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TRIBUNA PLURAL

La revista científica

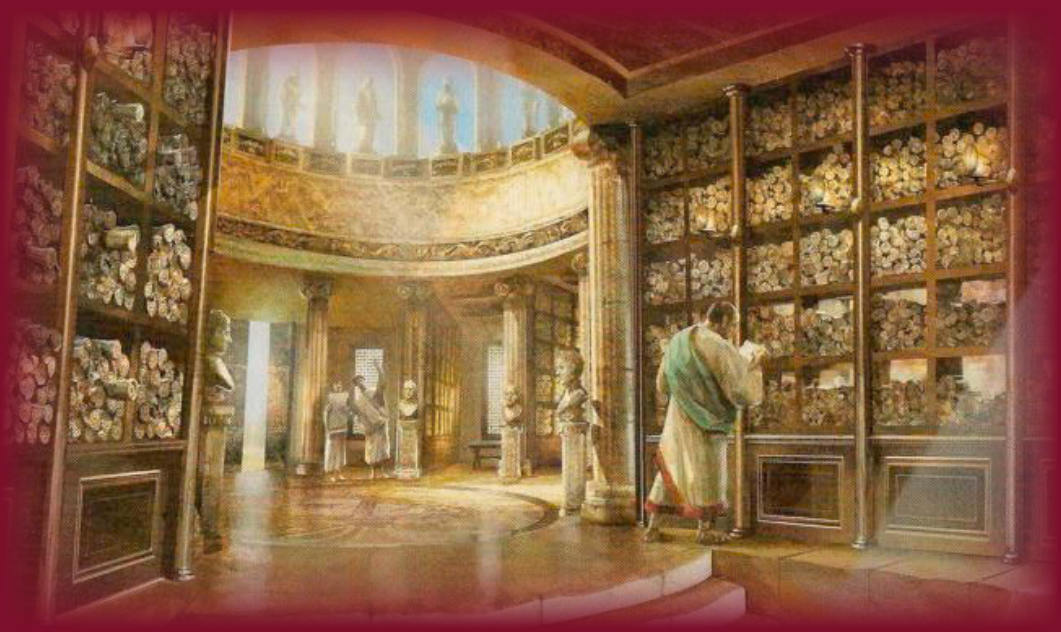
Homenajes Núm. 2 **4/2016**

Aaron Ciechanover
Premio Nobel de Química

Josep Maria Gil Vernet Vila
Catedrático Emérito de Urología

Björn O. Nilsson
Presidente de la Real Academia Sueca de Ciencias de la Ingeniería

Ismail Serageldin
Director de la Biblioteca de Alejandría



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Real Academia Europea de Doctores
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EDITADA POR

Real Academia Europea de Doctores

Sumari - Sumario

EXCMO. SR. DR. BJÖRN O. NILSSON

EXCMO. SR. DR. ISMAIL SERAGELDIN

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PRESENTACIÓ / PRESENTACIÓN / INTRODUCTION

PRESENTACIÓ / PRESENTACIÓN / INTRODUCTION

JOAN FRANCESC PONT CLEMENTE
ALFREDO ROCAFORT NICOLAU

Presentació

Els esforços per sistematitzar i difondre el coneixement es remunten a temps immemorials, tot i que és en el període hel·lenístic quan neixen les grans biblioteques llegendàries, com la Biblioteca d'Alexandria o la de Pèrgam, creades ja per aplegar tot el coneixement social del seu temps i posar-lo a l'abast dels erudits. La biblioteca com a gran centre d'investigació i d'aprenentatge emparenta amb la voluntat de les primeres acadèmies per compartir el saber artístic i científic. Savis notables que han contribuït i contribueixen a millorar les condicions de vida de la humanitat. En aquest segon número de la col·lecció *Homenatges* de *Tribuna Plural* ens apropem a quatre nous membres d'honor que, cadascun des del seu camp, aporten coneixements i mèrits per combatre malalties i augmentar la qualitat de vida de l'ésser humà. Aquest volum recull la participació en la solemne sessió conjunta de recepció, convocada el 20 de desembre de 2016, així com algunes de les seves obres escollides. Per a la nostra Corporació centenària és un honor poder contribuir a la difusió del coneixement en el segle XXI, el dels savis que aconsegueixen humanitzar la ciència.

El Dr. **Aarón Ciechanover** és un bioquímic israelià que ha destacat pels seus descobriments sobre com les cèl·lules del cos humà s'organitzen per degradar regularment les proteïnes inservibles: és el denominat sistema de la ubiqüitina o degradació proteica mitjançant la ubiqüitina. Per aquests treballs va rebre el Premi Nobel de Química de 2004, compartit amb dos investigadors

més. Els seus descobriments fan possible desenvolupar medicaments contra malalties com el càncer cervical i la fibrosi quística. El Dr. Ciechanover és Doctor en Medicina des del 1981 pel Technion - Institut Tecnològic d'Israel i és el director de l'Institut Rappaport Family per a la Investigació de les Ciències Mèdiques, ambdós a Haifa. Des del 1996 és membre del Consell de l'Organització Europea de Biologia Molecular (EMBO) i publica regularment a revistes científiques de prestigi com *Nature*, *Cell* i *Scientific American*.

El Dr. **Josep Maria Gil-Vernet** és Doctor en Medicina i Cirurgia per la Universitat de Madrid, Especialista Diplomat en Urologia, Catedràtic Extraordinari d'Urologia de la Universitat de Barcelona i *visiting professor* en nombroses universitats, entre les quals en destaquen la de Columbia (The College of Physicians and Surgeons), Johannesburg, Salisbury, Rochester, Missouri, el Medical Center de Nova York, la Universitat Lliure de Berlín i la Universitat René Descartes de París. És membre de la Reial Acadèmia de Medicina de Barcelona, membre d'Honor de l'Acadèmia de Medicina de l'extinta Unió Soviètica, i membre de 26 societats estrangeres d'Urologia. El Dr. Gil-Vernet, home innovador en la docència i l'ensenyament de la urologia, ha realitzat importants aportacions en el camp de la tècnica urològica que contribueixen a millorar el pronòstic de nombroses malalties i a aconseguir una especialitat més eficaç i segura.

El Dr. **Björn O. Nilsson** és el president de la Reial Acadèmia Sueca de Ciències de l'Enginyeria, es va doctorar en Bioquímica a l'Institut Reial de Tecnologia a Estocolm, on actualment exerceix de docent. El professor Nilsson ha ocupat i ocupa rellevants posicions executives i de gestió a les indústries farmacèutica i biotecnològica, principalment amb el grup Pharmacia. És el president del Consell d'Administració de l'empresa pública de biotecnologia Bioinvest International AV. El professor Nilsson ocupa càrrecs de rellevància en diverses organitzacions governamentals i no governamentals nacionals i internacionals en els camps de la ciència, l'educació i la innovació. El 2016, el professor Nilsson va ser elegit membre del Consell de Direcció de l'Institut Europeu d'Innovació i Tecnologia. D'altra banda, ocupa des de març de 2016 la presidència de l'Associació Sueca d'Atletisme.

El Dr. **Ismail Serageldin** es va graduar a la Universitat del Caire i doctorar a la de Harvard. Compta amb una trentena de doctorats *honoris causa* per universitats dels cinc continents. És el director de la Biblioteca Alexandrina i el president dels deu instituts de recerca associats a la Biblioteca. Serveix com Ambaixador davant l'Aliança de Civilitzacions i és el president del Comitè

Executiu de la Biblioteca Digital Mundial. Participa activament en diversos consells d'administració i consells assessors d'institucions acadèmiques, investigadores i científiques de caràcter internacional i contribueix als esforços de la societat civil per a la millora de les condicions de vida. Ha estat professor visitant en nombroses universitats i institucions, com el Col·legi de França, la Universitat de Wageningen als Països Baixos i la Universitat Americana del Caire. Va estar vicepresident del Banc Mundial fins al juliol de 2000, institució amb la qual va començar a col·laborar el 1972.

