コスタリカ大学医学部プロジェクトに対するエバリュエーション調査団報告書

昭和52年3月

国際協力事業団
医療協力部

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Ⅰ　はしがき

わが国のコスタリカに対する医療協力は、昭和４８年１０月２７日から同年１１月１４日までの間、コスタリカに派遣された大阪大学微生物学研究所深井教授を団長とする実施調査団とコスタリカ政府関係者との協議の結果、同国の基礎医学分野の研究に必要な機材の供与と専門家の派遣並びに研修員の受入れを組合せた形で進めていくことに双方意見の一致を見、同年１１月２日に取りかわされた合意議事録に基づいて、昭和４８年度から同５０年度まで行なわれてきた。

このような経緯のもとに、過去３年間の本プロジェクトの評価を実施することとなり、本エバリュエーション調査団が昭和５１年９月７日から同月１８日までコスタリカ国に派遣された。

本報告書はその調査報告書である。

Ⅱ　調査団の編成

団長	竹内　正	日本大学教授
団員	星野宗光	愛知県ガンセンター研究所超微形態学部長
団員	山本二郎	国際協力事業団医療協力部長

Ⅲ　調査団の日程表

昭和5 14年9月6日(月)　東京発　(20.00) JL012 → メキシコ着(2015)

9月7日(火)　メキシコ発(8.00) LR641 → サンホセ着(1130)

15.30～　ウイルブルグ・ヒメネス外務次官を表敬訪問

19.30～　大使公邸で行われた大使館員歓送会パーティに出席

9月8日(水)　9.00～12.00　大使館を表敬訪問、事前の打合せ

14.00～　クラウディオ・グティエレス コスタリカ大学学長を表敬訪問

15.15～　ヘルマン・ウエインストック厚生大臣を表敬訪問

9月9日(木)　9.00～12.00　コスタリカ大学において第1回会議

14.00～17.00　コスタリカ大学において第2回会議

9月10日(金)　9.00～12.00　コスタリカ大学において第3回会議

12.00～13.45　コスタリカ大学学長主催の昼食会

14.00～17.00　コスタリカ大学において第4回会議

9月13日(月)　9.00～11.00　日本で研修を受けたコスタリカ側専門家とコスタリカ大学において懇談会

11.10～12.00　コスタリカ大学歯学部訪問

9月14日(火)　10.00～12.00　日本供与の医療機材のコスタリカ大学における贈呈式、

18.00～　日本側主催夕食会

9月15日(水)　コスタリカ独立記念日のため休日

9月16日(木)　サンホセ発　(7.40) PA542 → ニューヨーク着(1800)

9月17日(金)　ニューヨーク発(13.10) JL005 → 東京着(18.05)

なお、山本部長のみは、サンホセからの帰途グァテマラに立寄り、9月23日に帰国。

Ⅳ　調査団とコスタリカ大学との会議

1　総　　論

(1)　Record of Discussions の主旨

まず、このプロジェクトのRecord of Discussionsについて述べてみたい。このプロジェクトは昭和４８年１１月２日に大阪大学微生物学研究所の深井教授とコスタリカ大学学長のDr. Rodriguezとの間に、向う３年間の有効期限を以て発足した。目的は、電子顕微鏡（以下ＥＭという）による基礎医学教育及び医学、生物学の研究を研究を行うことであった。従って中心となるものはＥＭで、これの供与と、これを用いる教育ということであり、事実機材供与としては、透過型ＥＭを昭和４８年に、また走査型ＥＭを昭和５０年に供与することが予定されていた。しかし、実際にはＥＭの据付の作業は遅れた。一方日本側からの専門家の派遣は、最終年度の昭和５１年２月になって長期滞在の専門家が派遣されたので、この点でも教育の実効をあげるスタートが遅れていたことは、そうなる実情はあったとしても、遺憾なことであったと思う。教育の面での研修生受入れは、わが国の大学等の協力によって順調に進んだ。

(2)　日本及びコスタリカ双方の協力の実情

本調査団は、コスタリカ滞在中、全２日間をコスタリカ大学側との対論に費やした。第一に注目すべきことは、コスタリカ大学側の極めて積極的な熱意である。コスタリカ大学は、Dr. Mata を中心とする衛生研究所（Instituto de Investigacions en Salud, INISA）を設立していた。これは同大学医学部敷地内に建設されたもので、ＥＭ Unitは、殊に独立平屋一棟をこれにあてており、Dr. Bolaños （日本で訓練ずみ）をＥＭ Unitの技術上の長として、以下に数名の訓練中のものがいた。日本側専門家である小塚芳道博士、赤堀宏博士及び福岡孝寿氏は、周到に、また、献身的に指導されていた。目下、小塚博士によるコスタリカ側専門家の訓

練日程表も作製されて、軌道にのろうとしている。ただ、将来への心配はこのようにコスタリカのみでなく、広く中央アメリカに名声が拡まることによる訓練志願者の増加に如何に対応するかであって、現在の透過型、及び走査型ＥＭでは、明らかに教育及び研究の二方面を満足させることは不可能であることを考慮すべきであろう。

(3) コスタリカ大学側の熱意

コスタリカ大学側の積極姿勢の最もよい現われは、このＥＭ Unit を利用しての研究計画にあると思う。元来ＥＭ（透過及び走査の両型）の技術を何に役立てるかは、コスタリカ側の何を知りたいのか、そして問題解決の対策が何かを確立することである。研究の推進を申出ているのは医学部のみでなく、農学部や、その他の学部である。

(4) ＥＭ Unit を場とする研究計画

医学部内にＥＭ Unit があり、研究構成員の中にも医学部職員が多いことから、当然研究は保健衛生及び疾病に関するものが取上げられている。それを裏づけるためにコスタリカのNational priority を調べてみると次の順位になっている。

1）子供の栄養失調

これは寄生虫症、腸管粘膜異常やその他の原因が疑われている。

2）死産の防止

人口問題の一環として、医学のみならず、その他の面からも広く取上げている。

3）下痢性疾患

赤痢などとは別に、一種の腸管ウイルスの感染が疑われている。これが緊急課題として多くの関心が払われている。

4）Health intervention

給水、下水処理、食品衛生及び予防注射等の活動を一括している。

5）慢性疾患予防

心臓疾患や高血圧やガン等の慢性疾患もまた問題となっている。

6）社会病理学

これはアルコール中毒その他である。

この中、ＥＭを用いる研究が１）、３）及び５）であって、国民的課題を解決するためにＥＭが如何に必要とされ、重要な役割を担うことを期待されているかが理解されよう。

(5) 本計画の将来を考える場合の資料として

本調査団は、協力計画の評価を目的としたもので、将来計画の検討をその本務とするものでないことはいうまでもない。しかし、過去の実績の評価は将来像の画き方によってかなり変化するものであり、将来像に言及することなしに判定することは、しばしば困難なことがある。それ故、non-committal な扱いとして聴取した将来計画を記述して今後の資料とする。要約すると次の如くになる。

1) ＥＭの供与、専門家の派遣、及び研修生受入れの過去の実績に対して、コスタリカ側はこれを高く評価し、自からもこれを発展させていく意欲はさかんであることを確認し、それの主体はＩＮＩＳＡである。

2) 発展の方向は国内の多方面からの要求に答えて研究の実績をあげることに努め、医学、生物学、農学等の分野を取扱いたい意向である。

3) 国内の充実が一定度に達すると、隣接諸国の要請にも応じうれば、協力したい考えであり、早晩ＥＭ研修生の中に外国人の志望者が入ることは明らかである。将来像としてはＩＮＩＳＡ当局の意向は、ここを中央アメリカのＥＭセンターとして広く門戸を開放したいという理想をもっていることが察知される。その考えを実現するために、今暫くは日本の援助を必要とするので、プロジェクトの更新を希望するという。

(6) 総論的小括

以上総論的小括として要約すると

1) 「コスタリカ大学プロジェクト」－ＥＭ供与を中心とした－に関して、コスタリカ大学側の熱意と、日本から派遣された専門家の熱意と技術が極めてよく適合して実績をあげていることを報告する。

2) これらの実績向上のために払われた現地日本大使館の真剣かつ温情ある協力を特に強調したい。

3) Follow-up の期限の延長（２年を３年に）が望ましい。それは主としてR. D. 決定後３年の間に、計画実施上の時間的遅れがあったことによる。

2 電子顕微鏡Unitの現状

(1) 運営と組織

EM Unitは、前述の如く、現在は、コスタリカ大学の主導のもとに設置されたＩＮＩＳＡの一部門となっていて、ＩＮＩＳＡは医学部、微生物学部、薬学部、歯学部などのいわゆる、health science area の中に位置している、ＩＮＩＳＡには、Dr Bolaños を主任として、小塚芳道、赤堀宏、及び福岡孝寿、専門家他数名がスタッフとして、EM Unitの実際上の運営にあたっている。このUnitは、従って、ＩＮＩＳＡの所長であるDr. Mataの管括下にあるわけである。

EM Unitの運営方針は、上記スタッフと、各専門分野の代表からなる委員会で行われており、この委員会の役割は、予算の具体的使用法の決定と、EMを利用する研究計画の採否の審査と決定にあたっている。その判断の規準は、主として研究計画がNational priorityにそったものかどうか、また、大学にとっても必要な研究かどうかということにある。更に、専門家の立場から、EM検索がその研究に有効か否か、また技術的に、妥当な計画か否か、というような適当なadvice と consultation が行われる。別添のV、資料１はその使用申込書である。

また後述の運営費の問題とからんで、利用者に予算的裏付けの用意があるかどうかもチェックする。

(2) 運 営 費

これは予算の出所と使途により、三大別できる。

1) 中央費的なもの

国家科学技術調査審議会の中からＥＭ Ｕｎｉｔの予算として支給され、ＥＭ Ｕｎｉｔ以外の費用に流用できない。主として、光熱、水道費として支払われる。

2) 利用者負担費

研究者個人の研究費の中からＥＭ Ｕｎｉｔに支払われるもので、いわば利用者負担金ともいうべきものであって、研究費の出所は、大部分が国家科学技術調査審議会からくる。

3) R. D. にもとづく援助資金

機器を除いて若干の材料費があるが、当初必要とされた材料（薬品、写真、材料等）の購入にあてられた。これは、ＥＭ Ｕｎｉｔのスタッフの使用にあてられているが、教育、研修に使われ、また、若干は研究者への材料の供給にもあてられている。

(3) ＥＭ Ｕｎｉｔの役割

1) ＥＭのmaintenance

2) ＥＭに関する教育と研修

3) 一般学生教育

4) ＥＭ試料作製技術の改良と開発

5) 研究者が現に行っているＥＭを使用する研究の円滑化のためのadviceやconsultation、共同研究等。

(4) 機材の現状

R.D.にもとづいて、援助計画期間中に漸次供与された機材は、別添Ⅴ、資料２のＢの通りである。これを通覧すると、機器の種類から考えて、形態学的研究のみにとどまらず、up-to-dateの細胞化学、細胞生物学的研究をも充分にカバーすることのできるだけの充分の機材がそろっていると評価できる。ただ、ＥＭについてだけいえば、後述のようにＥＭ Ｕｎｉｔが現

在計画しているような研修計画が軌道にのり出すと、本来の研究活動にEMをさくゆとりがないようにみうけられた。

消耗品についていえば、今後の補給方法が問題となろう。現在、援助予算で当初購入した薬品類や、写真材料のうち、すでに使いつくしたものがあるが、これの補給に時間がかかる。今しばらくは日本から、ある程度の供給をつづける必要があるが、将来は、コスタリカ側の自主的努力に期待すべきであると思う。

また、EMのmaintenanceについては、修理、管理にあたる技術者の養成と共に、部品の補給の問題がある。供給機材の中に含まれる消耗部品的な資材は、当分充分であるが、EMは注意深く使えば、長年にわたって使用できるので、部品の供給については、将来にわたる配慮をする必要がある。

(5) EM Unitの建物について

EM Unitの建物は、今や、各室がそれぞれの機能と目的をもって、供与機器が配置されている。誠に、能率良く、部屋の機能がととのえられていると感じた。ただ心配な点は、試料作製用のスペースは、一度に3～4名の人が同時に作業しうる程度の広さしかなく、Trainingと、研究用の試料作製の全作業をここで行うことは、不可能ではないかと思われる。大学当局は、部屋の増設を考えているようであるが、早急の実現が望ましい。

(6) 研修及び研究計画について

1) EMのmaintenance 要員

この援助計画の開始にあたって、maintenanceの要員として、2人の現地の技術者、研究者を日本に招いて研修を行ったが、本人達が帰国後、ここのEMが、必ずしも円滑に作動していたとはいい難いようであった。実際に、活発に動き出したのは、小塚、赤堀及び福岡専門家が着任してからであったということである。これは、日本の研究機関の場合には、メーカーの修理サービスがゆきとどいているから、特に専門の技術者がいなくても、実際上あまり不安と感じないで、maintenanceがで

きることと比して、大きな違いである。

したがって、今後の重要な課題は、前述の部品の供給の問題と共に、maintenanceに充分な能力を有する現地の人を、養成することである。しかし、これにはかなりの年数を要することもあって、コスタリカ大学側は派遣専門家の長期滞在を強く希望しており、同時に、エレクトロニクス専攻者の中から一人選んで、日本で研修を受けさせると同時に、すでにかなりの経験をつんだ者を、再研修に日本に派遣することを希望しており、fellowship の面での配慮を要望している。これに関連して、日本側として、早急に検討を要することは、現地のoperatorに、どこまでの修理技術を身につけさせることが現実的に可能であり、また望ましいかということをある程度はっきりさせることである。これは、派遣専門家の意見と、メーカー側の意見を充分つき合わせた上で決めるべきことであるが、他方、今後この種の援助計画を考える上で、良い前例を作ることにもなるものであろう。

2) EMの医学、生物学への応用に関する研修教育

小塚、福岡両専門家を中心に、EMの取り扱いと、試料作製技術等の研修計画と、カリキュラムが詳細にまとめあげられ、すでに軌道に乗りつつある。別添V　資料3はそのカリキュラムである。この研修教育の理念は、一日も早く、コスタリカ大学のスタッフだけの力で、EMの研究、教育の出来る態勢を作ることにあるといってよい。カリキュラムの概要は大別して、

a. 過去にEMに関する教育を全く受けていない人に、初歩から研修させる。

b. 基本的技術をすでに修得している人に、更に高度なEM技術を修得させると共に、a の教育にたずさわる要員とする。

の二つに分けられる。現在、後者に属する研修生として、8名位があげられ、前者に6名位のpostgraduate の研修が考慮されている。また、日本で研修を受けたDr. Bolaños 及びDr. Jimenéz は、すでに研究活

動も開始すると同時に、教育スタッフとしても活躍している。

また、これと平行して、一般の学生や教官にも、現在の細胞生物学、微細細胞学の講義の中に、ＥＭ Unit で実際に撮影したＥＭ写真を供覧し、より具体的な教育内容の充実が行われており、この面での小塚専門家の役割は大きい。

3) 研 究 活 動

ＥＭ Unit を中心とした研究活動は、すでに２～３のグループで成果をあげつつある。（別添のＶ　資料２のＥ、Ｈ及びＩ）

一つは Dr. Mata を中心としたグループによる幼児伝染性下痢症のウイルス学的研究である。特に Rotavirus（infantile diarrhea virus）に関する検索については、このウイルスは現在、世界的に話題を呼んでいるウイルスである。

