interfaces within the structure. There was no evidence of 60° dislocations crossing interfaces to form edge dislocations. Of the few edge dislocations that were observed, the majority of these resided in the interfaces. Further study showed that these edge dislocations were formed from 60° dislocations lying in the adjacent interfaces with a higher indium composition as shown in fig. 2. Fig. 3 shows the residual contrast associated with edge dislocations imaged in the g004 reflection residing only at interfaces. The edge dislocation density found in these samples is certainly less than that recently calculated for standard step-graded layers of InGaAs up to a nominal indium composition of x=0.3[7] where the density of edge dislocations was determined to be approximately 28%. Edge dislocation densities in the study samples were estimated to be less than approximately 10%.

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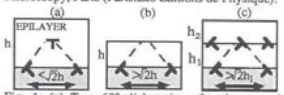

Fig. 1: (a) Two 60° dislocations forming an edge dislocation, (b) no edge dislocation formation due to {111} plane intersection outside the epilayer, and (c) no edge dislocation formation because of dislocation interactions and affects of change in elastic modulus.

Fig.2: Edge dislocation formation a) in the substrate and b) at the $In_{0.05}Ga_{0.95}/GaAs$ interface. Diffraction condition g220.

Fig. 3: Edge dislocations identified by residual contrast at the interfaces. Diffraction condition g004.

EFFECTS OF CHEMOTHERAPEUTIC DRUGS ON WILMS' TUMOUR CELLS

S. Bux, S.N. Govender, A.A. Chuturgoon and *G.P. Hadley

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Wilms' tumour (WT) is a complex neoplasm that resembles the embryonic kidney in histological appearance. In South Africa WT is the most common solid tumour[1]. Two opposing views exist in the management of WT viz., primary surgery and preoperative chemotherapy. The stage distribution and survival figures for both types of management are equally good but the question as to which form of treatment is better in our environment has not been answered. We examined the ultrastructural pathology of WT before and after chemotherapy, cultured WT and assessed the cytotoxicity of preoperative chemotherapeutic drugs on these cells.

Six patients were used in the study. Five patients were treated with preoperative non-stage 4 chemotherapy while one patient had no chemotherapy and served as a control as did the fine needle aspirations that were taken from the tumours before preoperative chemotherapy. Wedge biopsies were taken after treatment from both the tumour and residual kidney. Biopsies were immediately immersed in Karnovsky's fixative and processed for electron microscopy using conventional methods. For the MTT [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cytology assay[2] WT cell lines were established from surgically excised tumours from the above patients and 10^5 cells placed in 96 well microtitre plates. Using the MTT bioassay the cytotoxic effects of vincristine, 5-fluoro-uracil and mytomycin-C were assayed.

Light microscopy showed that four patients presented with classical triphasic (T) WT while 2 were diagnosed as anaplastic (A) WT. Both TWT and AWT comprised blastemal, embryonal tubular and glomeruloid structures but in the anaplastic variety many abnormal mitotic figures were present. Histologically, treated tumours showed large areas of amorphous material while the residual kidney in both the treated and untreated specimens showed compression, interstitial fibrosis and inflammation with tubular atrophy. Ultrastructurally, blastemal cells in pretreated WT contained large nuclei with slightly indented profiles, evenly distributed chromatin and small nucleoli; occasional strands of endoplasmic reticulum and mitochondria (Fig.1). In treated WT blastemal cells showed nuclear distortion, increased heterchromatin, large single or multiple nucleoli and several autophagosomes (Fig. 2). The epithelial component of treated WT showed convoluted basement membrane and occasional cells with swollen ER and areas of cytoplasmic lysis. Some areas showed focal degeneration of cells, histiocyte invasion and haphazardly arranged collagen fibres. A majority of specimens in treated WT, however, showed only large areas of amorphous material with several "foam" cells containing numerous fat droplets and autophagosomes (Fig. 3). The residual kidney in treated specimens exhibited several oedematous cells in the collecting tubules (Fig.4).

The dose response curves of the MTT assay showed that 5-fluorouracil, a potent antimetabolite, caused 73% of cell death while with vincristine (a vinc-alkyloid) and mytomycin C (tumour antibiotic) cell mortality was approximately 60%.