Per a l'Acadèmia és un honor molt especial rebre a les quatre personalitats que acabem de glossar; en aquesta ocasió –volem destacar– seran emparades per l'evocació de la Biblioteca d'Alexandria i tot el que simbolitza el nou projecte. La Biblioteca antiga fou un Temple del Saber condemnat a la destrucció, com el Temple de Jerusalem. És terrible la pèrdua d'allò que molt ha costat de construir, però si aquells murs acullen tot el saber conegut, la pèrdua és irreparable. Que la Biblioteca hagi renascut setze segles després, com el fènix que mor per renéixer amb més força, ens compromet amb la magnífica tasca de la Reconstrucció del Temple, un afany universal de l'ésser humà que ha aconseguit amansir la seva animalitat.

Arran del recent referèndum italià, podríem reflexionar també sobre com aconseguir reconstruir els temples simbòlics que ens han estat encomanats. Més enllà de les propostes de reforma constitucional –que pocs electors havien llegit i que no estaven mancades de fonament sòlid–, resulta forçós acceptar que els votants senten la necessitat de dir no a tot allò que se'ls proposa. Els electes i els governants farien bé de no mirar cap a una altra banda: algú ha de ser capaç de formular propostes mereixedores d'un sí. Potser l'hivern que avança lentament sigui el moment idoni per pensar serenament sobre allò que construïm i allò que desconstruïm. Que el solstici d'hivern li sigui propici, amic lector, en companyia dels seus.

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Presentación

Los esfuerzos por sistematizar y difundir el conocimiento se remontan a tiempos inmemoriales, aunque es el período helenístico el que ve nacer las grandes bibliotecas legendarias, como la Biblioteca de Alejandría o la de Pérga-

mo, creadas ya para reunir todo el conocimiento social de su tiempo y ponerlo a disposición de los eruditos. La biblioteca como gran centro de investigación y aprendizaje emparenta con esa voluntad de las primeras academias por compartir el saber artístico y científico. Sabios notables que han contribuido y contribuyen a mejorar las condiciones de vida de la humanidad. En este segundo número de la colección *Homenajes* de *Tribuna Plural* nos acercamos a cuatro nuevos miembros de honor que, cada uno desde su campo, aportan conocimientos y méritos para combatir enfermedades y aumentar la calidad de vida del ser humano. Este volumen recoge la participación en la solemne sesión conjunta de su recepción, convocada el 20 de diciembre de 2016, así como algunas de sus obras escogidas. Para nuestra Corporación centenaria es un honor poder contribuir a la difusión del conocimiento en el siglo XXI, el de los sabios que logran humanizar la ciencia.

El Dr. **Aarón Ciechanover** es un bioquímico israelí que ha destacado por sus descubrimientos sobre cómo las células del cuerpo humano se organizan para degradar regularmente las proteínas inservibles: el denominado sistema de la ubiquitina o degradación proteica mediada por ubiquitina. Por estos trabajos recibió el Premio Nobel de Química de 2004, compartido con otros dos investigadores. Sus descubrimientos hacen posible desarrollar medicamentos contra enfermedades como el cáncer cervical y la fibrosis quística. El Dr. Ciechanover es Doctor en Medicina desde 1981 por el Technion - Instituto Tecnológico de Israel y es el director del Instituto Rappaport Family para la Investigación de las Ciencias Médicas, ambos en Haifa. Desde 1996 es miembro del Consejo de la Organización Europea de Biología Molecular (EMBO) y publica regularmente en prestigiosas revistas científicas como *Nature*, *Cell* y *Scientific American*.

El Dr. **Josep Maria Gil-Vernet** es Doctor en Medicina y Cirugía por la Universidad de Madrid, Especialista Diplomado en Urología, Catedrático Extraordinario de Urología de la Universidad de Barcelona y *visiting professor* en numerosas universidades, entre las que destacan la de Columbia (The College of Physicians and Surgeons), Johannesburgo, Salisbury, Rochester, Missouri, el Medical Center de Nueva York, la Universidad Libre de Berlín y la Universidad René Descartes de París. Es miembro de la Real Academia de Medicina de Barcelona, miembro de Honor de la Academia de Medicina de la extinta Unión Soviética, y miembro de 26 sociedades extranjeras de Urología. El Dr. Gil-Vernet, hombre innovador en la docencia y enseñanza de la urología, ha realizado importantes aportaciones en el campo de la técnica urológica que contribuyen a mejorar el pronóstico de numerosas enfermedades y a conseguir una especialidad más eficaz y segura.

El Dr. **Björn O. Nilsson** es el presidente de la Real Academia Sueca de Ciencias de la Ingeniería, se doctoró en Bioquímica en el Instituto Real de Tecnología en Estocolmo, donde en la actualidad ejerce como docente. El profesor Nilsson ha ocupado y ocupa relevantes posiciones ejecutivas y de gestión en las industrias farmacéutica y biotecnológica, principalmente con el grupo Pharmacia. Es el presidente del Consejo de Administración de la empresa pública de biotecnología Bioinvest International AV. El profesor Nilsson ocupa cargos relevantes en diversas organizaciones gubernamentales y no gubernamentales nacionales e internacionales en los campos de la ciencia, la educación y la innovación. En 2016, el profesor Nilsson fue elegido miembro del Consejo de Dirección del Instituto Europeo de Innovación y Tecnología. Por otra parte, ocupa desde marzo de 2016 la presidencia de la Asociación Sueca de Atletismo.

El Dr. **Ismail Serageldin** se graduó en la Universidad de El Cairo y se doctoró en la de Harvard. Cuenta con una trentena de doctorados *honoris causa* por universidades situadas en los cinco continentes. Es el director de la Biblioteca Alexandrina y presidente de sus diez institutos de investigación asociados. Sirve como Embajador ante la Alianza de Civilizaciones y es el presidente del Comité Ejecutivo de la Biblioteca Digital Mundial. Participa activamente en diversos consejos de administración y consejos asesores de instituciones académicas, investigadoras y científicas de carácter internacional y contribuye a los esfuerzos de la sociedad civil por la mejora de las condiciones de vida. Ha sido profesor visitante en numerosas universidades e instituciones, como el Colegio de Francia, la Universidad de Wageningen en los Países Bajos y la Universidad Americana de El Cairo. Fue vicepresidente del Banco Mundial hasta julio de 2000, institución con la que empezó a colaborar en 1972.

Para la Academia es un honor muy especial recibir a las cuatro personalidades recién glosadas; en esta ocasión –queremos destacar– van a estar arropadas por la evocación de la Biblioteca de Alejandría y todo lo que el nuevo proyecto simboliza. La antigua Biblioteca fue un Templo del Saber condenado a la destrucción, como el Templo de Jerusalén. Es terrible la pérdida de aquello que ha costado mucho construir, pero si esas paredes acogen todo el saber conocido, la pérdida es irreparable. Que la Biblioteca haya renacido dieciséis siglos después, cual ave fénix que muere para renacer con más fuerza, nos compromete con la magnífica tarea de la Reconstrucción del Templo, un afán universal del ser humano que ha conseguido domeñar su animalidad.

Al hilo del reciente referéndum italiano, podríamos reflexionar también sobre cómo conseguir reconstruir los templos simbólicos que nos han sido en-

comendados. Más allá de las propuestas de reforma constitucional –que pocos electores habían leído y que no carecían de fundamento sólido–, resulta forzoso aceptar que los votantes sienten la necesidad de decir no a cuanto se les propone. Los electos y los gobernantes harían bien en no mirar hacia otro lado: alguien tiene que ser capaz de formular propuestas merecedoras de un sí. Quizás el invierno que avanza lentamente sea el momento idóneo para pensar serenamente sobre lo que construimos y lo que desconstruimos. Que el solsticio de invierno le sea propicio, amigo lector, junto a los suyos.