もう一つのグループは、Dr. Gamez を中心とするグループの植物ウイルスの研究であって、これは、従来からの Dr. Gamez の研究テーマであったが、ＥＭ的な検索は、ＥＭ Unit の設立によって、著しい成果をあげ得たものである。

(7) 結　　語

比較的短時日の間に、コスタリカ大学のＥＭ Unit の発展は、めざましいものがあり、なお、いろいろの困難な問題があるにせよ、着実にこの国に定着しつつあるように見える。

これは、現地に優秀な研究者がいて、この人達が中心となって、多くの人を引っぱっていることが大きいと思われる。また、派遣専門家とコスタリカ大学側との人間関係が非常にうまくいっていることもあずかって力あることである。

専門家に対する信頼も厚く、小塚専門家は、客員教授の待遇を受けることになったことは、これを如実に示しているものである。またＥＭ Unit の設立が、周辺に対する好ましい波及効果をもたらしつつあることも指摘せねばならない。コスタリカ大学は、ＥＭ Unit の設置によって、予算的に

も、機構の上でも、また、他分野の研究活動をも改変する役割を果しつつあることである。また、EM Unitについていえば、今までやりたくとも出来なかったような研究課題を、可能ならしめたといえる。

3. プロジェクトの将来方向

(1) コスタリカ側の説明と要望

1) 総　括

コスタリカ大学側からは、次のような将来方針が述べられた。将来方針の詳細は、別添のV　資料2のJに述べられているが、その活動の範囲は、研究の進展と、科学的訓練と、科学の進歩のための科学的協力とに大別される。

2) 研究の進展

研究の進展については、コスタリカ大学では、研究と技術に強調がおかれ、例えば、研究問題担当の副学長の制度が創設されている。コスタリカ大学における研究目標の再編成は、保健衛生と生物医学、及び生物科学の分野である。

まず、保健衛生と生物医学の分野における研究としては、コスタリカでは心臓疾患や高血圧やがん等が問題となっている。一方では、子供の下痢性疾患や栄養失調が見られる。研究のpriorityについては、すでに総論　1において述べられた。また生物科学の分野における研究については、保健衛生の領域における研究の一般的な組織化によって、各種の研究活動がおこってきた。

一方、研究の進展をはかるためには、医学の分野において研究及び訓練を遂行すべき機構の強化が必要であり、このためには、EM Unitの強化が必要であることが指摘された。とくにEM Unitについては、このUnitは、将来の活動にあたっては、よりスペースが入要であり、またEMや他の機材の追加が必要であること、またEM Unitの付属建物の建

設について必要のあることが述べられた。

3) 科学的訓練

科学的訓練に関しては、まず日本におけるコスタリカ専門家の訓練については、この継続が必要であり、将来においては、EMの問題について基本的な理解を既に持っている人々の訓練が必要であることが指摘された。

コスタリカにおける訓練としては、コスタリカ及び中央アメリカからの訓練された科学者(組織学者、医師、細胞学者等)で、EMの施設をまだ用いたことのない人々のためのコースが必要であることが述べられた。

4) 科学の進歩のための科学的協力

科学の進歩のための科学的交流に関しては、これは、日本人科学者のコスタリカ大学訪問と、コスタリカ科学者の日本訪問等によって行われるものである。さらに今後必要とされる機材のリストは、別添のV 資料2のKに示された。

5) コスタリカ側の提案

以上の説明の後に、コスタリカ側から、この医療協力事業を1977年から1982年まで、5年間延長することが提案された。

(2) 日本側の対応

このコスタリカ側の提案に対して、本調査団は、日本大使館と相談の上で次のように答えた。

コスタリカ側のEM Unitの利用に関する熱意と誠意及び今後の計画は十分に理解出来るものであるが、R. D. に定められた昭和48年度から昭和50年度までの3年間の期間はすでに終了していることであり、ここで、コスタリカ側の要望するように、R. D. の期間を更新することは、日本の予算の体系上残念ながらでき難い。しかしFollow upの形において、今後2年間継続することができる。このFollow upの制度によって、今後日本側専門家の派遣、コスタリカ専門家の日本での研修を続けることがで

き、また機材の供与もR.D. の期間に比較すれば総額は低下するが、供給することはできるので、このFollow up を続けたい。

これに対して、コスタリカ側は、日本側の医療協力の実施が事実上遅れたこともあって、若干割切れないとの態度を示しながらも、了解した。

ただし、Follow up の2年間はあまりにも短かすぎるとして、3年間のFollow up を強く要請し、これについては、コスタリカ側のEMの使用に関する熱意と誠意に答えるためにも、本調査団も実現の方向で検討する旨を述べた。

さらに、コスタリカ側は、既に述べたような将来計画があるので、同構想が固まった段階で新規プロジェクトとして日本側に提案したい旨述べた。

これに対して、本調査団側は、新案件を検討する立場にない旨先方に説明するとともに、新規案件は、将来、外交ルートを通じて交渉すべきである旨答えた。

V 資 料 1

Universidad de Costa Rica

Instituto de Investigaciones en Salud (INISA)

Unidad de Microscopía Electrónica (UME)

PROTOCOLO DE INVESTIGACION

Investigador *responsable* (chief investigator)

Investigador (es) asociado (s) (associated investigator)

Unidad o Institución (department an applicant belongs to)

Dirección (director) Tel.

Título de la investigación: (title of an investigation)

Objectivos (object of a proposed research project)

Justificación de la investigación:

(Significance of a proposed research project)

Personal que usará las facilidades de la Unidad

(person who uses the EM facility)

Breve descripción de la investigación (introducción, material y métodos, manera de analizar la información, etc.)

(background information of a proposed project)

Nota: Use por lo menos 2 páginas adicionales para describir adecuadamente el proyecto de investigación.

Indique qué conocimientos en microscopía electrónica posee su personal

(extent of knowledges an applicant have about EM)

Costo total de la investigación

(estimated cost needed for an accomplishment of a proposed project)

Fuentes de financiamiento

(sources of a fund used for an proposed project)

Cantidad total disponible a nivel de la Unidad de Microscopía Electrónica

(Amount of money which will be transferred to EM unit from the budget)

Duración a nivel de la Unidad de Microscopía Electrónica

(a period of time needed for EM examination)

Fecha de inicio

(Date of commencement of EM examination)

Equipo que empleará y horas mensuales

(Type of equipments needed for a proposed project)

Reactivos y otros materiales que necesita, y cantidad aproximada

(Reagents needed for a proposed project)

UNIVERSIDAD DE COSTA RICA

V 資料2

INSTITUTO DE INVESTIGACIONES
EN SALUD (INISA)

CIUDAD UNIVERSITARIA
RODRIGO FACIO
COSTA RICA

Documents
for the
Members of the Japanese
Government Scientific Mission

Dr. Tadashi Takeuchi, Chief
Dr. Munemitsu Hoshino
Dr. Jiro Yamamoto

Ciudad Universitaria Rodrigo Facio
September 9 and 10, 1976

CONTENTS

A Significance of the Electron Microscopy Unit in the University of Costa Rica

SIGNIFICANCE OF ELECTRON MICROSCOPE UNIT IN THE UNIVERSITY OF COSTA RICA

The electron microscope unit has established in 1974 under the medical cooperation program between the Government of Japan and the University of Costa Rica. The Installation of transmission electron microscope and related equipments enhance the research activities which made rapid progress in the research on virology and biomedical sciences. The number of research workers interested in the application of electron microscope grow rapidly in the past few months. The installation of scanning electron microscope is under way and the more people expected to join in the ultrastructural research group. The application of electron microscope in the biomedical science and other non-biological field would expect much precise and detail analysis on respected area. However, the advantages of the application of electron microscope made possible only under the condition of well trained research workers having ample knowledge on the operation of instruments, specimen preparation and interpretation of the results.

I think the electron microscope unit in the University of Costa Rica responsible for not only the application of electron microscope to the research work in the field of life science but also the training of the people who would expect to apply the electron microscope in the future. It is worth to consider at this moment that "What is the significance of the electron microscope unit in the university of Costa Rica."

Followings are some suggestion and hope to aided certain phase of discussion relating to the establishment of future plans and operation of electron microscope unit.

I. ESTABLISHMENT OF TRAINING PROGRAM

The objectives of the establishment of the training program is to preserve human resources for extended application of electron microscope in biomedical and even non-biological field, such as, minerology, paleontology, etc. The training course would tentatively set two different levels at two different period of time.

1. Those who has no previous experience in electron microscopy.

2. Those who have some experience in electron microscopy and wish to aquire new technique or improve his techniques for his own specific areas.

The former, the training course will comprises basic theory of electron microscope which does not necessary to aquire sophisticated modern electronic theory, the operation of equipment, general specimen preparation technique for biological specimen, i.e. Negative Staining, Shadow Casting, Replica, and Ultrathin Section Technique for cytology and histology in both plants and animals as they specified. I also with to emphasize that the handling of various chemicals, plastics in order to prevent unexpected accident in the laboratory due to their toxic nature and also the prevention of pollutions. For those who included in this group may have a chance to take alternative rather short period of training course for their own specific area together with basic electron microscope theory. It is applicable for limited research area such as bacteriology, virology, etc., not necessary to cover whole animal and plant materials.

The latter group of people would expect the application of electron microscope toward cytochemistry, histochemistry, autoradiography, freeze-etching and fracture technique. This group of people expected to work in the area connected to the biochemistry, molecular biology to ultrastructural cytology, histology to make much extended analysis on the ultrastructural level, and the work towards the analysis between form and functions of the living organisms.

Similar type of training should offer for the scanning electron microscope and all the course preferrably open to all research institute to Costa Rica and the Central American regions.

II. RESEARCH WORK TOWARDS THE ADVANCEMENT OF THE TECHNOLOGY OF ELECTRON MICROSCOPY

Current techniques applied to the specimen preparation for the transmission electron microscope and scanning electron microscope are fairely well established, however, there are still need to be improved in many ways. The constant improvement of the preparatory techniques is absolutely necessary and the work toward the improvement of technique may produce entirely new information which has been overlooked due to the application of conventional techniques. The electron microscope unit might be only the place to work out such problem and would be one of the

driving force for the advancement of the ultrastructure research in cell biology and life science.

III. LAISON FOR LIGHT MICROSCOPE AND ELECTRON MICROSCOPE

The study of cell biology may not be stand only by the knowledge based upon the electron microscope or light microscope alone. The light and electron microscope are equally important for the study of the cell biology, therefore, the installation of well equipped laboratory for light microscope is advised to the electron microscope unit. This type of laboratory for light microscope would particularly useful, and indispensable those who work on the field of histochemistry and cytochemistry at the electron microscope level.

IV. SUPPLIES FOR MATERIAL FOR THE EDUCATIONAL AND RESEARCH PURPOSES

A. The collection of electron micrographic slides, prints for the teaching aid would help students to visualize the actual ultrastructure for various cells and tissues. The slide or prints should be filed in appropriate order or system and open to public if necessary.

B. Supplies of up-to-date information on the ultrastructure research is considered as the another phase of activity of the electron microscope unit. The establishment of filling system of information comprised the collection of books, reprints and index card system would help to reduce the time consuming reference hunting in the library.

V. ELECTRON MICROSCOPE UNIT AS A SERVICE FACILITIES

It may takes a quite a while to aquire whole course of electron microscopic techniques. Therefore, it must be great help if we open the door to those who is not a electron microscopist need a electron micrograph for his part of work. The committee should carefully evaluate such a proposals, if submitted, and approve if necessary work as a cooperative basis. This type of service would enhance the research activity and will give correct knowledge of the application of the electron microscope in the research work.

Att: Dr. Mata, Dr. Gamez

Y. Kozuka

B List of Equipment and Materials donated to the University of Costa Rica by the Government of Japan

UNIVERSIDAD DE COSTA RICA
ciudad Universitaria "Rodrigo Facio"
Costa Rica, América Central

VICERRECTORIA DE INVESTIGACION

6 de setiembre de 1976
VI-1004-76

Señor Tetsusaburo Hitomi
Embajador del Japón
Embajada del Japón
Apartado 501
San José

Distinguido señor Embajador:

Tengo el agrado de dirigirme a usted para expresarle las muestras de nuestro más sentido agradecimiento por la distinción con que el Honorable Gobierno de la República del Japón ha asistido a la Universidad de Costa Rica en el desarrollo de la Unidad de Microscopía Electrónica y del Centro de Virología y Fisiología Celular, del Instituto de Investigaciones en Salud.

Cumplo con gusto la responsabilidad de comunicar al señor Embajador Hitomi, que la Universidad de Costa Rica acepta formalmente y agradece la donación del valioso equipo científico cuyo detalle aparece en la lista adjunta y el cual hemos catalogado en nuestro índice de equipo propiedad de la Institución.

Tenga la seguridad el señor Embajador de que la Universidad de Costa Rica hará el mejor uso posible de tan valiosa donación, la cual servirá para fortalecer una de las más apasionantes áreas de la actividad investigadora en que ahora se inician nuestros científicos.