Cellular pathology induced by chemotherapeutic drugs in malignant cells appear to be more severe than that observed in the residual kidney. Cell death as indicated by focal areas of degenerate cells and large areas of cellular debris in close proximity to "foam" cells was also pronounced in treated tumour specimens. This may be attributed to the tubulin (found in mitotic cells) binding characteristics of vincristine which would therefore, mainly affect malignant cells. The apparent absence of mitotic figures in the treated tissue suggests that the action of vincristine is rapid. As chemotherapy is more cytotoxic on malignant cells and as cellular damage in the residual kidney appears to be less severe the results of this study suggest that preoperative chemotherapy would be the treatment of choice for WT.

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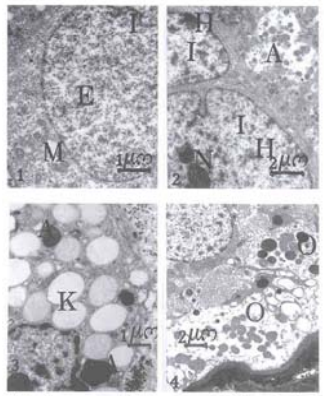

Fig.1 Blastemal cell in pretreated WT showing slightly indented nucleus (I) with evenly distributed chromatin (E) and mitochondria (M).

Fig.2 Treated WT blastemal cells showing nucleus (I) with increased heterchromatin (H) and prominent nucleoli (N) and autophagosomes (A).

Fig.3 "Foam" cell containing many fat droplets (K) and autophagosomes (A).

Fig.4 Oedematous cells (O) in the collecting tubules in treated WT.

..... others struggle for words!

LOCALISATION OF SAMORIN® IN *Trypanosoma congolense* BY FLUORESCENCE, IMMUNOELECTRON MICROSCOPY AND AUTORADIOGRAPHY

Internati , Kenya

Samorin® (isometamidium chloride) is the major compound recommended for chemoprophylaxis of bovine trypanosomiasis in sub-Saharan Africa. Although in use for over 30 years, very little is known about its mode or site(s) of action. Various workers have demonstrated that the compound interacts *in vitro* with a number of intracellular molecules[1–4], although whether these activities contribute to the compound's trypanocidal action *in vivo* is not known. Zilberstein *et al.*[5] demonstrated that isometamidium is transported rapidly into *Trypanosoma congolense* via a protein carrier in the plasma membrane.

The auto-fluorescent property of the drug when complexed with cell components[6] was utilized initially to study the uptake of Samorin into a sensitive clone of *T. congolense*. This was seen to be very rapid with a focus of fluorescence appearing in the region of the flagellar pocket after 2 minutes incubation with the drug. The fluorescence signal in this region increased at 5 and 15 minutes and appeared to become more diffuse throughout the posterior region of the trypanosome by 30 minutes.

Immunoelectron microscopy on sections of Lowicryl K4M embedded trypanosomes using a monoclonal antibody against Samorin[7] revealed diffuse labeling throughout the cytosol and nucleus of the cells with more intense labeling in the kinetoplast and mitochondrion. The endocytic organelles appeared unlabeled. It was not possible, however, to study shorter incubation times by this method. For this we employed EM autoradiography. Trypanosomes were incubated in medium containing 1.3µg/ml tritiated Samorin. The uptake was stopped and unbound drug removed by centrifuging the cells through a layer of silicone oil into the fixative mixture, then proces[illegible] by standard techniques. After incubation for 15 seconds there was a detectable signal within the kinetoplast. The signal was clearly localised only to this organelle at times of 2 and 5 minutes. Longer incubations gave a more diffuse label throughout the mitochondrion, cytosol and nucleus.

Having identified the early target organelles within *T. congolense in vitro*, we hope that this will lead to a better understanding of the mode of Samorin's trypanocidal action. With these techniques we hope to investigate further the uptake and possible efflux of Samorin *in vitro* and to compare resistant and sensitive clones to investigate possible differences in their uptake and localisation of the drug.

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QUANTITATION OF RAT PANCREATIC ISLET CELLS EMPLOYING DOUBLE LABELING IMMUNOCYTOCHEMISTRY AND COLOUR IMAGE ANALYSIS

K. W oote

Ex ey

Pathophysiological insults to the pancreas such as non-insulin dependent diabetes (NIDDM), usually result in changes in pancreatic function and morphology[1]. These include changes in pancreatic islet size, as well as changes in cell type distribution within islets. As a result, many approaches have been developed to study these changes[2]. In this study we introduce an approach that uses double labeling immunocytochemistry to identify islet cell type, and colour image analysis to quantify islet size and islet cell population.