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Presentation

Efforts to systematise and disseminate knowledge can be traced back to time immemorial, although it was the Hellenistic period that saw the birth of the great legendary libraries, such as those of Alexandria or Pergamum, which were created to bring together all the available knowledge of the day and place it at the disposal of scholars. The library, as a great centre of research and learning, is associated with the desire of the first academies to share artistic and scientific knowledge, from wise elders who have contributed - and continue to contribute - to improving the living conditions of humanity. This second part of the *Tributes* collection of *Tribuna Plural* deals with four new honorary members, each one of whom is providing, in his own field, knowledge and expertise to combat disease and improve the quality of human life. This issue covers the participation in the joint formal session and reception to be held on 20th December 2016, as well as some of the works chosen for inclusion. Our organisation, which is currently celebrating its first centenary, is honoured to be able to contribute to the dissemination of knowledge in the twenty-first century, thereby echoing the wise individuals who manage to humanise science.

Dr **Aaron Ciechanover** is an Israeli biochemist, best-known for his discoveries relating to the manner in which the cells of the human body regularly organise themselves in order to dispose of unusable proteins: the so-called proteasomal degradation pathway. He was awarded the 2004 Nobel Prize in Chemistry, shared with two other researchers, on the basis of this work. His discoveries have made it possible to develop drugs designed to combat such illnesses as cervical cancer or cystic fibrosis. Dr Ciechanover gained his medical degree in 1981 at Technion, the Israel Institute of Technology in Haifa.

He is likewise the director of the Rappaport Family Institute for Research in the Medical Sciences, also based in Haifa. He has been a board member of the European Molecular Biology Organisation since 1996, and publishes regularly in such prestigious scientific journals as *Nature*, *Cell* and *Scientific American*.

Dr **Josep Maria Gil-Vernet** is a doctor of medicine and surgery of the University of Madrid, a qualified specialist in urology, Extraordinary Professor of Urology at the University of Barcelona and visiting professor at various other universities, including Columbia (The College of Physicians and Surgeons), Johannesburg, Salisbury, Rochester, Missouri, the New York Medical Center, the Free University in Berlin and the René Descartes University in Paris. He is a member of the Barcelona Royal Academy of Medicine, an honorary member of the Academy of Medicine of the former Soviet Union and a member of 26 professional urology associations worldwide. Dr Gil-Vernet, an innovator in the teaching and dissemination of urology, has made important technical contributions to the field that have helped to improve the prognosis of numerous diseases and which have succeeded in making this specialist area of medicine safer and more efficient.

Dr **Björn O. Nilsson**, the President of the Royal Swedish Academy of Engineering Sciences, was awarded his doctorate in biochemistry by the Royal Institute of Technology in Stockholm, where he currently lectures. Professor Nilsson has occupied, and continues to occupy, various key executive and managerial positions in the pharmaceutical and biotechnological sectors, particularly in the Pharmacia Group. He chairs the board of directors of the public biotechnology company Bioinvest International AV. Professor Nilsson also occupies important posts in various governmental and non-government organisations in both Sweden and elsewhere, in the fields of science, education and innovation. In 2016, Professor Nilsson was appointed to the management board of the European Institute of Innovation and Technology. He has also been president, since March 2016, of the Swedish Athletics Association.

Dr **Ismail Serageldin**, a graduate of the University of Cairo, gained his doctorate at Harvard. He holds some thirty honorary doctorates from universities on all five continents. He is the Director of the Library of Alexandria, and chairs its ten associated research institutes. He serves as ambassador to the Alliance of Civilisations and chairs the executive board of the World Digital Library. He plays an active role in various management boards and advisory committees of different international academic, research and scientific institutions, and

contributes to the efforts of civil society in improving the quality of life. He has been a visiting professor at numerous universities and institutions, including the College of France, Wageningen University in the Netherlands and the American University in Cairo. He served as vice-president of the World Bank, an institution with which he had begun to collaborate in 1972, until the year 2000.

It is a very special honour for the Academy to receive four such outstanding personalities with recent achievements to their name, and we would like to evoke the spirit of the Library of Alexandria in stressing everything that this new project symbolises. The original Library was a temple to knowledge that was ultimately destined, like the Temple of Jerusalem, for destruction. It is terrible to contemplate the loss of something that cost so much to build, and the loss is irreparable if those walls really did contain all existing knowledge. But the Library was reborn sixteen centuries later, like the Phoenix that dies to re-emerge with even greater vigour, and we are now committed to the noble task of rebuilding this temple, as part of a universal human aim to tame our baser desires.

With the recent Italian referendum in mind, we could also reflect on how to reconstruct the symbolic temples with which we have been interested. Beyond the proposals for constitutional reform - which few voters read, but which did not lack solid foundations - it seems somewhat forced to accept that voters feel the need to reject what has been proposed to them. Elected representatives and governments would be well advised not to look the other way, as someone has to be able to formulate proposals that are worthy of a “yes” vote. The onset of winter is perhaps an ideal time to think calmly about what we build and deconstruct. So let the winter solstice be a conducive moment to you and yours in this respect.

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EXCMO. SR. DR. AARON CIECHANOVER

Discurso de presentación

Dr. Rafael Blesa González
Académico de Número de la Real Academia Europea de Doctores

It is my honor to introduce Professor Aaron Ciechanover, Distinguished Research Professor at the Technion-Israel Institute of Technology, Haifa, Israel and one of the recipients of the 2004 Nobel Prize in Chemistry. Dr. Ciechanover has had an illustrious career and spanning momentous events in history. His parents, Bluma and Yitzhak emigrated from Poland to the British Mandate of Palestine in the mid-1920s and were active in the Zionist movement. Aaron was born in Haifa in October 1947, one month before Israel was recognized as an independent state by the United Nations. Soon after Israels' War of Independence began while thousands of refugees from Europe poured into the new state of Israel. The following years saw many conflicts but Aaron's parents made sure that he and his older brother Yossi obtained the best possible education.

Early on the young Professor Ciechanover had a strong inclination towards biology, collecting flowers on Mount Carmel near Haifa, extracting chlorophyll from leaves, and using the microscope that was a gift from his brother. During high school in the early 1960s inspiring teachers introduced him to the wonders of biology, chemistry, physics and mathematics. As a result, Aaron chose to study medicine as, in his words, "medicine emerged as a compromise between the complexity and mysteries of biological mechanisms to what I thought are the already well founded mechanisms of physics and chemistry".

He started his medical studies at the Hebrew University of Jerusalem in 1964 and paraphrasing his own words, “Towards the end of the 4th year, serious doubts had begun to arise whether I made the right choice. The imbalance between phenomenology and pathogenetic mechanisms of diseases on one hand, and the lack of any mechanism-based treatment for most of the major killers on the other hand, made me seriously think that I was on the wrong trail. I started to realize how little we know and how descriptive is our understanding of disease mechanisms and pathology and as a consequence how most treatments are symptomatic in nature rather than causative.”

This revelation led Aaron to the biochemists, Jacob Bar-Tana and Benjamen Shapira with whom he investigated the mechanisms underlying carbon tetrachloride-induced hepatotoxicity. A love of biochemistry was born leading Aaron to join the Unit of Biochemistry at the newly established Faculty of Medicine at the Technion in Haifa. The director was a young Avram Hershko. During this time, Ciechanover completed medical school, received his PhD, fulfilled his military service in the Israeli Defense Forces and, with Hershko and Rose carried out the studies on how cells destroy redundant proteins that would lead to the discovery of the Ubiquitin system.