Con muestras de mi más alta consideración y aprecio, me suscribo del señor Embajador,

Manuel M. Murillo, Ph. D.
Vicerrector de Investigación

MMM : nbb
cc: Dr. Claudio Gutiérrez
Dr. Leonardo Mata
archivo

Date of Shipment: April 2, 1974
Port of Shipment: Yokohama
Destination: Puntarenas

Item	Quantity	Price (¥)
Electron Microscope Hitachi HU-12A	1 set	16,575,000
Sapre Parts for HU-12A	1	1,637,000
Tilting Device Hitachi HK-6	1	1,600,000
Vacuum Evaporator HUS-5	1	1,000,000
Ultramicrotome LKB 8800	1	2,750,000
Ultramicrotome Table 4606	1	160,000
Knifemaker LKB 7800	1	480,000
Glass Strips for LKB Knifemaker	20	280,000
Scoreing Wheel	2	18,000
Balance M-100	2	6,950
Magnetic Stirer	1	18,000
Refrigerator Hitachi R-2142	1	60,000
Vacuum Cleaner Hitachi CU-1500	1	17,600
PH Meter	1	173,000
Tool Set S-5	1	5,500
Epon 812	8	19,200
MNA	8	20,800
DDSA	8	13,600
DMP-30	4	800
Bioden Mesh Cement	3	3,000
Polyvinyl Formal	1	1,200
Kit for Microgrid Preparation	1	2,500
Beem Capsules	10	19,500
Gelatine Capsules	20	14,000
Carbon Rod for Evaporation	2	14,600
Chromium Metal for Evaporation	2	3,200
Tungsten Wire	10 m	3,000

Tungsten Basket	5	9,000
Metal Wire for Electron Microscope	3	18,000
Grid VECO 200	50	140,000
Grid VECO 300	50	140,000
Grid VECO 400	10	28,000
Negative Envelopes	200	8,000
Polystylene Latex Particles 0.109	1	38,000
Polystylene Latex Particles 0.234	1	38,000
Polystylene Latex Particles 0.357	1	38,000
Kit for Supporting Membrane Preparation	1	12,000
Sharp Point Tweezer #7	3	9,600
Sharp Point Tweezer #5	3	10,500
Forceps	5	7,500
Stand for Gelatine Capsules	10	25,000
SANWA Circuit Tester	1	8,010
4 Slit Mesh	5	3,650
Incubator Yamato D FM-41	1	350,000
Transformer	1	14,000
Electric Machine Tool Set Hitachi	1	37,800
Transformer	1	5,000
Enlarger Fuji 45	1	205,000
Transformer	1	5,000
Enlarging Lens 50 mm	1	10,500
Enlarging Lens 90 mm	1	20,500
Spare Lamps	10	4,500
Negative Carrier 45-S	1	2,000
Easel Mask for 45 S Fuji-1012	1	3,000
Easel Mask 3W	1	1,100
Focusing Device LPE #2	1	1,200
Electric Timer for enlarger	1	5,000
Slidac for Developing Bath	2	130,000

Stainless Steel Photo Tray	4	18,400
Stainless Steel Photo Tray	3	1,500
Stainless Steel Photo Tray	3	6,600
Stainless Steel Photo Tray	2	3,800
Developing Tank for 35 mm Film	3	6,000
Developing Tank Deep Bath	3	31,800
Film Hanger	6	130,000
Film Clip	10	3,000
Safe Light Lamp	2	8,000
Safe Light Filter #2	2	1,240
Safe Light Filter #4	2	1,240
Safe Light Filter #5	2	1,240
Dark Room Timer	1	6,400
Print Washer	1	78,000
Transformer	1	5,000
Print Dryer	1	219,000
Transformer		17,000
Washing Syphon Hanza	2	3,600
Metal Paper Cutter	1	10,800
Plastic Solution Container 1000 ml	4	1,800
Plastic Solution Container 2000 ml	4	2,400
Plastic Solution Container 5000 ml	4	3,400
Negative Blower Brush UN-100	2	1,040
Magnifier Box	3	6,200
Light Box Fuji	2	11,200
Film Fuji Neopan F	24	4,320
Film Fuji Minicopy	24	5,280
Film Fuji Minicopy 100 Ft. Roll	100	259,000
Photographic Paper #2	1	4,210
Photographic Paper #2	1	3,700
Photographic Paper #2	1	5,470

Photographic Paper #2	4	12,630
Photographic Paper #3	3	16,410
Photographic Paper #3	3	11,100
Photographic Paper #4	2	8,420
Photographic Paper #4	2	7,400
Photographic Paper #4	2	10,940
E. M. Film Developer Copinal 4 Lit.	40	18,400
Developer Microfine	20	2,600
Papitol	40	15,200
Stop Bath Fuji	2	5,000
Fixing Bath Fuji Fix	40	18,000
Hypo Remover	3	4,500
Drywell	3	6,630
Casette Hanger	3	7,500
	872 Sets	¥27,242,610
	Shipping Charge	70,698
	Ocean Freight	590,275
	Insurance Prem.	171,966
		¥28,075,549

Date of Shipment: May 11, 1974
Port of Shipment: Yokohama
Destination: Puntarenas

Item	Quantity	Price (¥)
Filter Paper No. 7	2 Sets	2,000
Syringe 1 ml	2	8,000
Syringe 5 ml	2	8,000
Syringe 10 ml	2	6,000
Petri Dish 10.5 cm	20	9,000
Measuring Flask 50 ml	10	13,000
Measuring Flask 100 ml	10	15,000
Measuring Flask 100 ml	5	10,000
Graduated Cylinder 100 ml	10	15,000
Graduated Cylinder 500 ml	5	15,000
Graduated Cylinder 1000 ml	5	20,000
Conical Flask 50 ml	30	9,000
Conical Flask 100 ml	30	9,000
Conical Flask 500 ml	15	7,500
Serological Pipette 1 ml	50	15,000
Serological Pipette 5 ml	30	13,500
Serological Pipette 10 ml	30	16,500
Weighing Bottle 5 g	30	16,500
Weighing Bottle 10 g	30	16,500
Specimen Preparation Kit	1	258,890
	319 Sets	483,390
	Shipping Charge	12,035
	Ocean Freight	50,659
	Insurance Prem.	3,369
		¥549,453

Date of Shipment: July 9, 1975
Port of Shipment: Yokohama
Destination: Puntarenas

Item	Quantity	Price (¥)
Automatic Photomicrographic Equipment Model LUR-Ke, Type 4W. FAMB, Polaroid Camera Adapter, Roll Film Holder, Photo Stand 115 V. 50/60 Hz. NIKON	2 Sets	1,698,000
Olympus Zoom-Stereo Microscope Model SZ III w/set. Acc.	4	497,000
Fluorescence Microscope Unit LUR-Ke NIKON	1	772,000
Microscope Model SUR-Ke Type 4 NIKON	2	854,000
Dark Field Condenser Nikon	2	49,000
Low-Power Condenser Nikon	2	8,000
F.I.T.C. Interference Filter Nikon	2	156,800
Halogen Lamp Illuminator Nikon	1	86,000
Hitachi Model 65 P Ultracentrifuge	1	11,704,000
Hitachi Model 18PR-3 Refrigerated Centrifuge	1	1,848,000
Cooling System for Electron Microscope	1	1,400,000
Ultra Low-Temp. Cabinet Ebara ESL-160	1	1,074,000
Ultramicrotome Porter Blum MT2-B	1	3,120,000
Distilling Apparatus	2	152,400
	19 Sets	23,420,000
	Shipping Charge	58,880
	Ocean Freight	467,531
	Insurance Prem.	147,576
		¥24,093,987

Date of Shipment: March 31, 1976
Port of Shipment: Yokohama
Destination: Puntarenas

Item	Quantity	Price (¥)
Scanning Electron Microscope Hitachi HHS-2R	1 Set	18,270,000
Specimen Cooling Device HH-CS2	1	722,000
Specimen Exchange Device HH-EX 3	1	609,000
Dual Magnification DMS-11	1	400,000
Display OM-11	1	652,500
Specimen Manipulator HH-SM 3	1	478,500
Spare Parts for HHS-2R	1	696,000
Critical Point Dryer Hitachi HCP-1	1	609,000
Ion Cleaner IB-3	1	521,000
Chemical Balance Analytical Himadzu L-D	1	300,000
Centrifuge Hitachi 03 P	1	226,600
Rotary Vacuum Pump Hitachi 3VP-C3	1	124,630
Rotary Vacuum Pump Hitachi 4VP-C5	1	129,780
Spurr Low Viscosity Resin Kits	5	90,000
Ultrasonic Cleaner Sharp UT-52	1	95,000
Roll Film 120 Neopan SS	100	17,500
Polaroid Film 105 NP	120	190,800
Electron Microscope Film Fuji Type FG	30	217,200
Nikomart EL Nikon	1	105,930
Slide Copier & Bellows PB-5, PS-5	1	17,380
Pyramitome LKB	1	1,586,200
Polymerizer Sakura EM-200 T	1	360,000
Penetrator Sakura VEM-16	1	155,000

Analytical Meter	1	500,000
	25 Sets	27,075,810
	Shipping Charge	72,310
	Ocean Freight	518,385
	Insurance Prem.	170,508
		¥27,837,031

Date of Shipment: May 21, 1976
Place of Shipment: Tokyo
Destination: San José (Air Freight)

Item	Quantity	Price (Y)
Electron Microscope Films	70 Sets	435,400
Copy Paper (Photographic) V 2	8	26,560
Copy Paper V 3	20	66,400
Copy Paper V 4	10	33,200
Copy Paper V 2	10	38,650
Copy Paper V 3	10	38,650
Copy Paper V 4	10	38,650
Copy Paper V 2	10	57,100
Copy Paper V 3	10	57,100
Copy Paper V 4	10	57,100
	10 Sets	848,810
	Shipping Charge	10,130
	Air Freight	258,200
	Insurance Prem.	3,783
		¥1,120,923

Date of Shipment: July 6, 1976
Place of Shipment: Tokyo
Destination: San José (Air Freight)

Item	Quantity	Price (¥)
Tabb Resin Kit	4 Sets	69,200
	Shipping Charge	4,510
	Air Freight	15,990
	Insurance Prem.	2,000
		¥91,700

The following items were carried by Dr. Akahori on August 14, 1976.

Item	Quantity	Price (¥)
Specimen Drying Basket Size L	5	4,000
Specimen Drying Basket Size S	5	3,000
Mica Plate	2	3,000
Specimen Stub for Scanning Electron Microscope	100	30,000
Nylon Gloves	2 Cases	1,600
Metal Polisher	1	250
Acrolein	5	20,000
2-4-6 Collidine	5	20,000
Methyl Methacrylate	5	16,000
n-Buthyl Methacrylate	5	14,000
Ethyl Methacrylate	5	14,000
Vestopal W	5	27,500
120 Roll Film Fuji Neopan SS	50	9,500
Fuji Neopan SS Roll Film 100 Ft.	1	3,150
Freeze Cracking Apparatus TF-1	1	120,000
		¥286,000

Period	Amount of Donation (¥)
1974	28,075,549
	549,453
1975	24,093,987
1976	27,837,031
	1,120,923
	91,700
	286,000
Total:	¥ 82,054,643 =
	$ 279,097.42 =
	£2,383,491.88

C List of Japanese Specialists visiting the Electron Microscopy Unit

October 27, 1973 - November 14, 1973

Primary Mission from JICA

Dr. Konosuke FUKAI

Professor of Osaka University
Department of Preventive Medicine
Research Institute for Microbial Diseases
Osaka University

Dr. Takeshi YAMADA

Opthalmologist
Gunma Chuo Hospital
Research Staff of Gunma University

Mr. Eiryo SUMIDA

Staff. Medical Cooperation on Department
Overseas Technical Cooperation Agency

Mr. Hiroshi HIGUCHI

Staff. Second Technical Cooperation Section
Economic Cooperation Bureau
Ministry of Foreign Affairs

June 3, 1974 - July 8, 1974

Installation of transmission electron microscope Hitachi Model HU-12 A

Mr. Tomio MIYAMOTO

Engineer. Hitachi Naka Work

June 3, 1974 - August 14, 1974

Instruction for the operation of Hitachi HU-12 A electron microscope.

Dr. Hiroshi AKAHORI

Nissei Sangyo Co., Ltd. Tokyo

October 2, 1974 - November 26, 1974

Instruction on the application of electron microscope to the biomedical science.

Dr. Konosuke FUKAI

Department of Preventive Medicine
Research Institute for Microbial Diseases
Osaka University

November 2, 1975 - November 5, 1975

General check up and maintenance service for HU-12 A electron microscope.

Mr. Shusuke OKADA

Nissei Sangyo Co., Ltd. Benezuela Branch Office

November 29, 1975 - December 22, 1975

Installation of centrifuge Hitachi Model 65-P, 18-PR and related instruments.

Mr. Eichi KATO

Hitachi Koki, Katsuta

August 3, 1976 - September 4, 1976

Installation of scanning electron microscope Hitachi Model HHS-2R and accessories.

Mr. Tokinori MIYATA

Hitachi Naka Work

August 14, 1976 - September 15, 1975

Instruction of scanning electron microscope and general check of transmission electron microscope.

Dr. Hiroshi AKAHORI

Nissei Sangyo Co., Ltd. Tokyo

February 1976 --

The Japan International Cooperation Agency (JICA) has assigned two specialist at the period of two years for the Electron Microscope Unit in University of Costa Rica under Medical Cooperation Program effected in the year 1973. Both specialist has following duties and assume the responsibilities of following:

Dr. Yoshimichi KOZUKA

Department of Enzymology
Research Institute for Chemobiodynamics
Chiba University

1. Promotion of the application of the electron microscope toward the biomedical or biological science.

2. Give appropriate advice for the application of electron microscope or conducting cooperative research work with the research personels in the University of Costa Rica and other research institutions.

3. Establish the training program for those who interested in the application of electron microscope to their research work.

Mr. Takahisa FUKUOKA

Nissei Sangyo Co., Ltd. Tokyo

1. Maintenance of Electron Microscope and related facilities donated from Japanese Government to the University of Costa Rica.

2. Give appropriate training on the operation of equipment and related techniques on electron microscopy to the research personels in the University of Costa Rica and other research institute in Costa Rica.

D Costa Rican Trainees in Japan

COSTA RICAN TRAINEES IN JAPAN

Dr. Roberto Ortiz Brenes
Jefe del Depto. de Cirugía
Hospital Nacional de Niños
October - November, 1973

Dr. Gil Reinaldo Con Wong
Hospital México
April 1973 - March 1975

Sr. Rodolfo Bolaños Alfaro
Director Centro de Microscopía Electrónica
Universidad de Costa Rica
March - June, 1974

Dr. Víctor Jiménez Brenes
Profesor de Patología
Universidad de Costa Rica
March - June, 1974

Dr. Gerardo Serrato Chávez
Profesor de Virología Médica
Universidad de Costa Rica
April - December, 1975

Dr. Ricardo Luis Lizano Aguiar
Hospital San Juan de Dios
February - June, 1975

Dr. Pedro Rafael Goyenaga
Hospital Calderón Guardia
February 1975 - August 1976

Dr. Francisco Mirambell Solís
Hospital Nacional de Ninos
February - May, 1976

Dr. Marco Vinicio Bolaños
Caja Costarricense de Seguro Social
Hospital México
From March 4, 1976, for one year

Dr. Olga María Arroyo Gutiérrez
Instituto Clodomiro Picado
Universidad de Costa Rica
From March 4, 1976, for one year

E Research Projects - Electron Microscopy Unit

RESEARCH PROJECTS - ELECTRON MICROSCOPY

ROTAVIRUSES

Title:	Rotaviruses in acute childhood diarrhea.
Researchers	L. Mata, F. Hernández, C. Lizano, E. Mohs, T. Fukuoka, M.E. Peñaranda and M.E. López.
Initiation date:	January 1976.
Objectives:	To determine the prevalance of rotaviruses in hospitalized children with and without diarrhea, and to relate the findings with results of clinical, parasitological and bacteriological investigations.
Summary of progress (Sept. 1976):	Specimens collected from 90 children of several wards of the National Children's Hospital revealed rotaviruses in about a third of the instances during February and March. More viruses were discovered in cases than in control cases.
Termination date:	January 1977.

ROTAVIRUSES

Title:	Sero-epidemiology of family infections with rotaviruses.
Researchers:	F. Hernández and L. Mata.
Initiation date:	August 1976.
Objectives:	To study the serological response to rotavirus infection; to determine the occurrence of silent rotavirus infection; to work patterns of spread of rotaviruses within the family.
Summary of progress (Sept. 1976):	None.
Termination date:	December 1977.

ROTAVIRUSES

Title: Morphology and structure of human rotavirus.

Researchers: T. Fukuoka, Y. Kozuka, F. Hernández and L. Mata.

Initiation date: October 1976.

Objectives: To determine the exact size of the virion; to study the various kinds of particles; to explore the structural features of the rotavirus particle.

Summary of progress (Sept. 1976): None.

Termination date: December 1977.

GASTROENTEROLOGY

Title:	Scanning electron microscopy of normal and malnourished intestinal mucosa.
Researchers:	R. Bolaños, L. Mata, Y. Kozuka and M.E. López.
Initiation date:	August 1976.
Objectives:	To describe the characteristics of normal human mocosa, and to compare the features with those of malnourished mucosa. To provide the basis for interpretation of host-parasite relationships at the mucosal level.
Summary of progress (Sept. 1976):	None.
Termination date:	December 1977.