Rat pancreata were fixed in Bouin's fixative overnight and then prepared for paraffin wax embedding and sectioning. Tissue sections (3-5µm thick) were attached to glass slides and prepared for immunocytochemistry. Slides were immunostained with polyclonal glucagon antiserum (DAKO) using the ABC method and DAB as chromogen. The same sections were immunostained with monoclonal insulin antiserum (Sigma) using the APAAP method and new fuchsin as chromogen. Sections were counterstained with haematoxylin and mounted in glycerol jelly aqueous mountant.

Sections were viewed with a light microscope attached to a PC with a video camera, colour frame grabber (Data Translation) and HLImage++ image analysis software (Western Vision). The number of insulin and glucagon producing cells per islet were calculated by counting the nuclei of positively stained cells for insulin and glucagon. The images were captured as colour RGB images using the 10x objective and stored to disk. The stored images were used to determine islet size and cell area on other computers also loaded with the image analysis software. Islet size was determined by tracing around the perimeter of the islet with a mouse and measuring the enclosed area. Glucagon cell area was determined by thresholding for brown (DAB) (Fig. 1) and insulin cell area was determined by thresholding for red (new fuchsin). The system was calibrated so that all acquired measurements were expressed in µm^2. Insulin and glucagon cell size was extrapolated by dividing the total cell area for each cell type by the number of nuclei counted for that specific cell. All data was entered directly into Excel for data analysis.

By this approach, using a single section, we were able to count the number of islets; measure islet size (area); determine the proportion of insulin and glucagon cells in each islet; determine the proportion of insulin to glucagon cells per islet; and calculate the size of insulin and glucagon producing cells.

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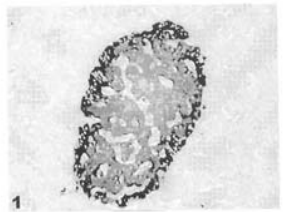

Fig. 1. Section of pancreas double immunostained for glucagon and insulin. Glucagon producing cells appear black.

..... others struggle for words!

ULTRASTRUCTURE OF CABOT RINGS IN HUMAN RED BLOOD CELLS

R. :vens

Depa Pretoria

Cabot rings have been found in human red blood cells in a variety of haematological disorders, particularly megaloblastic anaemia, but are also seen in sideroblastic anaemia and thalassaemia. They are infrequently seen in the peripheral blood smears of such patients because they are uncommon and probably also because they are not searched for.

The significance of Cabot rings has long been controversial. Some authors consider these red blood cell inclusions to be artifacts[1]. However, subsequent reports published support their existance[2]. The one ultrastructural report of these red blood cell inclusions did not unequivocally resolve the dilemma[3]. We report Cabot rings clearly showing both the ring and the associated granules.

Venous blood was drawn in EDTA tubes. Ten patients with a macrocytosis were found to have Cabot rings on their peripheral blood smears. Perls' and supravital stains were also performed. Two samples had sufficient Cabot rings present to warrant further electron microscopic investigation. The red blood cells were washed, fixed in 2.5% gluta
0.5% osmium tetroxide. Aft
the samples were embedded
Philips 301 transmission ele

Light microscopy showed
loops both in the Wright's (F
TEM (Fig. 2) shows the cal
structure with no identifiable membran
structures with alternating dark and lig
close to the ring and diffusely spread
cell. One of the two samples was teste
this could not be detected in a scanning
electron microscope (JOEL 200 CX) us
energy dispensive spectrometer.

We conclude that Cabot rings are definite
structures with associated dense granules. Th
these rings and granules remains unknow
currently under investigation.

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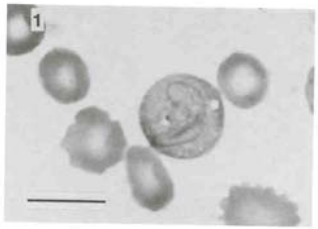

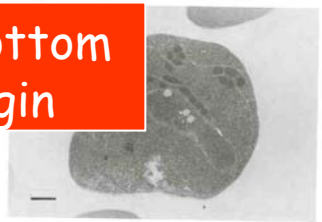

Fig. 1. Light micrograph of Cabot rings from a Wright's stained smear (Bar = 10μm).
Fig. 2. TEM view of Cabot ring and dense granules. (Bar = 1μm).