By the end of the 1990s it was clear that ubiquitin-proteasome-dependent proteolysis was a highly sophisticated process involved not just in protein turnover but also in the regulation of key biological functions such as cell division, transcriptional control, DNA repair, antigen presentation, and degradation of abnormal proteins. The recognition of the ubiquitin system as perhaps the most important post translational modification regulating the stability and functional activity of proteins resulted in the awarding of the Nobel Prize in Chemistry in 2004 to Aaron Ciechanover, Avram Hershko and Irwin Rose “for the discovery of ubiquitin-mediated protein degradation”.

In addition to the Nobel Prize, Professor Ciechanover has received other prestigious awards over the years, including the Albert Lasker Award, The Israel Prize and the Hans Krebs Medal, among others. He is an honorary member of more than 30 Academies and has received honorary PhDs from 30 universities throughout the world, and Honorary Citizenship from more than 10 capital cities of the world. He has published more than 250 articles, 50 book chapters, and 10 books.

In addition to his scientific work, and among many other interests and passions, Dr. Ciechanover is a committed educator. He credits his parents for instilling in him an early love of education ranging from secular topics to Jewish studies and cultural life. In his biography written for the Nobel Committee Dr. Ciechanover reminds us of the importance of mentors by giving thanks to his, including Ernie Rose for showing him and I quote "that methodic thinking is not always necessary in science, and is even interfering at times, and that being erratic and disordered, even absent minded, thinking in a most unconventional manner, can yield wonderful ideas and results" and to the great cell biologist, Harvey Lodish, with whom he trained at the Massachusetts Institute of Technology, and again I quote "for being a wonderful spiritual mentor in a different way we tend to think of mentors. He gave me complete freedom to choose my own way, but did not let me fall. He used to gently comment on my approach when he felt I got derailed, and helped redirect me".

We also thank his mentors and Dr. Ciechanover for his contributions. I am honored to present as a Honorary Member of the Royal European Academy of Doctors.

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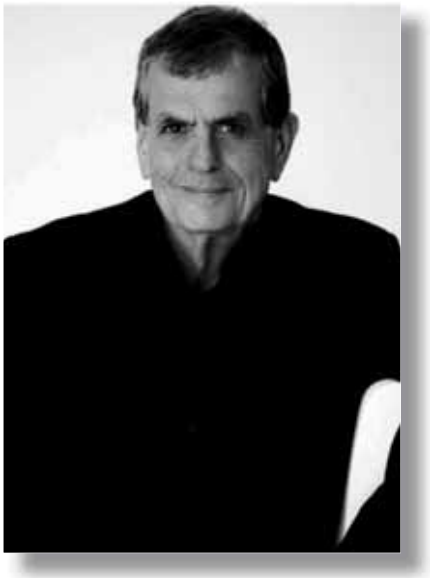

Dr. Aaron Ciehanover, Yaacov Hod and Avram Rershko[1]
Technion-Israel Institute of Technology, School of Medicine, Haifa, Israel
Received March 8,1978.

December 20, 2016

Dear Chairman of the Royal European Academy of Doctors,
Dear Colleagues, Dear Friends – buenas noches a todos ustedes

It is with a great feeling honor, but at the same time with great sense of humbleness, that I receive from you today the Degree of Academic of Honor in the Royal European Academy of Doctors – here in Barcelona on that day of December 20th 2016.

Most of my recognition and accolades thus far have come from scientific institutions. Even the Nobel Prize is in Chemistry. That because our basic fundamental discovery of the mechanism of removal of wasteful proteins form the living organism, is rooted deeply in biochemistry and molecular and cell biology. But there are two important facts I keep on carrying with me all along the time, and which tie me strongly to this evening's event.

The first, I am a physician by education, and as a matter of fact, with a stint in general surgery. My science came much later, but my first love has remained medicine – the patients, their families, and their diseases. Being a physician, I learnt however that in order to maximize our ability and to impact the progress of medicine, in particular nowadays, in the evolving era of personalized, precision medicine, we need to better explore the mechanisms that underlie the

pathogenesis of diseases, which will enable us to develop better therapeutic modalities to combat them. This was the thinking that was behind my initial foray into scientific research – yet, making sure to never forget my roots, and never remove my eyes from the patient's bed. And indeed, the system we have discovered – the ubiquitin-proteasome system - has been converted in recent years – with the efforts of many – into a fundamental platform for drug development. Velcade® to Carfizomib®, the most powerful and useful proteasome inhibitors used to treat Multiple Myeloma paved the road and have saved the lives of myriad of patients and improved and changed the quality of lives of many others. They were followed by Thalidomide®, Lenalidomide®, and Pomalidomide® that have done the same, and in combination with the proteasome inhibitors, were even more miraculously. These drugs are being used to treat certain lymphatic system malignancies. Yet, behind the corner are waiting many other cancers, neurodegenerative, inflammatory, vascular and heart diseases with new drugs developed to fight them. For me as a physician that went to develop a career in basic research, witnessing this exciting development, and I would say revolution, is heart-warming, and importantly, a closure of a cycle.

The second fact is quiet obvious – there is no science without medicine, and there is no medicine without science. The two are inter-wined with one another in a Gordian knot that cannot be untied. One feeds the other, one leads to the development of the other, one improves the other – and all is for one aim – bettering and improving human lives wherever people are living. We should make sure that the great achievements of BioMedical research do not remain the property and privilege of the rich, of us who live in developed countries, of those who can afford - but rather become available to all of us – the inhabitants of earth.

I would like to close by thanking again, on behalf of my family and myself, all my colleagues and friends who thought of me as deserving this prestigious accolade, and made it – along with this beautiful evening - possible. This accolade, I feel, belongs to all of us – scientists, physicians and patients, who work relentlessly to cure diseases. I am here just to represent them.

Last but not least, special thanks to my old times good friend and colleague, Professor José Ramón Calvo and his wife Cecilia for their long term loyalty.

Muchas gracias y buenas noches

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Trabajos aportados por el nuevo Académico de Honor

A HEAT-STABLE POLYPEPTIDE COMPONENTO F AN ATP-DEPENDENT PROTEOLYTIC SYSTEM FROM RETICDLOCYTES

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Received March 8,1978.

SUMMARY: The degradation of denatured globin in reticulocyte lysates is markedly stimulated by ATP. This system has now been resolved into two components, designated fractions I and II, in the order of their elution from DEAE-cellulose. Fraction II has a neutral protease activity but is stimulated only slightly by ATP, whereas fraction I has no proteolytic activity but restores ATP-dependent proteolysis when combined with fraction II. The active principle of fraction I is remarkably heat-stable, but it is non-dialysable, precipitable with ammonium sulfate and it is destroyed by treatment with proteolytic enzymes. On gel filtration on Sephadex-G-75, it behaves as a single component with a molecular weight of approximately 9,000.

INTRODUCTION: Intracellular protein degradation in various tissues depends upon the continuous supply of cellular energy (for reviews, see 1, 2). We have been studying the energy-dependence of protein degradation in reticulocytes, as a convenient model system (3). The major protein synthesized in these cells, hemoglobin, is normally stable, but abnormal globin molecules containing certain amino acid analogs (4), mutant globin chains (5) or puromycin peptides (6) are rapidly degraded. The degradation of abnormal globin molecules is energy-dependent (3, 7) and we have shown that energy is required at an early stage of the process (3).

Recently, Etlinger and Goldberg have reported that the degradation of analog-containing protein in reticulocyte lysates is stimulated by ATP (7). Crude lysates also carry out the ATP-dependent degradation of heme-free denatured globin (8). The elucidation of the mode of action of ATP obviously requires the separation and characterization of the enzyme(s) involved. Goldberg and coworkers have purified an endopeptidase from reticulocytes, but the stimulation by ATP was lost on purification (8). We now report that the ATP-dependent cell-free system is composed of complementing species, and describe the properties of one of the components.