BIOLOGY OF REPRODUCTION

Title:	Effects of vasectomy on the ultrastructure of hamster testis.
Researcher:	F. Ureña C.
Initiation date:	January 1976.
Objectives:	To investigate ultrastructural changes occurring in the hamster testis upon vasectomy, in parallel with a study of the testosterone levels in plasma. To search for changes in the germinal epithelium (spermatogonia, spermatocytes, spermatids or sertolis) resulting from vasectomy.
Summary of progress (Sept. 1976):	The morphology of the normal hamster testis has been analyzed in order to control for alterations observed in the vasectomized animals.
Termination date:	June 1977.

GENETICS

Title: Ultrastructure of eucariotic chrosomes and chromatin.

Researchers: P. León and M. de los A. Villalobos.

Initiation date: July 1976.

Objectives: To characterize the ultrastructure of meiotic chromosomes, particularly the "diffuse diplotene" of amphibians and other vertebrates. To clarify the meaning of the diffuse and lampbrush diplotenes.

Summary of progress (Sept. 1976): Nucleosome preparations of the same dimensions as those described by others were obtained. Structures with diameters which are multiples of the nucleosome size have been seen.

Termination date: June 1977.

GENETICS

Title:	Scanning electron microscopy (SEM) study of lampbrush chromosomes.
Researchers:	Y. Kozuka and P. León.
Initiation date:	September 1976.
Objectives:	To determine the polarity of lampbrush loops; the orientation of loops with multiple asymmetries; and the appearance of double loop bridges.
Summary of progress (Sept. 1976):	None.
Termination date:	June 1977.

PLANT VIRUSES

Title: Purification, morphology and ultrastructure of maize rayado fino virus.

Researchers: R. Gámez, T. Fukuoka, Y. Kozuka and A.M. Espinoza.

Initiation date: April 1976.

Objectives: To develop a suitable method for obtaining purified virus preparations for characterization of biochemical composition and for serology and electron microscopy. To determine the multipartite nature of the virus. To establish the size and morphology of the virus and the number of morphological and structural subunits of the viral capsid.

Summary of progress (Sept. 1976): The virus has been purified through celarfication of crude plant extracts by low speed centrifugation, precipitation with PEG and sucrose density gradient centrifugation. Three distinct components have been shown in the gradient columns. The top component contains empty particles, and the middle and bottom components contain full isometric particles 27 ± 2nm in diameter.

Termination date: December 1977.

PLANT VIRUSES

Title: Morphology of legume viruses.

Researchers: R. Gámez, T. Fukuoka, Y. Kozuka and A.M. Espinoza.

Initiation date: August 1976.

Objectives: To determine the morphology of the chrysomelid-transmitted legume virus, as part of the characterization studies on those viruses. To acertain the feasibility of the utilization of transmission electron microscopy for the localization of the virus in insect tissues and hemolymph.

Summary of progress (Sept. 1976): None.

Termination date: August 1977.

PLANT VIRUSES

Title: Maize rayado fino virus: intracelular location and ultrastructural changes associated with infected plants.

Researchers: E. Flores, A.M. Espinoza, Y. Kozuka and R. Gámez.

Initiation date: August 1976.

Objectives: To determine the intracelular location of the virus in infected cells and possible replication sites; the ultrastructure of infected cells and tissues and its relationship to symptomatology of the disease; and the sequential infection of different organs of the maize plant.

Summary of progress (Sept. 1976): None.

Termination date: December 1977.

MOLLECULAR BIOLOGY

Title: Electron microscopy of transferable plasmids.

Researchers: M.E. Peñaranda, T. Fukuoka, Y. Kozuka and L. Mata.

Initiation date: October 1976.

Objectives: The TEM will be used to visualize plasmids extracted from bacterial cells. This will aid with electrophoresis analysis in the identification and characterization of plasmids from different origins.

Summary of progress (Sept. 1976): None.

Termination date: December 1977.

MEDICAL BACTERIOLOGY

Title:	Scanning electron microscopy of intestinal indigenous bacteria of small children.
Researchers:	L. Mata, Y. Kozuka, T. Fukuoka and M.E. Peñaranda.
Initiation date:	October 1976.
Objectives:	The indigenous flora of breast-fed and artificially-fed infants will be characterized by SEM. Distinctive features are expected to appear using modern techniques.
Summary of progress (Sept. 1976):	None.
Termination date:	December 1977.

OTHER

Title: Development of the Unit of Electron-microscopy of INISA.

Responsible: L. Mata.

Initiation date: January 1976.

Objectives: To develop and expand the Unit of Electron Microscopy to serve the University Community and other national institutions.

Termination date: Indefinite.

F List of Persons working in the Electron Microscopy Unit

LIST OF PERSONS WORKING IN THE ELECTRON MICROSCOPE UNIT

Name	February	March	April	May	June	July	August
Dr. Leonardo Mata (INISA)	Rotavirus						
Gerardo Serrato (INISA)	Rotavirus						
Francisco Hernández (INISA)			Rotavirus				
Dr. Rodrigo Gámez (Agr.)	Plant Virus (Maize and Legume)						
Dr. Francisco Ureña (Med.)		Developmental Biology of Hamster Testis					
Ana Mercedes (Agr.)			Plant Pathology (Maize and Legume)				
Dr. Fernández Pacheco (Med.)			Ultrastructure of Leptomonas sp.				
M.E. Peñaranda (INISA)				Bacterial Conjugation			
Elena Campos (INISA)				Morphology of Anaerobic Bacteria			
Mansirio Odio (Med.)				Preliminary °			
Miguel Solano (Med.)				Preliminary °°			
Dr. Pedro León (Med.)				Ultrastructure of Chromosome			

_____ Indicated the time of start and continuation of work.

°, °° : Cerebrical Cortex Ultrastructure Preliminary Work.

G Course on Basic Electron Microscopy

COURSE ON BASIC ELECTRON MICROSCOPY

Credit:	3
Duration:	1 Semester
Number of Students:	6
Requirement:	Students must have at least a Bachelor of Science or the equivalent, or be graduate students in Biology, Microbiology, Pharmacy, Medicine, Dentistry or Agriculture.

TOPICS OF THE ELECTRON MICROSCOPE TRAINING COURSE

1. Historical background and modern instruments
   - 1-a Early history
   - 1-b Modern electron microscope (EM) up to high voltage EM
   - 1-c Difference between light microscope and EM
     - 1-c-1 Construction of instrument, resolution, etc.
   - 1-d Transmission (TEM) and Scanning (SEM) electron microscopes: differences in construction and function

2. Construction of Transmission and Scanning Electron Microscopes
   - 2-a General Construction of TEM
   - 2-b Evacuation system
   - 2-c High voltage supply system
   - 2-d Electron source
   - 2-e Lens system
   - 2-f Specimen chamber
   - 2-g Recording system (Camera chamber)
   - 2-h General construction of SEM
   - 2-i -- 2-n Same as 2-b -- 2-g

3. Instruments necessary for TEM and SEM specimen preparation

   3-a Vacuum evaporator

   3-b Ion coater IB-3

   3-c Critical point dryer HCP-1

   3-d Ultramicrotome

      3-d-1 Mechanical feeding system

      3-d-2 Thermal feeding system

   3-e Knife maker

   3-f Pyramitome

4. Photography

   4-a Developing and developer

   4-b Handling of photo sensitized materials

   4-c Enlarger and enlarging lens

   4-d Calibration of magnification

5. Applications of TEM and SEM

   5-a Negative staining technique

   5-b Shadow casting technique

6. Specimen preparation I. Virus, bacteria and other small objects

   6-a Grids and supporting membrane

   6-b Negative staining technique; shadow casting technique

   6-c Analysis of images

7. Specimen preparation II. Ultrathin section technique

   7-a Fixation: aldehyde, osmium tetroxide and other fixatives

   7-b Choice of fixative according to the materials

   7-c Embedding plastics. Choice of plastics

8. Specimen preparation III. Ultrathin section
   - 8-a Ultramicrotomy: glass knives, handling of specimen
   - 8-b Staining: block staining, section staining
   - 8-c Contamination during staining and its correction

9. Specimen preparation IV. Scanning Electron Microscope
   - 9-a Fixation, dehydration and critical point drying
   - 9-b Factors influencing the final image

10. Image analysis and interpretation
   - 10-a Quality of printed picture
   - 10-b Trouble shoting for specimen preparation
   - 10-c Improvement of overall techniques
   - 10-d How much can we get from the electron micrograph?

COURSE ON ELECTRON MICROSCOPY MICROGRANISMS

Credit:	3
Duration:	1 Semester
Number of Students:	3 to 6
Requirements:	Basic Electron Microscopy Course, Microbiology and Parasitology

1. Specimen preparation. Virus, bacteria and other small objects
   - 1-a Grids and supporting membrane
   - 1-b Negative staining technique, shadow casting technique
   - 1-c Analysis of images

2. Specimen preparation. Ultrathin section technique
   - 2-a Fixation: aldehyde, osmium tetroxide and other fixatives; buffer system, pH and tonicity
   - 2-b Choice of fixative according to the materials
   - 2-c Embedding plastics; choice of plastics

3. Specimen preparation. Ultrathin section
   - 3-a Ultramicrotomy
   - 3-b Staining: block staining, section staining
   - 3-c Contamination during staining and its correction

4. Specimen preparation: Scanning electron microscope
   - 4-a Fixation, dehydration and critical point drying
   - 4-b Factors influencing the final image

5. Image analysis and interpretation

   5-a Quality of printed picture

   5-b Trouble shooting for specimen preparation

   5-c Improvement of overall techniques

   5-d How much can we get from the electron micrograph?

COURSE ON ELECTRON MICROSCOPY OF CELLS AND TISSUES

Credit:	3
Duration:	1 Semester
Number of Students:	3 to 6
Requirement:	Basic Electron Microscopy Course: Cytology, Cell Biology and Histology

1. Specimen preparation
   - 1-a Grids and supporting membrane
   - 1-b Fixation: aldehyde, osmium tetroxide and other fixatives; buffer system, pH and tonicity
   - 1-c Choice of fixative according to the materials
   - 1-d Embedding plastics; choice of plastics

2. Specimen preparation
   - 2-a Ultramicrotomy: glass knives, handling of specimens
   - 2-b Staining: block staining, section staining
   - 2-c Contamination during staining and its correction

3. Specimen preparation: Scanning electron microscope
   - 3-a Fixation, dehydration and critical point drying
   - 3-b Factors influencing the final image

4. Image analysis and interpretation
   - 4-a Quality of printed picture
   - 4-b Trouble shooting for specimen preparation
   - 4-c Improvement of the overall techniques
   - 4-d How much can we get from the electron micrograph?

H Manuscripts prepared with Data Obtained in the Electron Microscopy Unit

THE LEAFHOPPER TRANSMITTED MAIZE RAYADO FINO VIRUS IN CENTRAL AMERICA

Rodrigo Gámez

Centro de Investigación en Virología y Fisiología Celular

INISA

Universidad de Costa Rica

THE LEAFHOPPER-TRANSMITTED RAYADO FINO MAIZE VIRUS IN CENTRAL AMERICA

HISTORY AND DISTRIBUTION

The rayado fino disease has been known in Central America since 1961. It was originally considered one of four different types of "corn stunt" present in this area and transmitted by the leafhopper Dalbulus maidis Delong & Wolcott (Ancalmo and Davis, 1961). Gámez (1969, 1971) demonstrated that the disease was caused by a virus transmitted by D. Maidis-Delong & Wolcott (Ancalmo and Davis, 1961). Gámez (1969, 1971) demonstrated that the disease was caused by a virus transmitted by D. maidis in a persistent manner, and showed that the leafhopper could simultaneously transmit the rayado fino virus (RFV) and the corn stunt spiroplasma.

Rayado fino has been found in México, Guatemala, El Salvador, Honduras, Nicaragua, Panamá, and Perú (Gámez, 1969, 1971, and unpublished data). The corn streak virus (BCSV) described in Brazil (Costa, et al., 1971) Kitajima, et al., 1975b) is serologically identical to RFV and similar to it in the symptomatology of the infected plant, the morphology of the virus particle, and the type of ultrastructural changes induced in the infected cells. BCSV and RFV are considered identical or closely related viruses (Kitajima, et al., 1975a). The Colombian maize stripe virus (CMSV) (Martínez-López, et al., 1974) is also similar to RFV in symptomatology of the infected maize plant. Preliminary tests have shown these viruses to be serologically identical (Gámez and Martínez-López, unpublished data).

The virus occurs in areas where the leafhopper vector is present and has been observed from the warm coastal plains to the high, cool mountain valleys and plateaus of Central and South America.

SYMPTOMS

When infection occurs during the plant's early growth stages, disease symptoms appear within 8 to 14 days. At first a few chlorotic dots develop at the base and along the veins of the young leaves; in the following new leaves the dots gradually become more numerous and occasionally some of them fuse; and, finally, a characteristic fine stripple stripping of the vein

prevails (Fig. 1A). Long continuous stripes are seldom found in the more tolerant varieties, but this symptom is frequently observed in the more susceptible genotypes, which may also show varying degrees of stunting and chlorosis (Gámez, 1969).

Disease symptoms are more conspicuous during the first 3 or 4 weeks after infection; in tolerant varieties they gradually become milder and eventually, as the plant reaches maturity, are absent from the newly formed leaves. Plants infected late may show only inconspicuous stipple stripping at the base of their leaves, or, frequently, novvisible symptoms at all. Plants infected early produce ears of reduced size.

TRANSMISSION

The virus is transmitted by the leafhopper D. maidis (Gámez, 1969, 1971), its only known vector (Fig. 1B). Attempts to transmit the RFV by the planthopper Peregrinus maidia Asch, by sap inoculation, or through seeds from infected plants were unsuccessful. Insects become viruliferous after injection of the virus into their homocoel, or by artificially feeding on virus preparations through membranes (Gámez, 1971, and unpublished data; Paniagua and Gámez, 1976).

Transmission of RFV occurs in a manner characteristic of viruses that multiply in their leafhopper vector. Insects transmit the virus after incubation periods of 8-37 days (Gámez, 1969, 1971; González and Gámez, 1974). Males have shorter innubation periods than females, but die sooper; also, females are more efficient vectors than males. Among females to 75% may be active transmitters as compared to 4.0 to 20% of the males. No appreciable differences were observed between nymphs and younger adults in their ability to transmit the virus. (Gámez, 1971; González and Gámez, 1974; Paniagua and Gámez, 1976).

Infectivity is retained by insects for 1-20 days; transmission by most insects is intermittent, and inoculativity by D. maidis decreases with time but the virus may be recovered from insects that have lost their ability to transmit (Gámez, 1971). The shortest acquisition and feeding periods are 6 and 8 hours respectively. The number of active transmitters in a colony varies from 11 to 34%. Colonies with 23 to 40% transmitters in the F_2 generation may be developed through controlled matings and the selection of viruliferous progeny, but these more active colonies return to the normal level of transmission following a

few generations of fandom mating. Puncturing the abdomen of nymphs immediately before or after acquisition results in slight increases in the ability of D. maidis to transmit RFV (Paniagua and Gámez, 1976). Insects retain infectivity after moulting, but the virus is not transovarially transmitted to the offspring of infected females.