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THE THERMAL DECOMPOSITION OF CAESIUM PERIODATE

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Departmen , Pretoria, 0002,
South Africa.

Using a heating rate of 5 °C.min^{-1} $CsIO_4$ decomposes between 300 and 333 °C according to the following reaction :

$2CsIO_4(s) \rightarrow 2CsIO_3(s) + O_2(g)$

Decomposition of the solid is initiated at regions on the crystals as nuclei of the solid product are formed. These nuclei then grow until only the solid product remains [1]. Models based on the different possibilities of these processes have been derived [2]. Kinetic analysis involves the relation of experimental α,t (mass fraction gaseous products formed, time) values to the models and determination of th equation that best describes t mechanism of the reaction.

$CsIO_4$ was heated at con temperatures ranging from 280 t 5 °C in a Stanton Redcroft T nal Analyzer in a nitrogen atm ere. Fitting the α,t data to the 19 r els of decomposition it was observ hat the reaction can clearly be divid nto two parts, but it proved difficu to obtain just one model that show he best fit for both parts. The first p t between α = 0 and 0.2 can be de cribed by the contracting area equat n, $1-(1-\alpha)^{\frac{1}{3}} = kt$, a power law equat n and a second order equation [1]. Th second part can be described by th contracting area equation or the Avrami-Erofe'ev equation with n = 4 [1]. To distinguish between these equations (and thus mechanisms) electron micrographs at α-values between 0 and 1 (both included) were obtained on a JEOL 840 scanning electron microscope. In the first two micrographs shown in Figure 1 the formation at a constant rate of a limited number of the nuclei as well as a very slow growth are observed. For α-value

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uclei are nuclei is graph in α-values he two- ei. Using sible to n between the proposed isms. The decomposition of to $CsIO_3$ proceeds via the tion of nuclei at a constant rate a maximum number of ± 14 clei per μm^2 is formed. These nuclei row according to the contracting area equation until only $CsIO_3$ remains.

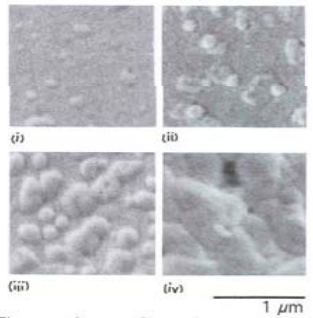

Figure 1 : Secondary electron micrographs of the decomposition of $CsIO_4$ at α-values of (i) 0; (ii) 0.1; (iii) 0.4 and (iv) 1.0.

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ULTRASTRUCTURAL CHANGES IN THE PLATELETS OF PATIENTS ON CHEMOTHERAPY

R.A.M. Mattana and L. du Plessis

Department of Haematology, University of Pretoria

Platelets play a vital role in the maintenance of haemostasis but these anuclear cells are also involved in the pathogenesis of malignant disease. Adhesive interactions between circulating tumour cells and host platelets represent one of the indispensable determinants of metastasis[1]. Haemostatic abnormalities, usually detectable by laboratory tests, are present in patients with metastatic spread of the tumour. Cells of the human breast carcinoma cell line, MCF-7, have been shown to produce a protein which is immuno-related to GPIbα-receptor and which participates in the tumour-induced platelet aggregation process.

This pilot study was initiated to investigate the ultrastructural changes in platelets of breast carcinoma patients on chemotherapy. Six patients were studied. Venous blood was collected for various platelet studies and for TEM (prepared via the standard method for TEM). Case number 1 was a newly diagnosed patient who had not yet received chemotherapy. All the other five patients had received chemotherapy. Case 6 was currently receiving only radiotherapy (radio), but had previously received chemotherapy. The results are expressed in Table 1. (A = Adriamycin, P = Promethazine, Ta = Taxotere, G = G-CSF [Granulocyte - Colony Stimulating Factor], C = Cyclophosphamide, M = Methotrexate, F = 5-Fluorouracil)

Table 1 : Platelet count and aggregation.

	Metastatic spread	Treatment	Plt	Estrogen Receptor	Plt Agg
1	Negative	none	N	Negative	N
2	Negative	APTaG	N	Negative	N
3	Positive	APTaG	N	Positive	N
4	Negative	none	N	Negative	N
5	Positive	CMF	N	ND	ND
6	Negative	radio	N	ND	ND

Plt=Platelet count, N=Normal, ND=not done, Plt Agg = platelet aggregation.