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METHODS: Reticulocyte Lysate. Reticulocytosis of 70–80% was induced in rabbits by injections of phenylhydrazine (4). The cells were washed twice with ice-cold saline and incubated (37°C, 2 h) with 0.2 mM 2,4-dinitrophenol and 20 mM 2-deoxyglucose, to deplete cellular ATP (3). Following additional washing, the cells were lysed with 1.6 volumes of water containing 1 mM dithioerythritol and particulate material was removed by centrifugation at 40,000 x g for 90 min.

Fractionation on DEAE-Cellulose: All operations were at 0–4°C. 30 ml lysate was applied to a 4.5 x 12 cm column of DEAE-cellulose (Whatman, DE-52) equilibrated with 3 mM potassium phosphate, pH 7.0. Nonadsorbed protein was eluted with 50 ml of the above buffer containing 1 mM dithioerythritol. The last two-thirds of the nonadsorbed material containing hemoglobin were collected - designated fraction 1. Adsorbed protein was then eluted with 70 ml of a solution containing 10 mM Tris-Cl (pH 7.1), 0.5 M KCl and 1 mM dithioerythritol. Solid ammonium sulfate was added to 90% saturation (65 g per 100 ml of solution) and the suspension was stirred for 30 min. Precipitated protein was collected by centrifugation, dissolved in 4–5 ml of 10 mM Tris-Cl (pH 7.1), 1 mM dithioerythritol, and dialyzed for 20 h against 2 lit of the same buffer; this preparation was designated fraction I.

Partial Purification of Fraction I: The nonadsorbed material of the DEAE-cellulose column was heated at 90°C for 10 min, stirred up and chilled on ice. Denatured protein was removed by 2 repeated centrifugations at 35,000 x g for 20 min. Solid ammonium sulfate was added to 90% saturation and after stirring for 30 min, the precipitate was collected by centrifugation, dissolved in 1.3% of the original volume and dialyzed overnight against 10 mM Tris-Cl, pH 7.6. If residual hemoglobin color remained, the preparation was heated again (90°C, 15 min) and centrifuged as before; the final preparation was practically colorless. In a typical preparation, the partially purified material contained 0.1% of the initial protein, the recovery of activity was around 40%, and thus the purification was approximately 400-fold. Protein was determined by the method of Lowry et al. (9).

Preparation of [^{3}H]-labeled Globin: A 50% suspension of reticulocytes in Krebs-Ringer solution (3) was incubated at 37°C for 60 min with L-[4,5-^{3}H]leucine (38 Ci/mmole, 60 μCi/ml) and 0.25 mM each of the other 19 unlabeled amino acids. Hemoglobin was purified by DEAE-cellulose chromatography and heme-free globin was precipitated with HCl-acetone, as described (10). The purified globin had a specific radioactivity of 50,000 dpm/mg of protein, and was devoid of any proteolytic activity. Before use, it was denatured by heating in 0.01 N NaOH at 60°C for 30 min, to increase its susceptibility to ATP-dependent proteolysis (3).

Assay of ATP-Dependent Degradation of [^{3}H]Globin: The reaction mixture contained in a final volume of 0.5 ml: 100 mM Tris-Cl (pH 7.6), 5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithioerythrito., 80 ug/ml [^{3}H]globin, 0.5 mM ATP, 10 mM phosphocreatine, 100 ug/ml creatine phosphokinase (Boehringer) and the enzyme fractions as indicated. The ATP-generating system was added to prevent the formation of ADP that inhibits the reaction (3). Control experiments showed that creatine phosphate and kinase had no influence on proteolysis in the absence of ATP. Following incubation at 30°C for 60 min, the reaction was stopped with 1 ml of 5% trichloroacetic acid and following centrifugation, 10 mg of bovine serum albumin was added to the supernatants and the samples were centrifuged again. Radioactivity in the clear supernatants was estimated and the percentage of the degradation of [^{3}H]globin to acid-soluble products was computed.

RESULTS AND DISCUSSION: In our initial attempts to purify the ATP-dependent proteolytic activity from reticulocyte lysates, we have employed the widely used

TABLE I: Resolution of the ATP-Dependent Cell-Free Proteolytic System into Complementing Activities

Enzyme fraction	Degradation of [^{3}H]globin percent/h	
	-ATP	+ATP
lysate	1.5	10.0
fraction I	0	0
fraction II	1.5	2.7
fraction I and fraction II	1.6	10.6

Enzyme fractions were separated by DEAE-cellulose as described under "Methods" and supplemented at the following amounts (ug of protein/ml reaction volume): lysate, 28; fraction I, 45; and fraction II, 3.5. Where indicated, ATP was added together with phosphocreatine and creatine phosphokinase.

method of stepwise separation on DEAE-cellulose (II): at neutral pH and at low ionic strength, most non-hemoglobin proteins are adsorbed on the resin, while hemoglobin and a few more basic proteins are not retained. As shown in Table I, the protein fraction adsorbed on DEAE-cellulose and eluted with 0.5 M KCl (fraction II) contained a neutral proteolytic activity, but it was stimulated only slightly by ATP, as compared to the effect of ATP in the whole lysate. The unadsorbed fraction (fraction I) had no proteolytic activity either in the presence or absence of ATP. However, the supplementation of fraction I to fraction II restored to a large extent the stimulation of [^{3}H]-globin degradation by ATP. This action of fraction I appeared to be specific for the ATP-dependent system, since it had no influence on the ATP-independent proteolytic activity in fraction II. It should be noted that in different preparations, considerable variations were observed in the amount of ATP-independent proteolytic activity in fraction II (up until 10% [^{3}H]globin degraded/h). However, the extent of the additional proteolysis stimulated by fraction I and ATP was much more constant.

Figure 1 shows the dependence of ATP-stimulated protein degradation on the

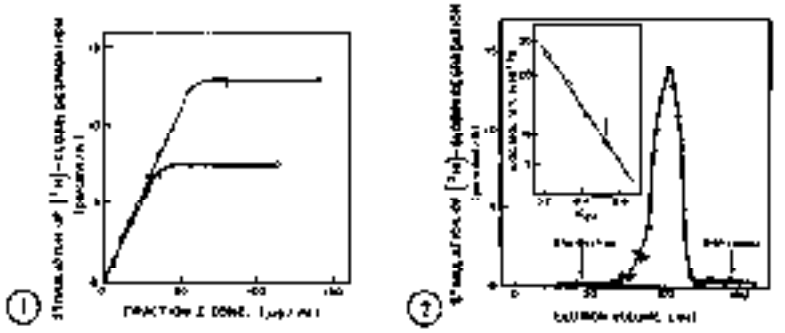

Fig. 1. Concentration dependence of the effect of fraction I on ATP-dependent proteolysis. The degradation of [^{3H}]globin was assayed with increasing amounts of partially purified fraction I, in the presence of the following concentrations of fraction II (mg/ml): open circles, 2.2; closed circles, 5.5. The activation of [^{3H}]globin degradation was computed by subtracting the breakdown values obtained without fraction I (fraction II with ATP); these were 3.9 and 5.6 percent/h for the low and high concentrations of fraction II, respectively.