RFV causes no observable deleterious effected on its vector, and longevity of transmitters and nontransmitters is similar (González and Gámez, 1974).

THE VIRUS

The particles of RFV are isometric and 25-30nm. in diameter (Gámez and Ramírez, 1975; Kitajima, et al., 1975a; and Gámez, Fukuoka, and Kozuka, unpublished data). Fig. 1C.

The virus may be extracted and isolated from infected maize leaves in 0.01M. phosphate buffer pH 6.9 using methods which involve a polyethylene glycol 6000 (PEC) precipitation and sucrose density gradient centrifugation. Empty and full particles have been separated in density gradient columns. Infectivity of the preparations has been determined by injecting or artificially feeding the vector through membranes (Gámez and Ramírez, 1975; Gámez, Fukuoka, and Kozuka, unpublished data).

Partially purified preparations have been used to prepare RFV antiserum which has permitted its detection in plants and the determination of its serological relationships to BCSV and CMSV (Gámez and Ramírez, 1975; Gámez and Martínez-Lopez, unpublished data; Kitajima et al., 1975a). Electron microscopy of ultrathin sections of infected maize leaves has revealed the presence of RFV particles in vacuoles of parenchimatous and epidermal cells (Kitajima, et al., 1975a). Obvious alteration in cell organelles has not been observed.

HOST RANGE AND EPIDEMIOLOGY

Only two species of Gramineae are presently known to be susceptible to RFV: Z. mays and Z. mexicana. Other species of wild and cultivated grasses tested as host of RFV and found immune include: Oryza sativa L.; Secale cereale L.; Triticum vulgare L.; Eragrostid sp.; Axopopus scoparius (Flugge) Hitch.; Digitaria sanguinalis (L.) Scop.; Paspalum conjugatum Bergius;

Sacharum officinarum L.; Sorghum halepense (L.) Pers.; Tripsacum laxum Nash; and Coix lachryma-jobi L. Other species of common wild herbs or grass-like herbs tested include Setaria geniculata (Lam) Beaw; Cenchrus sp. and Cyperus tenuifolius (Stend.) Dandy (Gámez, 1971; Paniagua and Gámez, 1976).

Maize and teosintle are the only species known to be susceptible to the virus and are the only plants with which D. maidis may successfully complete its life cycle (Barnes, 1954; Pitre, 1967). Other species of grasses may serve as feeding host to the vector in the United States (Pitre, 1967); and certain unknown species of wild grasses probably serve as hosts to D. maidis and RFV between planting seasons in tropical and subtropical areas of Latin America; but maize is presently the only known host plant for both virus and vector in South and most parts of Central America, since the distribution of teosintle is restricted to Northern Guatemala and México.

ECONOMIC IMPORTANCE

Yield losses on individual early infected plants of local Central American varieties may be 40-50% of the weight of the mature ear, but may reach 100% in introduced foreign or newly developed maize varieties (A. Díaz, personal communication; Gámez R.; unpublished data). Disease incidence is variable depending on maize genotypes, geographical area, and seasonal and climatic conditions. Locally adapted varieties usually show infection in 0-20% of the plants, but incidence may reach 100% in the more susceptible genotypes. Although the incidence of RFV appears to be gradually increasing in many areas of Central and South America, it has not attained the prevalence and economic importance that the corn stunt has here.

CONTROL

Limited information is available on the behavior of maize materials in relationship to RFV. Observation carried out at experimental stations in Costa Rica and El Salvador have shown that introduced lines and varieties are more susceptible that the local ones. No immune varieties are presently available.

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PANIAGUA, R. and GÁMEZ, R. (1976). El virus del rayado fino del maíz: estudios adicionales sobre la relacion del virus y su insecto vector. Turrialba 26, 39-43.

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VIRAL INFECTIONS DURING PREGNANCY AND IN EARLY LIFE[1, 2]

Leonardo Mata[3], Juan J. Urrutia[4],
Gerardo Serrato[3], Edgar Mohs[5]
and Tom D. Y. Chin[6]

INSTITUTO DE INVESTIGACIONES EN SALUD (INISA)
Universidad de Costa Rica
San José, Costa Rica

[1]Presented at the Fourth International Symposium on Intestinal Microecology, Columbia, Mo., May 24-25, 1976.

[2]Supported in part by USPHS-NICHD Contract N° NO1-DH-2-2737, and by the University of Costa Rica, the National Children's Hospital and CONICIT, Costa Rica.

[3]Instituto de Investigaciones en Salud, Universidad de Costa Rica, Costa Rica.

[4]Instituto de Nutrición de Centro América y Panamá, Guatemala.

[5]Hospital Nacional de Niños, Costa Rica.

[6]University of Kansas School of Medicine, Kansas.

Abstract

There is evidence that fetal antigenic stimulation and intrauterine infection is much more frequent in developing rural populations than in industrialized societies. A similar contrast is observed as far as postnatal intestinal infection is concerned, in that rates are significantly greater in the less developed areas. The differences are explained by the divergence in environmental sanitation and personal hygiene. Intestinal infection is important in that diarrheal disease is one of the main factors leading to malnutrition. It is apparent that for developing nations to attain a better nutrition, much of the present burden of intestinal infection needs to be controlled.

Under optimal conditions the fetus is well protected from the infections environment of the mother and her surroundings. However, a variety of factors, some only suspected, permit occassional infection of the membranes or the fetus itself. The development of technology to identify viral infections in the mother and in the unborn has demonstrated an increasing number of viral agents causing antenatal infection, and has shown that, after all, the fetus is not so invulnerable as we once thought.

Often, infection in the mother does not cause clinical manifestations in the fetus, although interruption of pregnancy, pre-term delivery, fetal growth retardation, embryopathy, overt disease and sequelae on growth and performance may occur[1-5].

After birth there is an increased opportunity for infection and the beginning of a series of manifestations of host-parasite interactions. Microbial invasion, including viral infection, influences the development of immune competence of the host, and at the same time, exerts profound effects on his nutrition, growth and development and survival[6-8].

This paper will touch on some aspects of antenatal and early postnatal infection with special reference to viruses, and will give particular attention to the phenomenon as observed or expected in developing, pre-industrial societies. Part of the data to be presented have been obtained from the long-term prospective observation of children of an Indian village in Guatemala[9-11] and from prevalence observations of hospitalized children in Costa Rica[12]. The problem of viral infection in the fetus and the infant and pre-school child will be discussed first in more advanced, industrial societies, and then in the less developed ones, placing emphasis on the latter.

1. Antenatal viral infection

The concept of fetal sterility has been challenged with the apparent finding that "C" particles are a common occurrence in human fetal tissues[13] and that they may be transmitted vertically to determine, in utero, risk of degenerative disease in later life. Aside from this biological possibility, viruses have a relatively well defined course of action in the human host, to which the response is inflamation, cell proliferation and necrosis, with the corresponding sequelas[1-5, 14, 15].

a. Industrialized countries

Studies in societies living under conditions of good sanitation have shown that synthesis of immunoglobulin M (IgM) by the fetus, frequently a response to intrauterine infection, is a rare event[16, 17]. Detailed prospective observations in the lower socioeconomic strata, however, indicate that intrauterine infection is not so rare[18]. Evidently there is quite an impressive incidence of maternal infection which becomes evident through systematic serologic or clinical prospective study[19, 20]. Many antenatal viral infections are silent in the mother and without apparent consequences on the fetus, although a considerable number of infants in industrial nations are born handicapped or will become so as a result of antenatal infection[21-23]. A large number of viral agents (Table 1) have been found to cause intrauterine infection and damage to the fetus[1-5,21, 23]; the list has a good possibility of enlarging.

b. Pre-industrial countries

Of the very few studies done on this aspect in developing nations, most were circumscribed to the hospital environment and did not adequately represent the rural population. In one rural Indian village of Guatemala, prospective observations were carried out which are probably representative of the situation in similar rural areas of the region. The description of the village and its population and of the methodologies employed have been given elsewhere[8-11,24], and some of the findings will be discussed here.

Viral infection in the small village setting is determined in great part by the deficient environmental conditions influencing women from the moment of conception. Even though rural communities are relatively isolated, particularly in nonindustrial, agricultural societies, present transportation facilities provide effective means of communication with the larger

population centers. Thus, a continuous supply of viral and microbial agents is insured[24]. Agents are brought into the community and spread by person to person contact and other mechanisms until susceptibles become immunized and transmission is arrested. Some agents, however, tend to persist, either as latent or silent infections (healthy and convalescent carriers) or in association with mild recurrent disease. Still others, like cytomegalovirus infection, probably have always been transmitted from mother to child in the villages. Nevertheless, it is possible to conceive that some isolated communities were free from these and similar viral agents in ancient times.

The village mother does not escape the force of infection. Table 2 summarizes the infectious disease experience of a cohort of 82 village women studied prospectively[25]. It is striking that in 30 per cent of the pregnant women, lower respiratory tract infection was noted, and in 36 per cent, diarrhea or dysentery. Undoubtedly, morbidity rates are much greater than those found in industrial societies[20].

It is not surprising that just as the village women have greater opportunities for infection during pregnancy than women in industrial societies, so too, their fetuses are stimulated by antigenes more frequently. Table 3 shows the distribution if IgM and IgG values in umbilical cord serums of 250 consecutive village newborns. Specimens with IgA values above 0.09 mg/ml were removed from the tabulation, on suspicion that they might have been admixed with maternal blood at the time of collection, which was done by two traditional Indian midwives. It is striking that 40 per cent of the specimens had "elevated values of IgM[24,25]. In fact, 10 per cent of the cases had values of 0.40 mg/ml or greater, clearly indicating fetal antigenic stimulation. Still, some of the specimens could have had admixture with maternal blood. Then a more detailed study of consecutive newborns in four rural lowland mestizo communities which had health problems similar to those in the highland village, was conducted. This time blood was obtained from the femoral vein within 3 or 4 days of delivery, and IgM concentrations were determined as in the preceding study[26,27]. Again, a large number of infants, specifically 15 per cent, had unquestionable evidence of having been stimulated by antigens *in utero.*

A prospective serological study of 61 of the 82 pregnant women mentioned above revealed a 12 per cent conversion rate to cytomegaloviruses, herpesviruses and *Toxoplasma gondii*, Table 4. No antibody responses were noted against rubella or syphilis; no testing was made for any of the several other dozen agents that

may cause prenatal infection[3,18,23]. What the data indicate is the frequent occurrence of infection in the pregnant woman, and a likely infection and/or antigenic stimulation of the fetus. The events are apparently more frequent than described for societies living under better conditions.

2. Postnatal infection

From birth onwards the child can be infected with viruses mainly through the respiratory and alimentary routes. The risk and probability of infection are determined by the nature of the ecosystem in which the child lives.

a. Industrial countries

Due to a viriety of conditions that determine cleanliness, early infection with intestinal viruses is a relatively rare event in societies of industrial nations and in well-to-do sectors of the urban areas of developing countries. This fact undoubtedly contributes to the slower rate of "maturation" of the serum immunoglobulins in children from industrial societies[28]. On the other hand, this circumstance favored the assessment of pathogenicity of certain viruses in the diarrheal syndrome, in that viral infections occurred in relative isolation from other infections which could make it difficult to interpret the results. Thus, several "filterable agents", which, unfortunately, are not now available for identification, were described in the pre-tissue culture era[20]. The advent of cell cultures permitted recognition of many serotypes of enteroviruses and adenoviruses in the causation of diarrhea[30-33]. It became apparent later that infection with enteroviruses and fecal shedding was a relatively common event, particularly during the summer[34] and among institutionalized individuals[35]. However, an intestinal "viral flora" does not seem to exist in individuals living in highly developed environments.

The identification of the calf diarrhea virus[36] and its study by the electron microscope (EM) was the beginning of a new era in intestinal virology. The application of immune sera to viral preparations permitted agglutination of the virions and easier observation in the EM[37]. Examination of fecal preparations of young children by the EM revealed reovirus-like agents or rotaviruses[38,39] and several other viruses, such as the Norwalk agent[40], astroviruses[41] and caliciviruses[42]. The agent of hepatitis A was also demonstrated in fecal extracts[43], as well as coronaviruses[44], adenoviruses, picornaviruses and

bacterial viruses[45].

The relationship of these agents to diarrheal disease is summarized in Table 5. Rotaviruses and 27nm particles are agents which appear to be important in the etiology of outbreaks of diarrhea in industrial countries, particularly in winter months.

b. Pre-industrial countries

No village child escapes from early intestinal infection. During delivery, which in the village occurs without any elaborate preparation of the mother, the infant has a good opportunity of acquiring intestinal viruses which she harbors; for instance, one study revealed that 25 per cent of the women may carry enteroviruses[8]; maternal defecation was detected in almost all of the deliveries[24].

Early infection of the child's intestinal tract is expected under these conditions, Table 6. Fecal extracts were inoculated in primary human amnion, primary human kidney (post mortem) and HEp-2 cells[24]. Among a cohort of 79 infants, one showed an infection with echovirus 7 in the first day of life; the viral concentration was 10^2 $TCID_{50}$ per gram. Seven per cent of 54 infants were shedding viruses in the second day of life; one child had poliovirus 1 at a titer of 10^5 $TCID_{50}$ per gram; eight per cent of 61 children showed infections on the third day of life, and these had larger virus concentration in their feces.

Enteric viral infection in newborns increases as a function of age; they become chronic virus shedders by age six months. Figure 1 depicts the natural history of viral infection in 18 infants during their first year of life. Some were practically free of infection for about three months, while others had infections in the first weeks of life. The prevalence of fecal excretion of viruses is in Table 7. About 20 per cent were shedding viruses in the first six months of life; 42 per cent, in the second half of the first year of life, and 53 per cent in the third six months. The prevalence was over 50 per cent during the second and third years of life. Most of the cytopathogenic viruses isolated were echo-like. The remainder were polio-, coxsackie- and adenorivuses.

A recent study of hospitalized children in Costa Rica revealed frequent infection of the intestine with the ubiquitous rotaviruses[12] (Figure 2). Fecal extracts prepared by treatment with fluorocarbon were examined in formvar-coated grids

after negative staining. No antibody treatment was necessary and viruses were easily detected by EM. The preliminary results indicated that rotaviruses behave similarly to the enteroviruses as far as age distribution is concerned. The agents were not rare in young infants, and they were more prevalent as age increased, attaining a prevalence of about 50 per cent in children 12 to 17 months old, Table 8.

In view of the frequent difficulty of showing a clear cause-effect relationship, the significance of virus infection in diarrheal disease has been questioned. The clear cytopathogenic and lytic capacity of viruses make it untenable to disregard their participation in diarrhea, malabsorption and colitis. Part of the problem, for instance, resides in the finding of as many enteroviruses in cases of diarrhea as in the controls. Nevertheless, studies of well defined outbreaks clearly show an association of several serotypes of enteroviruses and adenoviruses, and of rotaviruses and other agents with diarrheal syndrome.