Two distinctive platelet inclusions were found. The first shows the presence of villus projections from within dilated canalicular system (cases 5 and 6) a seen in Fig. 1. The second involves a large oval ar with a sponge-like appearance (Fig. 2). Th phenomenon was seen in cases 1, 2, 3 and 4. Case last received treatment in 1993 and is now back on treatment and case 1 was untreated at the time of this study. Similar structures have been reported in Wistar Furth rats and in humans treated with vincristine[2]. They were also seen in one of the four female control patients who were on no medication.

This pilot study suggests that structural changes take place in the platelets of patients on chemotherapy. Larger studies are required to confirm these results and to evaluate the possible clinical effects. We wis to thank the Department of Oncology for the referr of the patients for consultation.

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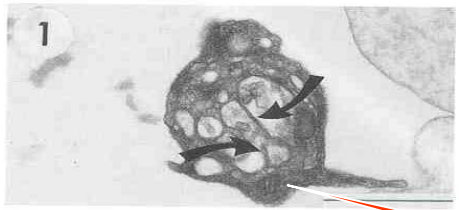

Fig. 1. Case 5 - Villus structure protruding from the inside of the markedly dilated canalicular system. Bar = 1 μm.
Fig. 2. Case 4 - Oval spongy area of probably delicate dilated canalicular system. Bar = 1 μm.

Acknowledgements

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Micrographs

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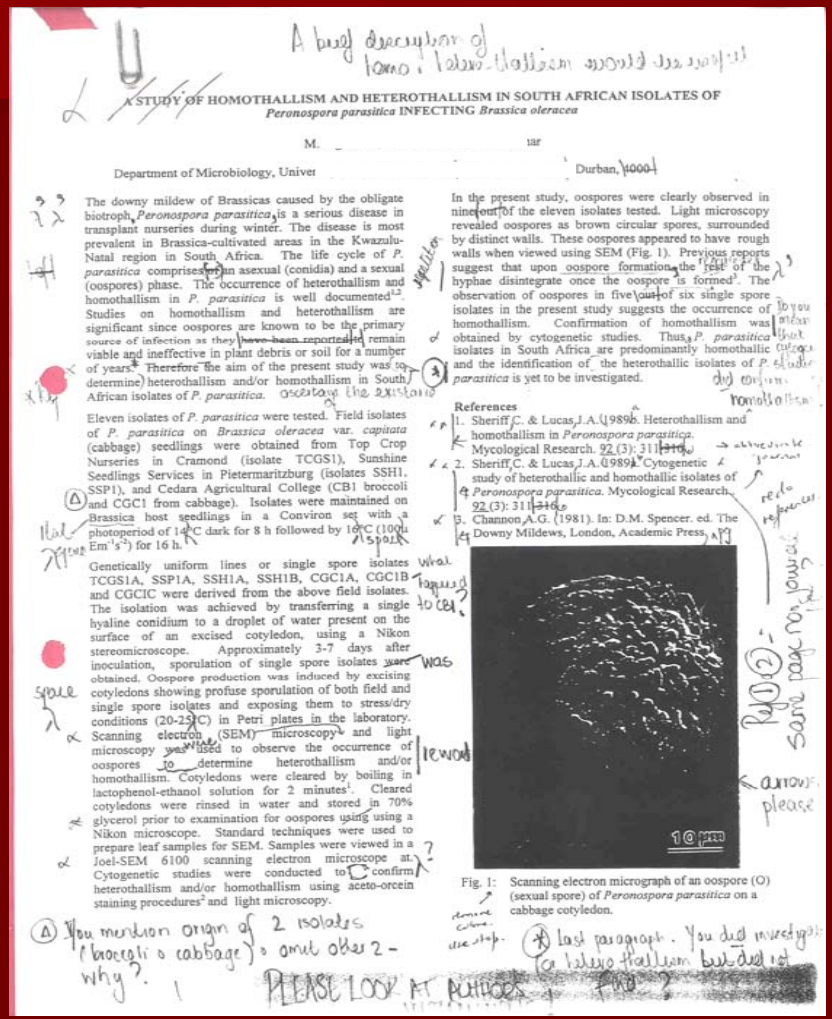

A STUDY OF HOMOTHALLISM AND HETEROTHALLISM IN SOUTH AFRICAN ISOLATES OF *Peronospora parasitica* INFECTING *Brassica oleracea*