Fig. 2. Gel filtration analysis of the heat-stable polypeptide. A 7.0 ml sample of partially purified fraction I was introduced into a column (1.5 x 86 cm) of Sephadex-G-75 which had been previously equilibrated with 10 mM Tris-Cl (pH 7.1). Elution was with the same buffer, fractions of 2 ml were collected and aliquots of 150 µl of each fraction were assayed for the activation of [^{3H}]globin degradation with 5.0 mg/ml of fraction II. The results are expressed as the increase in [^{3H}]globin degradation above that obtained with fraction II and ATP, that was 6.2%/hr. Insert: Estimation of molecular weight. The Sephadex-G-75 column was calibrated with the following marker proteins (open circles): α-chymotrypsinogen (mol. wt. 25,000); myoglobin (mol. wt. 17,800); and cytochrome C (mol. wt. 12,400). The closed circle indicates the elution position of heat-stable polypeptide activity.

maximal concentrations of fractions I and II. Proteolysis increased in a linear fashion with increasing concentrations of fraction I until it attained a constant maximal level. At this stage, the response to fraction I was clearly limited by the level of fraction II, since increasing the concentration of fraction II allowed a further stimulation by high concentrations of fraction I.

Preliminary characterization of the activity in fraction I revealed some rather unusual features. The active factor is remarkably stable at high temperatures that would inactivate most proteins, and retains considerable activity even following heating at 90°C for 60 min (Table 2). However, it appears to

TABLE 2: Properties of Fraction I

Treatment of Fraction I	Increase of ATP-dependent proteolysis	
	[^{3}H]globin degraded (percent/h)	% of control
Experiment 1		
Control	6.6	100
Heated 96°C, 15 min	6.3	95
Heated 96°C, 60 min	4.6	70
Dialyzed (20 h)	6.7	102
Pronase-treated	0	0
Chymotrypsin-treated	0	0
Experiment 2		
Control	6.7	100
$(NH_4)_2$ SO_4 precipitate	6.1	97
$(NH_4)_2$ SO_4 supernatant	0	0

Fraction I was heated at 87°C for 10 min and centrifuged as described in "Methods". Portions of this preparation ("control", 0.38 mg protein/ml) were then further heated in closed tubes at 96°C for the time periods indicated and centrifuged at 100,000 x g for 90 min. Other portions were subjected to dialysis for 24 h or precipitated with ammonium sulfate (90% saturation) and then dialyzed. Treatments with pronase (Sigma, 25 µg/ml) or α-chymotrypsin (Boehringer, 25 µg/ml) were at 37°C for 90 min at pH 7.6 or 7.8, respectively. The incubation was terminated by boiling for 15 min; control experiments showed that this was sufficient to destroy completely added protease activities, whereas the activity of fraction I decreased only slightly. The reaction mixtures contained 3.9 mg/ml fraction II, 80 µg/ml fraction I (or equivalent amounts after the respective treatments) and other ingredients as described in "Methods". The increase of ATP-dependent proteolysis was calculated by subtraction of the values of [^{3}H]globin degradation without fraction I (fraction II with ATP); these were 4.6%/h for experiment 1 and 9.9%/h for experiment 2.

be a polypeptide by a number of criteria: it is not dialyzable, it is precipitated by ammonium sulfate and furthermore, the activity is completely destroyed by treatment with proteolytic enzymes such as pronase or chymotrypsin (Table 2). By the combination of drastic heat-treatment and ammonium sulfate precipitation, an approximately 400-fold partial purification of the heat-stable polypeptide was achieved (see "Methods"). Gel filtration analysis of the partially purified factor on Sephadex-G-75 columns showed a single component with a molecular weight of approximately 9,000 (Fig. 2).

The heat-stable polypeptide does not contain any peptide bond cleaving activity, tested not only by the formation of acid-soluble products (Table 1), but also by

che possible cleavage of [^{3}H]globin to large fragments, analyzed by polyacrylamide gel electrophoresis. Furthermore, a number of reticulocyte aminopeptidase activities were also absent in partially purified fraction I, but were completely recovered in fraction II (K. Heller and A. Hershko, unpublished results).

As opposed to the properties of fraction I, the activity in fraction II is remarkably unstable and is destroyed by relatively mild heat-treatment (42°C, 30 min). The function of fraction I does not appear to be to stabilize fraction II, since it does not protect the latter against heat inactivation (data not shown).

The elucidation of the roles of the heat-stable polypeptide, or of ATP, depends upon the characterization of further component(s) in fraction II. This relatively small polypeptide might be an easily dissociable subunit of a larger enzyme complex, an activator of an enzyme present in fraction II, an inhibitor of an antagonistic factor, or it may interact directly with the substrate or ATP. Our previous studies on the degradation of amino-containing globin chains in intact reticulocytes indicated that ATP is required at or before the initial cleavage of the complete globin molecule (9). It might well be that the heat-stable polypeptide participates in such an early event that precedes the actual proteolytic reactions.

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ESSAY

The unravelling of the ubiquitin system

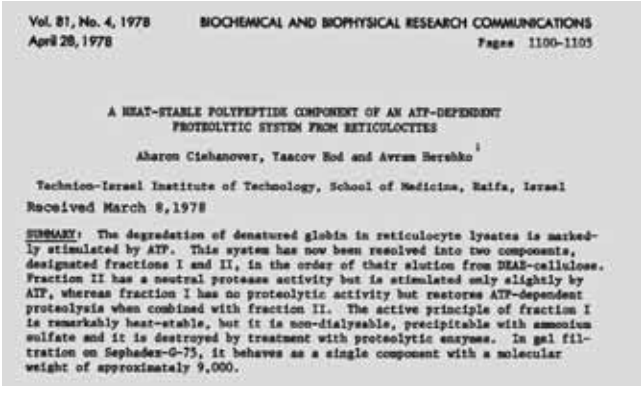
Aaron Ciechanover

Abstract | Today, many scientific discoveries are made using a top-down experimental approach. The ubiquitin system was discovered using a 'classic' bottom-up approach to tackle the question: 'how are cellular proteins selectively degraded?' A simple proteolytic assay, which used a crude cell-extract, was all that was required to address this question; it was followed by fractionation and reconstitution experiments to decipher the role of the components in this multi-step process. This 'biochemistry at its best' approach, which was published in a periodical that today would not be regarded as highly visible, provided magnificent findings.

Both the ubiquitin–proteasome proteolytic system and post-translational modifications by ubiquitin and ubiquitin-like proteins are involved in nearly all cellular processes. Aberrations in this system give rise to numerous diseases, including different malignancies and neurodegeneration. Interestingly, the modification of proteins by ubiquitin and the important part that these modifications play in targeting proteins for degradation were not discovered by screening, data mining or a systems biology approach. Rather, they came to life through the old 'classical' scientific approach of asking a biological question, which in this case was 'how are intracellular proteins degraded?' and, in particular, 'what is the identity of the mechanism (or mechanisms) that endows the degradation process with its high selectivity and specificity?'

The pioneering studies of Rudolph Schoenheimer taught us that cellular proteins are turning over[1], yet the mechanism (or mechanisms) underlying this had remained elusive. With the discovery of the lysosome by Christian de Duve[2], the proteolytic apparatus was thought to have been identified. However, the mechanism of degradation by the lysosome that was known at the time — involving small portions of the cytosol that contain an aliquot of the entire cytosolic proteome undergoing what is now known as microautophagy — could not explain the substrate specificity and selectivity of the proteolytic process. For example, one could not explain how misfolded, mutated or otherwise damaged proteins are recognized and removed, while the vast majority of functional proteins are spared, or how regulatory proteins such as cell cycle regulators or transcription factors are destroyed in a timed and programmed manner when they are not needed but are kept intact and active when they are. Another unsolved mystery was the role of metabolic energy in the process[3] — why is an investment of energy required for the hydrolysis of peptide bonds, which is an exergonic process? This finding could not be explained in simple thermodynamic terms. Although it was known that the acidification of the lysosome required ATP[4], the fact that a role for lysosome-mediated microautophagy in selective protein degradation had been ruled out, along with the finding that energy is also required for protein degradation in prokaryotes that do not have lysosomes[5], raised the hypothesis that ATP is required for a regulatory step (or steps) in the proteolytic process. The most direct evidence that the lysosome does not mediate selective intracellular proteolysis came from an experiment by Brian Poole[6], showing that lysosomotropic agents (that is, agents that preferentially accumulate in lysosomes) such as chloroquine — which neutralize lysosomal pH and thus inhibit the activity of lysosomal proteases — abolished the degradation of extracellular proteins that reach the lysosome via different endocytic routes but had no effect on the degradation of intracellular proteins. Brian Poole summarized his findings by predicting the existence of a non-lysosomal system that degrades intracellular proteins: "the exogenous proteins will be broken down in the lysosomes, while the endogenous proteins will be broken down wherever it is that endogenous proteins are broken down during protein turnover" (REF. 6).