In endemic diarrhea the role of enteroviruses is difficult to demonstrate[46]. The rotaviruses appear to have a much greater pathogenic potential since they are found more frequently in diarrhea episodes than in periods of good health, Table 8. About 38 per cent of the cases of diarrhea in the hospital had rotaviruses, while only 20 per cent of the controls had them[12]. It should be emphasized that in this study the controls were children of comparable age from the same wards where the cases occurred. Thus, the probability of cross infection was a factor in making it more difficult to establish the role of rotaviruses in the diarrheal syndrome.

Comment

The technological advance in virology and immunology has permitted demonstration that the human fetus is infected with viruses with relative frequency[18,22,23]. Fetal infection and antigenic stimulation seem to be directly related to low socioeconomic development and appear greater in pre-industrial tropical regions[25,47] than in industrial nations[16-18]. The practical consideration is that the phenomenon of antenatal infection contributes to fetal wastage, fetal malnutrition, premature delivery and sequelae. In fact, a very high incidence of fetal growth retardation has been recorded in the Indian village from which much of the data presented here were derived[48]. However, how much of the overall problem of low birthweight is

due to infection and to factors related to maternal nutrition and infection background, remains unknown. Maternal malnutrition, as reflected by her height, appears to be one of the most important factors in causation of fetal malnutrition[48]. Infection, in turn, is a determining component of the nutritional status of the mother by a variety of mechanisms, including the restriction of calorie intake in the anorexia that so often accompanies infectious disease.

The cell-dependence and cytopathogenic properties of viruses appear incompatible with the concept of a "normal" viral flora in postnatal life. Children in industrial nations have a significantly lower incidence of intestinal viral infection[34, 49] than their counterparts in developing countries[46,50,51]. The reasons are a better hygiene and the seasonality of the climate in those countries. Childhood populations in underdeveloped areas are normally infected with intestinal viruses, particularly in the first years of life. The phenomenon is probably related to the precocious "maturation" of the serum immunoglobulins in rural populations[28].

Frequent viral infection, together with bacteria and parasites, is a factor in the high morbidity observed in traditional societies living in poverty. In the Guatemalan Indian village, surveillance of families by a physician and nurses permitted accurate collection of morbidity data[24]. High rates of diarrhea and other illnesses were observed since early infancy, but especially during the weaning process (6 to 24 months). Breast-feeding in the first months was an important factor in protection against some of the infection observed[52]. During the period of exclusive breast-feeding, children were relatively free of disease, and symptoms were milder; mortality, however, was very high in pre-term and small-for-gestational age infants[24, 26,48]. Infectious disease has an associated negative effect on the nutritional state of the child, which becomes more important after 3 to 6 months of age in breast-fed village infants. The damage of infection, particularly when diarrhea is present, is weight loss, arrest in height, metabolic alterations in cell function, nutrient wastage and nutrient diversion[6-11]. Weight loss in diarrheal disease is acute and detectable within a few hours or days from the onset of disease. It results from dehydration and loss of tissue mass, and may become aggravated or persist for weeks or even months. Anorexia and despondency are symptoms commonly observed among ill weanlings[24,53]. They are the main factors in determining reduction of calorie and (to a significantly lesser extent) protein intake[8,53,54].

In a recent analysis we have shown that diminished calorie intake during illness is one of the most important determinants of malnutrition in the village[53,54]. The decrease in intake evident in the analysis was mainly due to infectious diseases such as diarrhea, often accompanied by anorexia. These accounted for the more drastic low calorie intakes among village children. On the other hand, repetitive infections with enteric agents are undoubtedly related to the abnormalities in the intestinal mucosa acquired by individuals in highly infections, tropical environments[55].

Lowering the rates of intestinal infection and diarrheal disease becomes increasingly important and prioritary in the process of national development. It seems evident that this can not be accomplished by any means but by an improvement in living conditions, particularly personal hygiene and environmental sanitation. Actions along this line will have a direct effect on the nutritional status. On the other hand, there is no evidence that an increase in food intake will have an effect on reducing intestinal infection and its associated clinical features [53].

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Legend to the Figures

Figure 1

Viruses shed in feces of 18 of 45 village infants observed during their first year of life[11,24] in Santa María Cauqué. Circles are enteroviruses and triangles, adenoviruses. Data are from weekly cultures of fecal extracts inoculated onto primary human amnion, primary human kidney (post mortem) and HEp-2 cells. The prevalence of adenoviruses is likely higher than shown; the greater lythic capacity of enteroviruses lowers the probability of isolation of adenoviruses.

Figure 2

Rotaviruses in a fecal extract of a Costa Rican child. Formvar preparation negatively stained with phosphotungstic acid, 160,000X.

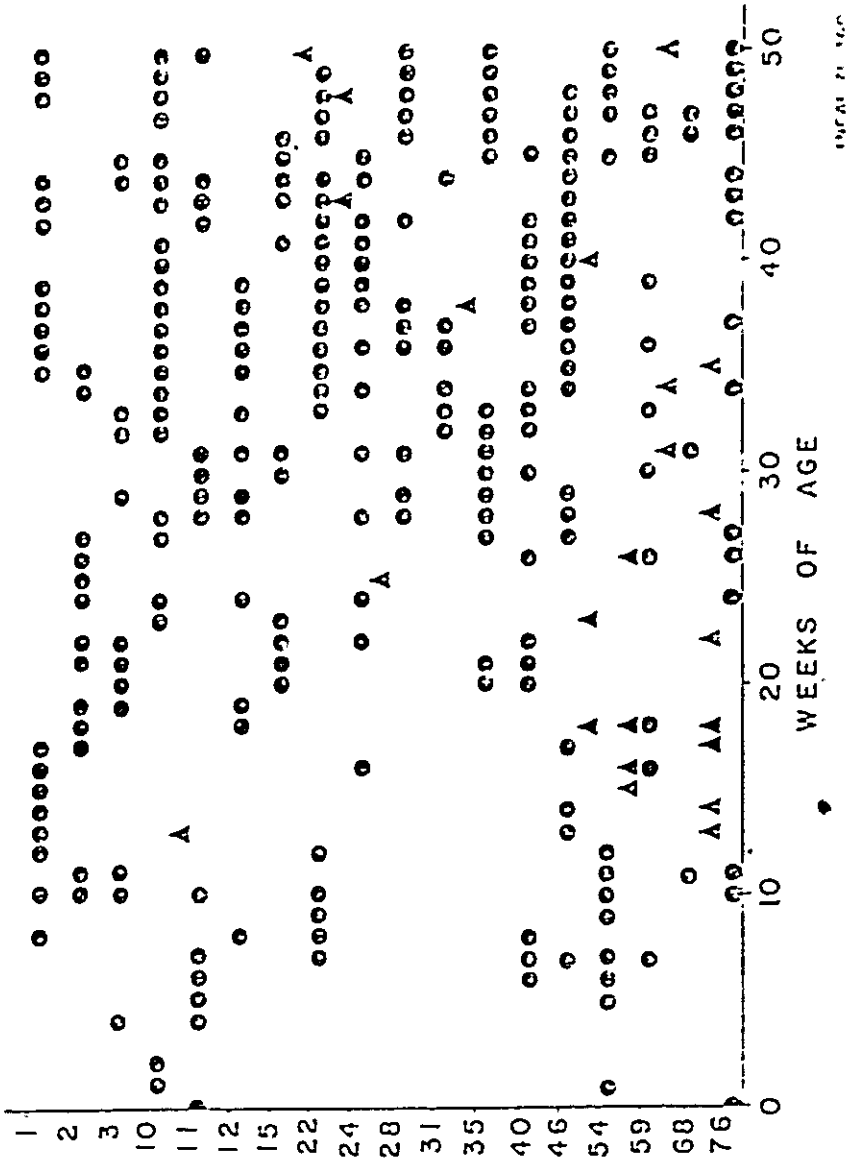

Figure 1

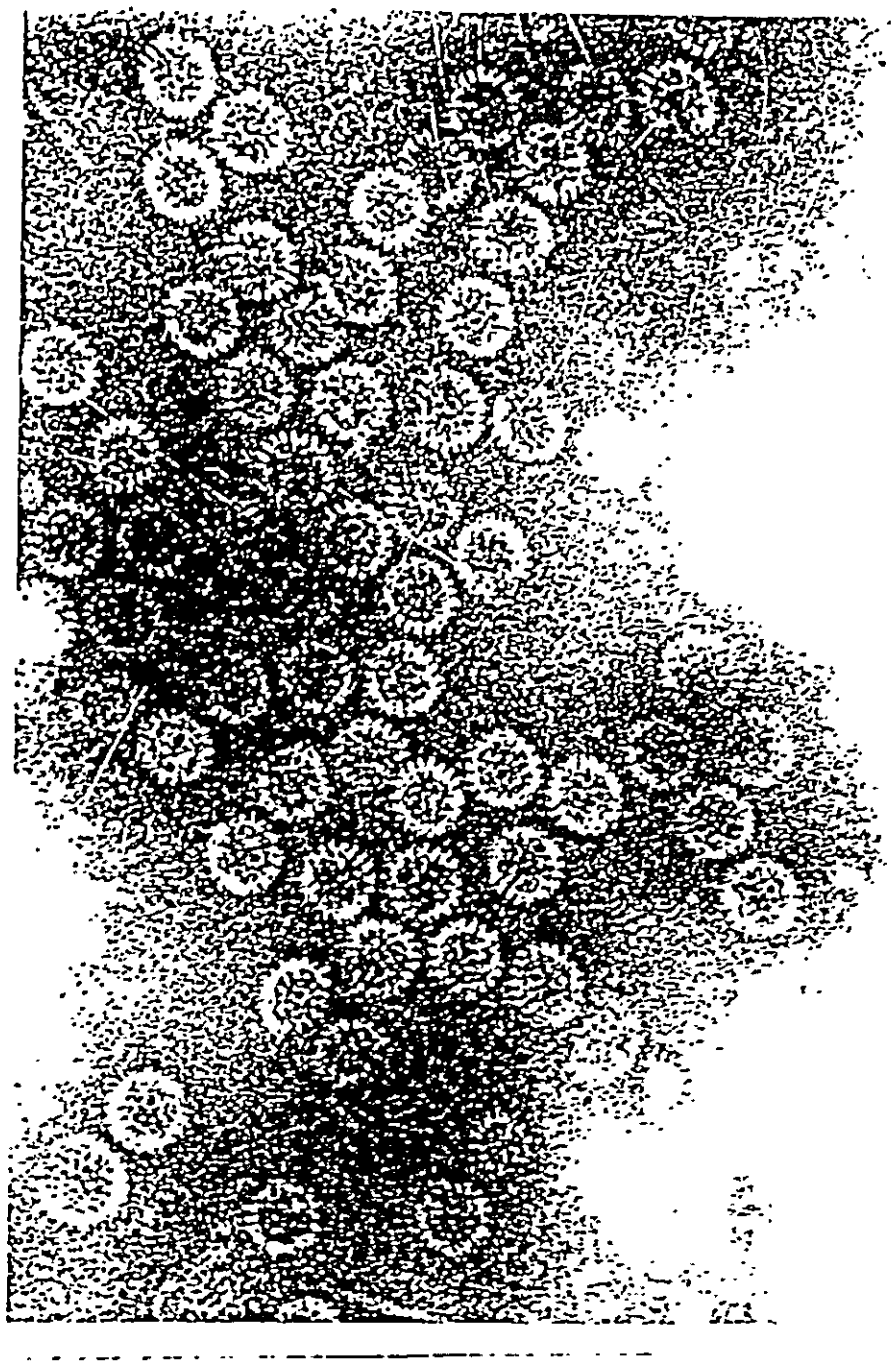

Figure 2

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TABLE 1

VIRAL AGENTS INFECTING THE FETUS[a*]

Polio, coxsackie A, coxsackie B, echovirus
Western-, eastern-, Venezuelan-equine encephalitis
Lymphocytic choriomeningitis
Influenza, mumps, measles
Rubella
Cytomegalovirus, herpes simplex, varicella-zoster,
Smallpox, vaccinia
Hepatitis A, hepatitis B

*[a]Virologic, serologic or epidemiologic evidence.

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TABLE 2

INCIDENCE OF INFECTIOUS DISEASE DURING PREGNANCY, 82 WOMEN, SANTA MARIA CAUQUE, 1972-1973

Trimester of Pregnancy	Urinary tract bacterial infection[a]	Diarrhea and dysentercy	Respiratory tract infection		Other Illnesses[c]
			Upper	Lower	
1st	8(10)[b]	7(9)	37(45)	5(6)	7(9)
2nd	8(10)	9(11)	26(32)	6(7)	5(6)
3rd	6(7)	13(16)	41(50)	14(17)	8(10)
% incidence per 100 pregnancies	27	36	127	30	25

[a] $\geq 10^5$ colony-forming units per ml urine
[b] Episodes (percentage)
[c] Conjunctivitis, otitis media, skin infection, stomatitis
From Urrutia et al. (1975).

TABLE 3

IgM AND IgG, 250 UMBILICAL CORD SERUMS,
SANTA MARIA CAUQUE, 1964-1971

ng/ml	IgM cases(%)	mg/ml	IgG cases(%)
<0.20	149 (59.6)	<9	36 (14.4)
0.20-0.39	76 (30.4)	10-14	148 (59.2)
0.40-0.59	16 (6.4)	15-19	46 (18.4)
0.60-0.79	7 (2.8)	20-24	16 (6.4)
0.80-0.99	2 (0.8)	25-29	4 (1.6)

NOTE: All serums with IgA≥0.10 mg/ml were not tabulated.
From Mata and Villatoro (1976).

TABLE 4

INCIDENCE OF ANTIBODY RESPONSES TO SPECIFIC AGENTS,[a]
61 WOMEN STUDIED THROUGH PREGNANCY
SANTA MARÍA CAUQUÉ, 1973

Agent (test)	Number of Women	Number of Seroconversions (%)
Cytomegaloviruses	51[b]	3 (5.9)
Herpes virus (CF)	60[b]	3 (5.0)
Rubella virus (HI)	61	0
T. pallidum (VDRL)	61	0
Toxoplasma (FA)	61	1 (1.6)
Total	61	7 (11.5)

[a] 4-fold (or higher) rise in antibody titer.
[b] Anticomplementary sera excluded.
From Urrutia et al. (1975).

TABLE 5

VIRAL AGENTS IN FECES OF CHILDREN DETECTED BY ELECTRON MICROSCOPY

Agent	Association with diarrhea
Rotaviruses (reovirus-like, duo-, orbiviruses)	Yes, endemic, outbreaks
27nm particles (Norwalk)	Yes, outbreaks
Coronaviruses	Yes, endemic
Astroviruses	Suspected
Caliciviruses	Not known

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TABLE 6

VIRUSES IN MECONIUM AND FECES WITHIN THE FIRST THREE DAYS OF LIFE, SANTA MARIA CAUQUE, 1964-1966

Day of life	Number of children	Number and % positive	Viruses isolated	Virus concentration $\log_{10}$ $TCID_{50}$ per gram
1st	79	1 (1.3)	E7	2
2nd	54	4 (7.4)	P1	5
			E6	3
			E6	3
			E6	3
3rd	61	5 (8.2)	P1+E6	4
			E6	5
			E7	5
			E9	5
			E11	5

TABLE 7

FECAL EXCRETION OF ENTEROVIRUSES AND ADENOVIRUSES BY 6-MONTH INTERVALS, 45 CHILDREN OBSERVED FROM BIRTH TO THREE YEARS OF AGE, SANTA MARÍA CAUQUÉ, 1964-1969

Age, months	Number of fecal specimens	Enteroviruses		Adenoviruses	
		Number positive*	%	Number positive*	%
0-5	1116	230	20.6	34	3.1
6-11	1162	483	41.6	46	3.9
12-17	917	481	52.5	33	3.6
18-23	953	438	45.9	60	6.3
24-29	908	446	49.1	58	6.4
30-35	867	530	61.1	48	5.5

*Isolation in primary human amnion, primary human kidney (post mortem) and HEp-2 cell cultures.