M. ... ar

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The downy mildew of Brassicas caused by the obligate biotroph, *Peronospora parasitica*, is a serious disease in transplant nurseries during winter. The disease is most prevalent in Brassica-cultivated areas in the Kwazulu-Natal region in South Africa. The life cycle of *P. parasitica* comprises an asexual (conidia) and a sexual (oospores) phase. The occurrence of heterothallism and homothallism in *P. parasitica* is well documented[1,2]. Studies on homothallism and heterothallism are significant since oospores are known to be the primary source of infection as they have been reported to remain viable and ineffective in plant debris or soil for a number of years[3]. Therefore the aim of the present study was to determine heterothallism and/or homothallism in South African isolates of *P. parasitica*.

Eleven isolates of *P. parasitica* were tested. Field isolates of *P. parasitica* on *Brassica oleracea* var. *capitata* (cabbage) seedlings were obtained from Top Crop Nurseries in Cramond (isolate TCGS1), Sunshine Seedlings Services in Pietermaritzburg (isolates SSH1, SSP1), and Cedara Agricultural College (CB1 broccoli and CGC1 from cabbage). Isolates were maintained on Brassica host seedlings in a Conviron set with a photoperiod of 14°C dark for 8 h followed by 16°C (100 Em^{-2}s^{-1}) for 16 h.

Genetically uniform lines or single spore isolates TCGS1A, SSP1A, SSH1A, SSH1B, CGC1A, CGC1B and CGCIC were derived from the above field isolates. The isolation was achieved by transferring a single hyaline conidium to a droplet of water present on the surface of an excised cotyledon, using a Nikon stereomicroscope. Approximately 3-7 days after inoculation, sporulation of single spore isolates were obtained. Oospore production was induced by excising cotyledons showing profuse sporulation of both field and single spore isolates and exposing them to stress/dry conditions (20-25°C) in Petri plates in the laboratory. Scanning electron (SEM) microscopy and light microscopy was used to observe the occurrence of oospores to determine heterothallism and/or homothallism. Cotyledons were cleared by boiling in lactophenol-ethanol solution for 2 minutes[4]. Cleared cotyledons were rinsed in water and stored in 70% glycerol prior to examination for oospores using using a Nikon microscope. Standard techniques were used to prepare leaf samples for SEM. Samples were viewed in a Joel-SEM 6100 scanning electron microscope at. Cytogenetic studies were conducted to confirm heterothallism and/or homothallism using aceto-orcein staining procedures[2] and light microscopy.

In the present study, oospores were clearly observed in nine out of the eleven isolates tested. Light microscopy revealed oospores as brown circular spores, surrounded by distinct walls. These oospores appeared to have rough walls when viewed using SEM (Fig. 1). Previous reports suggest that upon oospore formation, the rest of the hyphae disintegrate once the oospore is formed[5]. The observation of oospores in five out of six single spore isolates in the present study suggests the occurrence of homothallism. Confirmation of homothallism was obtained by cytogenetic studies. Thus, *P. parasitica* isolates in South Africa are predominantly homothallic and the identification of the heterothallic isolates of *P. parasitica* is yet to be investigated.

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Fig. 1: Scanning electron micrograph of an oospore (O) (sexual spore) of *Peronospora parasitica* on a cabbage cotyledon.