These open questions and experimental findings drove the search for the elusive non-lysosomal and ATP-dependent protease or proteolytic system that degrades intracellular proteins in a specific manner. Admittedly, only a handful of researchers were interested in the problem, as the attention of the scientific community was at the time (the 1970s) focused on deciphering the factors and regulatory mechanisms involved in the central dogma of biology — unlocking the genetic code of protein synthesis. Along with



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A HEAT-STABLE POLYPEPTIDE COMPONENT OF AN ATP-DEPENDENT PROTEOLYTIC SYSTEM FROM RETICULOCYTES

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SUMMARY: The degradation of denatured globin in reticulocyte lysates is markedly stimulated by ATP. This system has now been resolved into two components, designated fractions I and II, in the order of their elution from DEAE-cellulose. Fraction II has a neutral protease activity but is stimulated only slightly by ATP, whereas fraction I has no proteolytic activity but restores ATP-dependent proteolysis when combined with fraction II. The active principle of fraction I is remarkably heat-stable, but it is non-dialysable, precipitable with ammonium sulfate and it is destroyed by treatment with proteolytic enzymes. In gel filtration on Sephadex-G-75, it behaves as a single component with a molecular weight of approximately 9,000.

Figure 1 | **The title and abstract of the first manuscript that prompted additional studies and resulted in the discovery of the ubiquitin proteolytic system.** This study describes the fractionation of a crude cell-extract into two complementary fractions (see also TABLE 1). The active component in one fraction was identified as a small 8.5 kDa protein (later identified as ubiquitin). The finding that the activity in the crude extract is made of two complementing activities, rather than a single energy-requiring protease, prompted further fractionation, whch later resulted in the discovery of the cascade of conugating enzymes and the proteolytic machinery. Reprinted with permission from REF. 9, Elsevier.

Table 1 | **ATP and two enzymatic components are required for protein degradation**

Enzyme fraction	Degradation of ^{3}H-globin (percent per hour)	
	–ATP	+ATP
Lysate	1.5	10.0
Fraction I	0	0
Fraction II	1.5	2.7
Fraction I and fraction II	1.6	10.6

ATP and the enzymatic activity of two complementing fractions of the cell-free proteolytic system are required for degradation of the model substrate ^{3}H-globin. The energy-requiring proteolytic activity in crude reticulocyte lysate was resolved into two essential components using anion exchange chromatography: fraction I, which contains the proteins that did not adsorb to the resin, and fraction II, which contains the proteins that were adsorbed and eluted with high salt. Reprinted with permission from REF. 9, Elsevier.

Avram Hershko, my graduate studies mentor, we selected the reticulocyte, the terminally differentiating red blood cell, as our model system, because it was known that it expels its lysosomes during differentiation in the bone marrow but continues to degrade its proteins and protein-based machineries until its final maturation and ejection into the peripheral circulation[7]. Indeed, Etlinger and Goldberg[8] demonstrated that intact reticulocytes and, importantly, high-speed centrifugation supernatant prepared from them, degraded amino acid analogue-containing abnormal haemoglobin in an ATP-dependent manner by an unknown mechanism.

The first, and arguably the most important mechanistic clue came from experiments that were described in a short study published in 1978 in Biochemical and Biophysical Research Communications (BBRC)[9] (FIG. 1). The idea behind the crucial experiment was, obviously, to purify and then characterize the elusive ATP-dependent protease. Surprisingly, in the first attempt to purify it, we were already left without a paradigm: typically, the 'tango' of proteolysis is danced by two — a protease and a substrate. Here, fractionation of the crude lysate on an anion exchange resin revealed that the proteolytic activity required for the degradation of our model substrate was made of two necessary components and required ATP (TABLE 1). This finding raised the hypothesis that more than two components may be needed because the two fractions were crude and reflected the division of the entire cellular proteome according to protein behaviour on the resin. This indeed proved to be the case and, shortly after, using different chromatographic approaches and reconstituted cell-free assays, we began isolating additional factors. The first component was a small (molecular weight of ~9.0 kDa) heat-stable protein that we called ATP-dependent proteolysis factor 1 (APF1)[9], which we later found to be covalently attached to the target substrates by an ATP-requiring reaction; we hypothesized that APF1 probably signalled them for degradation by a downstream protease that had not yet been identified[10,11] (FIG. 2). Along with our collaborator, Irwin A. Rose, we proposed a model for the entire proteolytic cycle that has withstood the test of time and is accepted to be largely correct[11] (FIG. 3).

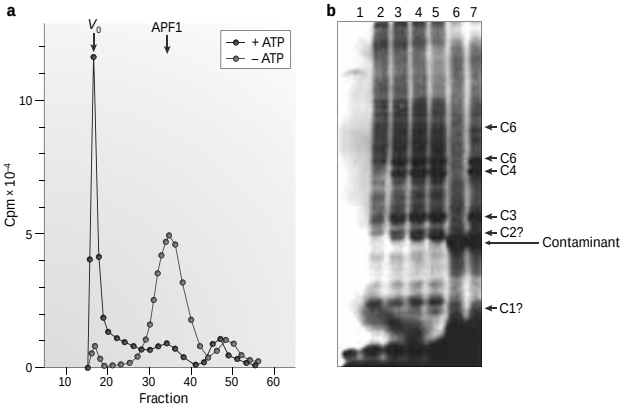

Figure 2 | **APF1 is covalently conjugated to proteolytic substrates, presumably marking them for degradation by a downstream protease. a** | ^{125}I-labelled ATP-dependent proteolytic factor 1 (APF1; later identified as ubiquitin) purified from fraction I (see TABLE 1) was incubated with crude fraction II in the absence or presence of ATP, and the mixtures were resolved by gel filtration chromatography. Shown are the radioactivity levels of the different fractions. The addition of ATP resulted in a shift of almost all the radioactivity to the high molecular-mass zone of the chromatographic separation. The arrow underneath APF1 points to the peak of radioactivity of the APF1 resolved from a system incubated without ATP (red), or to the remaining free APF1 left in a reaction mixture that was incubated with ATP (blue). **b** | ^{125}I-labelled APF1 purified from fraction I was incubated with crude fraction II in the absence (lane 1) or presence (lanes 2–5) of ATP. When increasing amounts of unlabelled lysozyme were added (lanes 3–5), new bands (conjugates, denoted by C1–C6) containing labelled APF1 appeared. To demonstrate that these newly formed bands also contained lysozyme, unlabelled APF1 was incubated with fraction II and ^{125}I-labelled lysozyme in the absence (lane 6) or presence (lane 7) of ATP. Bands of molecular mass similar to those in lanes 3–5 appeared. Presented is the autoradiogram of the SDS–PAGE-resolved reaction mixtures. The difference in molecular mass between adjacent conjugates is similar to the molecular mass of APF1, suggesting that multiple molecules of APF1 are conjugated to each protein target molecule. As we now know, this can be the result of either the generation of a polyubiquitin chain that is anchored to a single lysine residue or of the conjugation of several single ubiquitin moieties to multiple lysine anchors in the protein target (that is, multiple monoubiquitylations). Part **a** reprinted with permission from REF. 10, US National Academy of Sciences. Part **b** reprinted with permission from REF. 11, US National Academy of Sciences. Cpm, counts per million.