TABLE 8

PREVALENCE OF ROTAVIRUSES IN FECES OF HOSPITALIZED CHILDREN, BY AGE, SAN JOSÉ, COSTA RICA, JAN-MAR, 1976

Age Months	With diarrhea		Without diarrhea		Total	
	Number	Positive	Number	Positive	Number	Positive
1	4	1	8	1	12	2(17)
1-5	12	3	10	3	22	6(27)
6-11	23	8	19	4	42	12(29)
12-17	6	5	7	1	13	6(46)
Total	45	17(38)[a]	44	9(20)[a]	89	26(29)[a]

[a]Per cent positive in parentheses.

I Scientific Presentations given at the Meetings by Staff related to the Unit of Electron Microscopy

SCIENTIFIC PRESENTATIONS GIVEN AT MEETINGS BY STAFF LINKED WITH THE UNIT OF ELECTRON MICROSCOPY, 1975-1976

Dr. Rodrigo Gámez

1. "La importancia de los virus como factores limitantes en la producción de leguminosas en América Latina." Conference given at the Seminario sobre Patología de Luguminosas. CIAT. Cali, Colombia, December, 1975.

2. "Purificación, serología y microscopía electrónica del virus del rayado fino del maíz." Conference given at the Annual Meeting of the American Society of Phytopathology, December, 1975.

3. "Los insectos crisomélidos como vectores de virus de leguminosas." Paper presented at the XXII Annual Meeting of the Cooperative Program for the Improvement of Food Crops. San José, Costa Rica, July 1976.

4. "Serología - microscopía electrónica en el diagnóstico de virus del Maíz." Lecture given at the XXII Annual Meeting of the Cooperative Program for the Improvement of Food Crops. San José, Costa Rica, July 1976.

5. "La problemática de la caracterización de virus de leguminosas." Workshop at the University of Puerto Rico, San Juan, Puerto Rico, August 1976.

6. "Maize viruses transmitted by insects in Central America." World Conference on Maize Virus. Ohio Agricultural Research and Development Center. Wooster, Ohio, August 1976.

7. "The leafhopper transmitted maize rayado fino virus." Seminar given at the University of Illinois, August 1976 and at the University of Arkansas, August 1976.

8. "Transmission of viruses by chrisomelid insects." Workshop at the University of Arkansas, August 1976.

Dr. Pedro Léon

9. "Mollecular hybridization of 5S ribosomal RNA to salamander chromosomes." Paper presented at the V International Symposium on Toxins, San José, August 2, 1976.

Dr. Leonardo Mata

10. "Breast-feeding, weaning and the diarrhoeal syndrome in a Guatemalan Indian Village." Paper delivered at the Ciba Foundation Symposium on Acute Diarrhoea in Childhood, London, October 1975.

11. "Cultural and Nutritional Changes in Developing Nations: Focusing on Costa Rica." Paper presented at the Conference on Biological and Cultural Sources of Variability in Human Nutrition, University of California, Berkeley, Calif., December 1975.

12. "Breast milk and resistance to infection." Lecture presented at the Microsymposium on the Bifidus Flora and Maternal Milk, Zurich, Switzerland, October 1975.

13. "Rotavirus en la diarrea del niño." Paper delivered at the Conference for the XII Aniversary of the National Children's Hospital, San José, Costa Rica, May 1976.

14. "Shigella toxins." Panel discussion presented as part of the V International Symposium on Toxins, San José, Costa Rica, August 1976.

15. "Efecto de la infección sobre la alimentación y el estado nutricional." Conference presented at the International Nutrition Conference, Ministry of Health, San José, Costa Rica, August 1976.

16. "Viral infection of the fetus and the neonate." Paper presented at the IV International Symposium on Intestinal Microecology, University of Missouri, Columbia, Mo., May, 1976.

17. "Effect of Infection on food intake and the nutritional status: perspectives as viewed from the village." Paper presented at the Workshop on the Impact of Infection on the Host Nutrution, Arlie, Virginia, May 1976.

18. "Significación biologíca de la flora indígena." Conference presented at the III Latinamerican Congress of Physiological Chemistry, Caracas, Venezuela, May, 1976.

J Proposal for the Renewal of the Medical Cooperation Agreement between the Government of Japan and the University of Costa Rica

PROPOSAL FOR THE RENEWAL OF THE MEDICAL COOPERATION AGREEMENT BETWEEN THE GOVERNMENT OF JAPAN AND THE UNIVERSITY OF COSTA RICA

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PROPOSAL FOR THE RENEWAL OF THE MEDICAL COOPERATION AGREEMENT BETWEEN THE GOVERNMENT OF JAPAN AND THE UNIVERSITY OF COSTA RICA

I. BACKGROUND

In November, 1973, the Government of Japan and the University of Costa Rica signed a Medical Cooperation Agreement for the advancement of basic medical education and biomedical research through the use of electron microscopy.

As part of this agreement the government of Japan: a) donated to the University of Costa Rica one transmission and one scanning electron microscope and supporting equipment; b) sent personnel to install the equipment and to advise and collaborate in electron microscopy; and c) supplied fellowships to Costa Rican personnel for training in Japan. The University of Costa Rica created the Unit of Electron Microscopy and furnished it with its own building, now a part of the Health Research Institute of the University (INISA).

As a direct result of this cooperative program, the development of research in biomedical and biological areas has been greatly enhanced. Research projects and programs, which will make considerable use of the electron microscopy facilities at the University, are now being established or will become a reality in the near future. A collateral and logical development has been the training of personnel at several levels, an activity that will be significantly expanded in the near future.

Although the Medical Cooperation Agreement terminated in March, 1976, the University of Costa Rica considers that an extension of this association is desirable to further the development of research in the health, biomedical, and biological sciences. To this end we wish to propose to the Government of Japan the renewal of the Medical Cooperation Agreement according to the guideline that follows.

II. SCOPE OF ACTIVITIES

A. Promotion of Research

Great emphasis is being placed today on research and technology in Costa Rica as part of the present upward trend in national development. Considerable activity is being observed in

this regard in the University of Costa Rica, our leading center of research and education. For instance, the Vice-presidency of Research Affairs was recently created, and several groups of scientists have organized Institutes, Centers and Units of research.

One of the academic units of recent creation is the "Instituto de Investigaciones en Salud" (INISA), which conducts research on problems of human health and related sciences seeking solutions for their control and prevention. INISA is adscribed to the Schools of Pharmacy, Medicine, Microbiology, and Dental Medicine.

At present, INISA is growing very fast, and the priorities established are very realistic. Results obtained so far have been of importance in the knowledge of our health problems.

The scientific cooperation with Japan is extremely important in the events described. It has provided technical assistance, as well as equipment of invaluable help and has stimulated the present research development within the University of Costa Rica and the Nation as well. The current reorganization of research goals in the University gives certain emphasis to health, biomedicine and biological science. In these areas intensive use of electron microscopy and related technology is expected. The two electron microscopes donated by Japan to the University of Costa Rica places us in a special and unique situation, that of having advanced technology at the same time as having the health and biological problems typical of pre-industrial nations.

1. Research in Health and Biomedicine

The health situation in Costa Rica is evolving rapidly toward models presently observed in industrialized nations, for instance, in Japan and Western Europe. In this regard Costa Rica has an intermediate status, showing an increasing occurrence of coronary heart disease, hypertension, and cancer, while a relatively high rate of diarrheal disease and malnutrition during childhood is still observed. This makes it a most challenging country from the standpoint of research development.

The research priorities set by INISA are very much in accordance with the National Health Plan of our Government, and are:

Malnutrition in man
Viral diseases of humans, animals and plants
Diarrheal diseases of childhood
Mortality and demographic growth
Degenerative diseases (cancer, diabetes, hypertension)
Genetic disorders
Social pathology (family disruption, migration, alcoholism)

In the first stage, more emphasis will be given to the study of malnutrition, diarrheal disease and infant mortality, because significant improvements in that regard are to be expected in the next two decades. Evidently, the order of priorities will change as problems of childhood are diminished.

2. Research in Biology

Stimulated by the general organization of research in the health area, a variety of activities to utilize resources in the field of biology have also taken place. Some of the present interests in biological research are in direct relationship to medical and health problems, particularly as they pertain to nutrition and general welfare.

The research priorities established in this regard are:

Biology of the flora and fauna of Costa Rica with emphasis on those of importance to man

Diseases of food crops and animals, of stored grain and preserved foods

Alterations in the environment of man

3. Proposed Research Programs

By definition, INISA has three dependencies: the Center of Research in Human Development, the Center of Research in Virology and Cell Physiology and the Unit of Electron Microscopy. Plans exist to develop the Center of Epidemiological Research and a Laboratory of Nutritional Biochemistry.

Following are research programs to be conducted by INISA: The programs are, in general, multidisciplinary and have been designed according to important health problems in Costa Rica and the availability of resources in electron microscopy, epidemiology, and microbiology. The programs will be briefly described first. The necessary enlargement or development of the various dependencies of INISA will follow.

a. Host-parasite Relationships at the Mucosal Level

This investigation will integrate scientists of INISA, the Unit of Electron Microscopy and the National Children's Hospital, in an effort to describe the origin, evolution and significance of intestinal infection with viruses, bacteria and parasites. Emphasis will be placed on the study of children with acute or chronic diarrheal disease, or with malabsorption and energy-protein malnutrition. Hospitalized children will be assessed clinically, biochemically and microbiologically, focusing on malabsorption. Specific infections with rotaviruses, coronaviruses, parvoviruses, Shigella, Escherichia coli, Giardia, Trichuris and hookworm will be studied. Mucosal biopses will be obtained for investigation by scanning and transmission electron microscopy.

b. Etiology and Biology of Virus and Viral Diseases

More knowledge is required on the etiology and biology of many viral diseases of humans and cultivated food plants in Costa Rica. This research program will emphasize the characterization of selected viruses responsible for important diseases and the study of virus—vector and host—virus relationships. Special attention will be given to the morphology and biochemistry of the viral particles, the nature of the biological cycles of viruses in their vectors, the intracelular location and ultrastructural alterations induced by viruses, and physiological disturbances of cells and tissues induced by viral infections.

c. Biochemical Studies in Severely Malnourished Children

We need to know more about malnutrition, particularly in light of new knowledge on its etiopathogenesis. Studies of trace elements, endocrine balance and protein requirements are needed in order to improve treatment of malnutrition and to implement programs of prevention in rural areas. The program will be a collaborative effort of INISA, the National Children's Hospital and the Faculty of Medicine.

The aim would be to recruit infants and preschool children at the National Children's Hospital and the National Nutrition Clinic for prospective observation. Cases from rural areas will be investigated through a long-term prospective field study carried out by INISA. A variety of studies will be performed to characterize the nature of nutritional problems in various geographic regions.

d. Studies on Gastric Carcinoma

Cancer of the stomach is one of the leading forms of neoplasia in Costa Rica. There is little epidemiologic information regarding the reasons for the excess occurrence, although dietary habits are suspected. INISA will coordinate studies of the pathological examination of clinical material, including its electron microscopy. A bench—epidemiological study will determine geographic, age and sex distribution of gastric carcinoma and its relation to occupation, income, diet and background. These studies will parallel the organization of the Center of Epidemiologic Research of INISA programmed for 1977 and will utilize the facilities of the Unit of Electron Microscopy.

4. Strengthening Structures to Carry Out Research and Training in Health and Biomedicine

In order to promote research and training in the areas mentioned, and particularly, to develop the research programs outlined above, several structures need to be strengthened and further developed: a) the Unit of Electron Microscopy; b) the Center of Research in Virology and Cell Physiology; c) the Center of Research in Human Development; and d) the Center of Epidemiological Research. A description of the planned development of these units follows. A list of the equipment required is in the appendix.

a. Unit of Electron Microscopy

Although this Unit is unique in Central America, current and future activities indicate that more space will be required and that additional microscopes and other equipment will be needed to cope with the planned research and training at the regional level. Preliminary discussion with University authorities indicates excellent prospects for construction of an annex to the Unit while we expect that the equipment needed will be obtained through cooperation between Japan and Costa Rica.

b. Center of Virology and Cell Physiology Research

Viral diseases are likely to continue as the most common diseases of humans, cultivated plants and domestic animals. Viruses are not only important as pathogens; they are also biological systems depending on metabolic cellular activities and thereby causing disease, malfunction or genetic alterations. Much knowledge on the nature and mechanisms by which viruses induce such alterations is required. A comparative study of

the physiology of the virus-infected and non-infected cell may provide relevant information on normal cell function.

For these reasons, the Virology and Cell Physiology Research Center was established by the University of Costa Rica in 1976 as a multidisciplinary unit to promote scientific research in this field. The general objectives of the Center are: a) the study of properties and functions of viruses and viral diseases of humans, animals and cultivated plants; b) the study of the physiology and biology of normal and virus-infected cells and their organelles, with emphasis on the organization and function of the nucleus and of the genetic material; and c) the development of teaching and research training programs in virology and cell physiology.

The resources of the Center are six professionals aided by the collaboration of scientists from other academic units of the University. The resources include a physical plant of nearly $400m^2$ distributed in five laboratories for human virology, plant virology, cell physiology, ultra and analytical centrifugation, and cell and tissue culture, respectively. Special areas have been assigned for a cold room, sterilization and glassware washing, incubation and other general services.

The laboratory equipment presently available includes preparative ultracentrifuges, density gradient analyser, spectrophotometer and homogenizer. Facilities for electron microscopy are available at the adjacent Unit of Electron Microscopy established under the Medical Cooperation Agreement between Japan and Costa Rica.

Support required for the development of the Center up to the present has been obtained from the University of Costa Rica and the National Council of Scientific Research and Technology of Costa Rica. Further support is required to enable the Center to reach its objectives, as follows:

Laboratory equipment needed is listed in Appendix 1. An analytical ultracentrifuge is required to study molecular properties of viruses, subcellular organelles and components in centrifugal fields. By amino acid analysis the composition of proteins will be determined. Cell and tissue culture facilities are necessary for virus replication and protoplast cultures of plant cells.

c. Center of Research in Human Development

The Center of Research in Human Development has six professionals on its staff. The development of a Laboratory of Nutritional Biochemistry is a programmed activity of the Center. Basic equipment for working with proteins, amino acids and trace elements is needed. Also, equipment is required to determine the nutritive value of diets.

d. Unit of Epidemiological Research

This Unit is in the planning stage. There are two microbiologists and one epidemiologist in INISA conducting this kind of research. The work under way is dynamic and relevant despite the Limited resources at present. It deals with the epidemiological significance of plasmids in human enteric infections and with the problem of diarrheal disease. Again, basic laboratory equipment is required to extend our present observations on this problem, as shown in Appendix 2.