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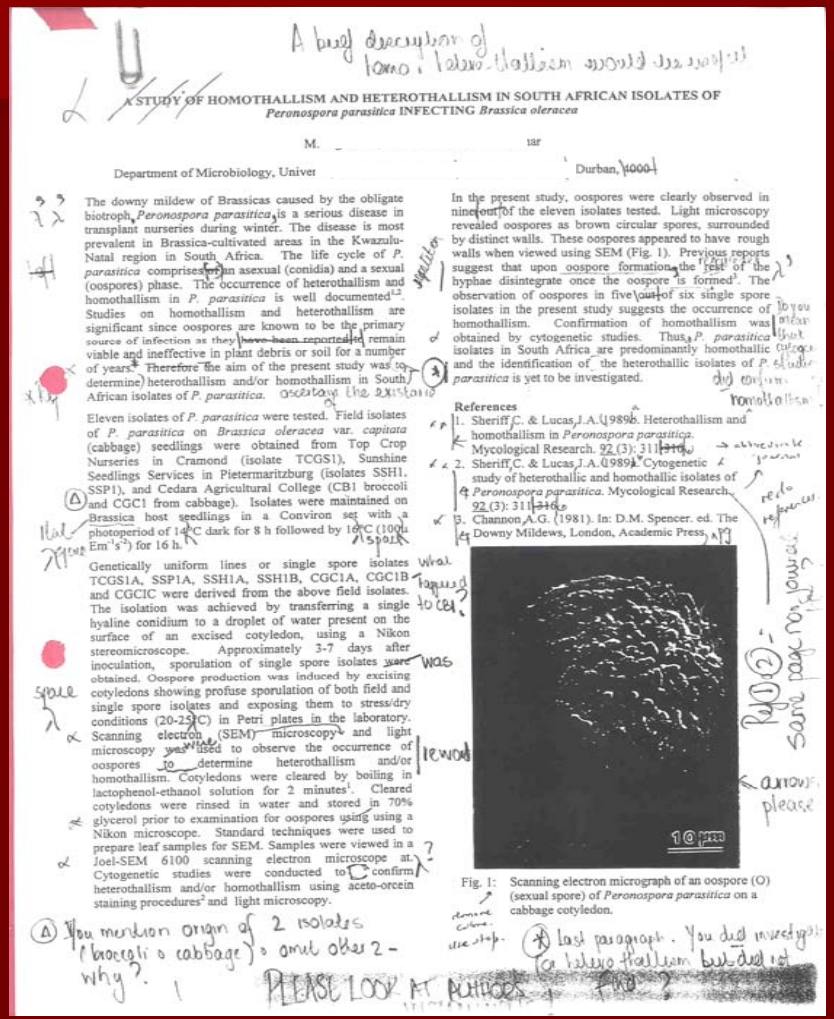
- Be open-minded about the method
  - there is more than one way to tackle a research problem!

Authors please .....!



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Eleven isolates of *P. parasitica* were tested. Field isolates of *P. parasitica* on *Brassica oleracea* var. *capitata* (cabbage) seedlings were obtained from Top Crop Nurseries in Cramond (isolate TCGS1), Sunshine Seedlings Services in Pietermaritzburg (isolates SSH1, SSP1), and Cedara Agricultural College (CB1 broccoli and CGC1 from cabbage). Isolates were maintained on *Brassica* host seedlings in a Conviron set with a photoperiod of 14°C dark for 8 h followed by 16°C (100 Em^{-1}s^{-2}) for 16 h.

Genetically uniform lines or single spore isolates TCGS1A, SSP1A, SSH1A, SSH1B, CGC1A, CGC1B and CGCIC were derived from the above field isolates. The isolation was achieved by transferring a single hyaline conidium to a droplet of water present on the surface of an excised cotyledon, using a Nikon stereomicroscope. Approximately 3-7 days after inoculation, sporulation of single spore isolates were obtained. Oospore production was induced by excising cotyledons showing profuse sporulation of both field and single spore isolates and exposing them to stress/dry conditions (20-25°C) in Petri plates in the laboratory. Scanning electron (SEM) microscopy and light microscopy was used to observe the occurrence of oospores to determine heterothallism and/or homothallism. Cotyledons were cleared by boiling in lactophenol-ethanol solution for 2 minutes[1]. Cleared cotyledons were rinsed in water and stored in 70% glycerol prior to examination for oospores using using a Nikon microscope. Standard techniques were used to prepare leaf samples for SEM. Samples were viewed in a Joel-SEM 6100 scanning electron microscope at. Cytogenetic studies were conducted to confirm heterothallism and/or homothallism using aceto-orcein staining procedures[2] and light microscopy.

In the present study, oospores were clearly observed in nine out of the eleven isolates tested. Light microscopy revealed oospores as brown circular spores, surrounded by distinct walls. These oospores appeared to have rough walls when viewed using SEM (Fig. 1). Previous reports suggest that upon oospore formation the rest of the hyphae disintegrate once the oospore is formed[3]. The observation of oospores in five out of six single spore isolates in the present study suggests the occurrence of homothallism. Confirmation of homothallism was obtained by cytogenetic studies. Thus, *P. parasitica* isolates in South Africa are predominantly homothallic and the identification of the heterothallic isolates of *P. parasitica* is yet to be investigated.

References

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Fig. 1: Scanning electron micrograph of an oospore (O) (sexual spore) of *Peronospora parasitica* on a cabbage cotyledon.

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