B. Scientific Training

1. In Japan

Continuation of training of Costa Ricans in Japan is desirable. So far, the emphasis has been on training professionals totally lacking in knowledge of electron microscopy; in the future the aim will be to send people who already have a basic understanding of the problem. In this way the training in Japan can be more intensive and of greater value while we can have greater assurance that the persons in training are already committed to investing considerable effort in the program.

2. In Costa Rica

In the last few months since the installation of the transmission electron microscope (TEM), research in virology and biomedicine has made significant progress. The installation of the scanning electron microscope (SEM) is underway and several staff are expected to join for ultrastructural research.

We believe that the Unit of Electron Microscopy constitutes a powerful means in the search for new knowledge in tropical biomedicine and allied fields. Following are some suggestions that will enable this Unit to develop its full research potential.

a. Training Programs

The objective is to prepare human resources for the application of electron microscopy to biomedical and related problems. The suggested programs consist of a sequence on a) introductory light microscopy, involving basic principles of optics and basic theory of electron microscopy (EM); b) the general principles for biological specimen preparations (negative staining, shadow casting, ultrathin section for cytology and histology in both plant and animal tissues), and operation of the equipment; and c) a course for individuals acquainted with the basics of EM which would include techniques of cytochemistry, histochemistry, and autoradiography. Students in this course will receive far wider training in the application of EM and are expected to work in the correlation of morphology and function. This type of training program will preferably be open to students in research institutes and to graduate students in the schools of microbiology, medicine, pharmacology, dentistry and biology. It should be indicated that graduate schools presently offering up to a Master's degree service students from the Central American region, Panama and Venezuela. Also, the extension of the Electron Microscopy services to the Graduate System of the University will assure wide support for future developments.

Trained scientists (histologists, physicians, cytologists, etc.) from Costa Rica and Central America, who have not used the EM facilities will be offered an intensive course (1 - 3 months). Trained scientists cannot usually enroll in a one or two semester sequence, such as the one described above. This type of training will undoubtedly respond to growing demands in the region for EM facilities which are rapidly becoming routine diagnostic and research tools.

b. Materials for Education and Research

A collection of electron micrographs and slides for teaching will be prepared and made available here. Reference materials, such as reprints and reference files will be prepared also. Other materials, like books, films and tapes could be included if feasible.

c. Scientific Exchange for the Advancement of Science

Scientific exchange is fundamental in the long-term plans of national development. This comprises the visit of Japanese scientists to our Institute and of Costa Ricans to Japan, as well as the celebration of international events in Costa Rica.

1. Exchange of Japanese and Costa Rican Scientists

Up to this moment this program has yielded important results, particularly with the vist of Japanese professionals to the University of Costa Rica. Other Japanese scientists could establish scientific links of mutually beneficial impact. The areas that need support at present are: biochemistry, epidemiology, biostatistics, nutrition and microbiology.

On the other hand, Costa Ricans will be selected for short- and long-term training in Japan in areas of relevant need. The overall aim is to have an active exchange of ideas and knowledge and to develop research projects of importance for our country in which the scientists from both nations would cooperate. It should be remembered that some of our current problems were present in Japan several years ago, for instance, antiobiotic-resistant shigellosis. Other problems, like gastric cancer, occur similarly in both nations. The exchange in the areas of health, biomedicine and biology will trigger scientific cooperation.

2. Organization of International Events

Staff from INISA have expertise in the promotion and organization of international conferences, symposia and workshops. This kind of activity is a mechanism for comparing, analyzing and diffusing information of interest to several nations; it promotes the exchange of specific information of mutual interest, and permits evaluation of techniques and methodologies that would result in a better understanding of research in progress and of approaches to solve those problems.

Several conferences or workshops are already envisioned with participation of Japanese and Latin American workers. For instance, meetings on "the biology of rotaviruses," "changes in human growth," "viruses of food corps," and "the human placenta." could take place in the near future.

The scientific events should be published in a series of INISA publications which will promote the research conducted in the various Centers and Units of the Institute.

III. DURATION OF THE AGREEMENT

We are proposing that this Cooperative effort should extend from 1977 through 1982, that is, five years. The period

is a continuation of the three years under the original Agreement (1974-1976).

K List of Equipment necessary for continued development

Year 1978

1.	Scintillation Counter Packard Tri-Carb	1
2.	Freeze Drying Apparatus	1
3.	Analytical Ultracentrifuge	1
4.	CO-2 Incubator for Tissue Culture	2
5.	Clean Bench	2
6.	Incubator Shaker	2
7.	Serological Water Bath	2
8.	Millipore Filteration Unit	6
9.	Incubator for Tissue Culture	2
10.	Autoclave	2
11.	Drying Oven Sterilizer	2
12.	Inverted Microscope with	2
	Phase Contrast Attachment	
	Test Tube Holder	
	Photomicrographic System	
	Photomicrographic Exposure Control Unit	
	Eyepieces for Photography	
	Objectives for Photography	
13.	Triocular Zoom-Stereo Microscope with Photographic Unit	1
14.	Cold Room	1
15.	Coolnics Circulator	1
16.	Double-Wavelength/Double Beam Spectrophotometer Hitachi Model 556	1
	with Flow Cell	2
	Gel Scanner	1
	Thin-layer Chromatography Accessory	1
	Base Line Corrector	1

Year 1979

1.	Electron Microscope Hitachi Model H-300	1
2.	Mini-SEM-6	1
	Large Sample Stage XRr-32	
	Goniometer Stage XYT-15	
	Display Unit	
3.	TV Scanning Device TV-3 for HHS-2R	1
4.	Freeze-Fracture/Etching Unit	1
5.	Recorder	1
6.	Vacuum Evaporator HUS-5GB	1
7.	Microtome Porter JB-4	1
8.	Microtome Jung	1
9.	Paraffin Oven	1
10.	Microtome Knife 250 mm	3
11.	Microtome Knife Sharper (Shandon)	1
12.	Cryostat (American Instrument)	1
13.	Amino Acid Analyzer	1
14.	Gas Chromatograph	1
15.	Coolnics Circulator	1
16.	Fraction Collector	1
17.	Refregerator (Large Capacity)	2
18.	UV Monitor for Fraction Collector	1
19.	Fluorescence Spectrophotometer Hitachi MPF-4	1
	Recorder QD-25	1
	Thin layer Chromatography Accessory	1
	Thermostat Cell Holder	1
	Micro Cell	4
	Fluorescence-free Cell	4
	Flow Cell	2
	Ultra-Micro Cell	4

Year 1980

1.	Electron Microscope Hitachi H-300	1
2.	Mini-SEM-6	1
	Display Unit	1
3.	Electrophoretic Apparatus with DC Power Supply	1
4.	Microdensitometer	1
5.	Digital Spectrophotometer Hitachi 102	2
	5 mm Quartz Cell Set	4
	10 mm Quartz Cell Set	4
	Temperature Controlled Cell Housing	2
	Test Tube Holder Set	2
	Recorder QD-25	2
6.	Vapor Pressure Osmometer Hitachi	1
7.	Complete Set of UNIMAT	1

Year 1981 - 1982

Miscellaneous supplies for the photometer and other equipments necessary for the replacement of parts, accessories, Cuvettes, recording chart papers and others for the maintenance of instruments and operation.

V 資 料 3

Proposed Curriculum

Group I.

A	Title:	Electron Microscope of Microoganisms I
	Credit:	6 Units
	Duration:	2 Semesters
	Number of Students:	Up to 6
	Requirements:	Fundamental knowledge on Microbiology and Parasitology.

B	Title:	Electron Microscopy of Cell and Tissues
	Credit:	6 Units
	Duration:	2 Semesters
	Number of Students:	Up to 6
	Requirements:	Fundamental knowledge on General Cytology and Histology.

The first one third of the above courses are dedicated to the discussion on the basic theory of electron microscope and the latter part will be separated either application to Microbiology or Cytology and Histology.

Group II.

A	Title:	Basic Electron Microscopy
	Credit:	3 Units
	Duration:	1 Semester
	Number of students:	6
	Requirements:	Those who hold at least B.A or equivalent and Graduate students on Biology, Microbiology, Pharmacology, Dentistry and Agriculture.

B Title: Electron Microscopy of Microoganisms II

Credit: 3 Units

Duration: 1 Semester

Number of Students: 3 up to 6

Requirements: Basic principles on electron microscopy Cytology, Cell Biology and Histology.

Group III

Title: Electron Microscopy of Tissue, Cell and Microoganisms

Credit: 9 Units

Duration: 2 Semesters

Number of Students: Max. 6

Requirements: Microbiology, Parasitology Cell Biology and Histology.

Coordinator of the Course: Dr. Y. Kozuka

Colaborators: Dr. R. Gámez

Dr. P. León

Dr. F. Ureña

Sr. R. Bolaños

Sr. T. Fukuoka

Probable candidates:

Ana Mercedes Espinoza

Francisco Hernández

Maria Elena Peñaranda

Jollyana Malavassi

Topics on the Electron Microscope Training Course.

1. Historical Background and Modern Instrument
   - 1-a Early History
   - 1-b Modern Electron Microscope up to high voltage E.M.
   - 1-c Difference between light microscope and electron microscope
     - 1-c-1 Construction of Instrument, Resolution, etc.
   - 1-d Transmission and Scanning Electron Microscope, difference in their construction and function.
   - 1-e Application of Electron microscope as a analytical instrument. X-Ray microprobe analysis.

2. Construction of Transmission and Scanning Electron Microscope.
   - 2-a General Construction (Transmission Electron Microscope)
   - 2-b Evacuation System
   - 2-c High Voltage Supply System
   - 2-d Electron Source
   - 2-e Lens System
   - 2-f Specimen Chamber
   - 2-g Recording System (Camera Chamber)
   - 2-h General Construction (Scanning Electron Microscope)
     - 2-c--2-g Same as above.

3. Instrument necessary for Transmission and Scanning Electron Microscope Specimen Preparation
   - 3-a Vacuum Evaporator
   - 3-b Ion Coater IB-3
   - 3-c Critical Point Dryer HCP-1
   - 3-d Ultramicrotome
     - 3-d-1 Mechanical Feeding System
     - 3-d-2 Termal Feeding System

3-e Knife Maker

3-f Pyramitome

4. Photography

4-a Developing and Developer

4-b Handling of Photo Sensitized Materials

4-c Enlarger and Enlarging Lens

4-d Calibration of Magnification

5. Application of Transmission and Scanning Electron Microscope to Biomedical Science

5-a Negative Staining Technique

5-b Shadow Casting Technique

5-c Replica Technique

5-d Ultrathin Section Technique

5-d-1 Enzyme Histochemistry

5-d-2 Histochemistry

5-d-3 Immuno Electron Microscopy

6. Specimen Preparation I (Virus, Bacteria and other small objects)

6-a Grids and Supporting Membrane

6-b Negative Staining Technique, Shadow Casting Technique

6-c Analysis of Images

7. Specimen Preparation II (Ultrathin Section Technique)

7-a Fixation: Aldehyde, Osmium Tetroxide, Other Fixatives. Buffer System, pH and Tonicity.

7-b Choice of Fixative according to the Materials.

7-c Embedding Plastics, Choice of Plastics.

8. Specimen Preparation III (Ultrathin Section Techniques)
   - 8-a Ultramicrotomy (Glass Knives, Handling of Sections)
   - 8-b Staining (Block Staining, Section Staining)
   - 8-c Contamination during staining and their correction

9. Specimen Preparation IV (Scanning Electron Microscope)
   - 9-a Fixation, Dehydration and Critical Poing Drying
   - 9-b Factors Influencing the Final Image

10. Image Analysis and Interpretation
   - 10-a Quality of Printed Picture
   - 10-b Trouble Shooting for Specimen Preparation
   - 10-c Improvement of the Overall Techniques
   - 10-d How Much we can get from the Electron Micrographs?

Schedule for Laboratory Work

I. Operation of Instrument (Transmission Electron Microscope)
   1. Start--Stop
   2. Adjustment of Optical Axis
   3. Adjustment of Objective Current to Light Axis
   4. Adjustment of High Voltage Alignment
   5. Correction of Astigmatism--Condenser
   6. Correction of Astigmatism--Objective
   7. Filament Exchange
   8. Specimen Exchange
   9. Focusing, Function of Image Wabbler, Control of Light Intensity for Photo Recording
   10. Exchange Cassets
   11. Film Loading and Exchange Film Reservors
   12. Phot Processing--Negative to Print

II. Operation of Instrument (Scanning Electron Microscope)
   1. Start--Stop
   2. Adjustment of Optical Axis
   3. Correction of Astigmatism
   4. Filament Exchange
   5. Focusing, Control of Brightness and Contrast
   6. Photo Recording and Loading the Film
   7. Development of the Negative and Prints

III. Operation of Vacuum Evaporator
   1. General Operation
   2. Metal Shadow Casting
   3. Carbon Coating

IV. Operation of Ion-Coater and Critical Point Dryer

1. Ion-Coating for Scanning Electron Microscope Specimen Preparation
2. Hydrophilic Treatment of Supporting Membrane
3. Operation of Critical Point Dryer

V. Ultramicrotomy (Porter Blum MT-2B, LKB 8800)

1. Handling of Blocks
2. Handling of Glass Knives

VI. Handling of Grids and Supporting Membrane

VII. Negative Staining

1. Negative Staining with Silico Tungustate
2. Negative Staining with Uranyl Acetate
3. Negative Staining with Phospho Tungustic Acid

Each canditates should submit Negative, Prints and brief discussion on every experiments. They are indicated below:

1. Comparison of STA, PTA, Uranyl Acetate Negative Staining

   Material: Flagellated Bacteria, and Polyethylene Latex Particle or Chroloplast

2. Shadow Casting Technique

   Material: Flagellated Bacteria and Polyethylene Latex Particles

   Shadowing Metal: Chromium

   Shadowing Angle: 15 and 45 Degree

   Calculation of the particle size.

3. Ultrathin Section

Plant Material:	Onion Root Meristematic Cell or Parechymal Cell of Leaves
Animal Tissues:	Rat Liver, Cardiac Muscle
Fixation:	Osmium Tetroxide Alone Glutaraldehyde—Osmium Tetroxide Double Fixation
Plastics:	Epon 812 of Luft Spurr's Low Viscosity Epoxy Plastic
Staining:	Uranyl Acetate Alone Uranyl Acetate and Lead Double Stain Lead Stain Alone
Magnification:	3,000 X, 5,000 X, 10,000 X, and 25,000 X at Direct Magnification
Photographic Enlargement:	4 X

Submit negative and corresponding prints and block together with detail preparation procedure and discussions.

4. Correction of Astigmatism

Material: Perforated Collodion Membrane

Photograph should be taken with Image Wabbler and Without Image Wabbler

Photographs to be taken over focus, infocus and under focus condition at the magnification at 100,000 X and 60,000 X.
Submit the negative and corresponding Prints

5. Scanning Electron Microscope

Material: Small Insects, Piece of Leaves

Procedure: 1. Air Dried Specimen

2. Critical Point Dried Specimen

All the specimen should either with fixation or without fixation

Photograph should be taken at least two different accelerating voltage and three different magnifications.