According to the model, n molecules of APF1 are covalently attached to the substrate, which marks it for recognition by a downstream protease that degrades the substrate and recycles APF1 for reuse. A regulatory function was also proposed, which involves the removal of APF1 from the substrate before its degradation, in case the substrate refolds to its native form or the modification (or modifications) that rendered it susceptible for destruction is removed or corrected.

Shortly after, APF1 was identified as ubiquitin, a previously-known protein that was, at that point, of unknown function[12,13]. Ubiquitin had previously been found to be covalently attached to fractions of both histone H2A and histone H2B. In this case, the link was identified as an isopeptide bond between the carboxy-terminal Gly76 residue of ubiquitin and an ε-NH_2 group of

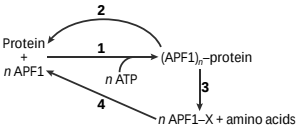

Figure 3 | **Model of the APF1 (ubiquitin)-mediated proteolytic pathway as proposed in 1980.** Step 1: a substrate protein is covalently conjugated by *n* molecules of ATP-dependent proteolytic factor 1 (APF1; later identified as ubiquitin) in an ATP-dependent reaction. It was later found that this step is catalysed by three types of enzyme that act in concert: a ubiquitin-activating enzyme (E1), ubiquitin-carrier proteins (E2 enzymes; also known as ubiquitin-conjugating enzymes (UBCs)) and ubiquitin-protein ligases (E3 enzymes). An E1 enzyme consumes two ATP molecules for each APF1 it activates. Step 2: APF1 can be removed (de-conjugated) in case the protein substrate was conjugated mistakenly, or in case it re-folds to its native form. This reaction was later found to be catalysed by deubiquitylating enzymes (DUBs). Step 3: the APF1-tagged substrate is degraded into free amino acids by a putative downstream protease, thereby releasing partially conjugated APF1 molecules (APF1–X; 'X' denotes a lysine residue or a short peptide derived from the substrate that is still bound to APF1). This reaction was later found to be catalysed by the 26S proteasome. We now know that the proteasome releases short peptides — not amino acids — that are later degraded to amino acids by cytosolic amino- and carboxy-peptidases. Step 4: the release of X by an amidase (now known as one of many DUBs) and the recycling of free APF1 for reuse by an E3 enzyme. Reprinted with permission from REF. 11, US National Academy of Sciences.

an internal lysine in the histone molecule[14]. Much later, this modification, which is mostly involved in gene silencing, was found to be a physiologically significant part of the 'epigenetic code' (REF. 15).

The convergence of identities of APF1 and ubiquitin, and the realization that ubiquitin can modify a protein by forming a peptide bond, helped us to understand the nature of the linkage between ubiquitin and the proteolytic target substrate, understand why ATP is required for this modification and to predict the existence of conjugation enzymes and machinery. From the terminally differentiating reticulocyte, the road took us to showing that the system is 'universal', and that ubiquitin mediates the degradation of proteins in nucleated cells as well[16]. The last two missing links in the chain of events leading to protein degradation were found shortly after. First, the conjugation machinery was shown to consist of three types of enzyme that act in concert: a ubiquitin-activating enzyme (E1), ubiquitin-carrier proteins (E2 enzymes; also known as ubiquitin-conjugating enzymes) and ubiquitin ligases (E3 enzymes)[17]. The second and last link was the identification of the 26S proteasome — a previously discovered protease complex with a then unknown function — as the proteolytic arm of the system[18,19]. Earlier, we had predicted this protease would specifically degrade ubiquitin-tagged proteins and recycle ubiquitin[11], a function that is now known to be carried out by deubiquitinases (DUBs), which are either integral to or associated with the proteasome, or by proteasome-independent DUBs.

The sequencing of the human genome in 2000 revealed the entire landscape of the ubiquitin system. It is made of ~1,500 components, many of which (~800) are E3 enzymes that recognize the myriad substrates of the system and endow it with its high specificity and selectivity. We now know that the modification of proteins by ubiquitin and ubiquitin-like proteins has important roles in almost all cellular processes, some of which are carried out by targeting proteins for degradation and others, such as the regulation of signalling, that are performed by non-proteolytic functions. Aberrations in the ubiquitin–proteasome system cause many diseases such as malignancies and neurodegenerative disorders. This has driven the development of drugs that modulate the activity of different components of the system.

The discovery of the ubiquitin system was the result of an attempt to solve a curiosity-driven question, of which many people either were unaware or did not consider important or biologically relevant. Technically, the experimental approach was based on embarrassingly simple biochemistry, involving setting the right output assay and purifying the basic components of the system. The core of success was the first experiment (TABLE 1), which taught us that it is not a 'classic' duo of protease–substrate that carries out the proteolysis, but rather a novel system that is potentially comprised of multiple components. We regard the BBRC article[9] as the most critical publication from which all other publications on the ubiquitin system emanated. Thinking of today's publication culture, one cannot help but conclude that it does not matter in which journal one publishes but rather what one publishes.

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Competing interests statement
The author declares no competing interests.

PROTEOLYSIS: FROM THE LYSOSOME TO UBIQUITIN AND THE PROTEASOME

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Premio Nobel de Química

Abstract | How the genetic code is translated into proteins was a key focus of biological research before the 1980s, but how these proteins are degraded remained a neglected area. With the discovery of the lysosome, it was suggested that cellular proteins are degraded in this organelle. However, several independent lines of experimental evidence strongly indicated that non-lysosomal pathways have an important role in intracellular proteolysis, although their identity and mechanisms of action remained obscure. The discovery of the ubiquitin–proteasome system resolved this enigma.

The concept of protein turnover is barely 60 years old. Previously, the protein components of the body were viewed as essentially stable constituents that were subject to only minor 'wear and tear', whereas dietary proteins, which were believed to function primarily as energy-providing fuel, acted as separate entities, independent from the structural and functional proteins of the body. This concept was challenged by Rudolf Scheonheimer who used ^{15}N-labelled amino acids to show that bodily protein components are turning over extensively — that is, they are continuously synthesized and degraded. In his book *The Dynamic State of Body Constituents*[1], he summarized his experiments in the following way: "The simile of the combustion engine pictured the steady state flow of fuel into a fixed system, and the conversion of this fuel into waste products. The new results imply that not only the fuel, but the structural materials are in a steady state offlux.The classical picture must thus be replaced by one which takes account of the dynamic state of body structure." However, the idea that proteins are turning over was not widely accepted, and was challenged as late as the mid-1950s. For example, Hogness and colleagues[2] studied the kinetics of β-galactosidase in *Escherichia coli* and summarized their findings by stating, "To sum up: there seems to be no conclusive evidence that the protein molecules within the cells of mammalian tissues are in a dynamic state. Moreover, our experiments have shown that the proteins of growing *E.coli* are static. Therefore it seems necessary to conclude that the synthesis and maintenance of proteins within growing cells is not necessarily or inherently associated with a 'dynamic state'."

This article reviews the revolution that occurred in the field of intracellular proteolysis.This includes the realization that proteins are, indeed, turning over extensively, that this process is specific, and that the stability of many proteins is regulated individually and can vary under different conditions. It also describes the search for the underlying mechanism (or mechanisms), the discovery of the lysosome, and the simple logical assumptions that led to the hypothesis that intracellular proteolysis probably occurs in this organelle. Finally, the emerging experimental data that strongly indicated that the degradation of most cellular proteins under basal metabolic conditions must be mediated by a non-lysosomal machinery — which led to the discovery of the ubiquitin

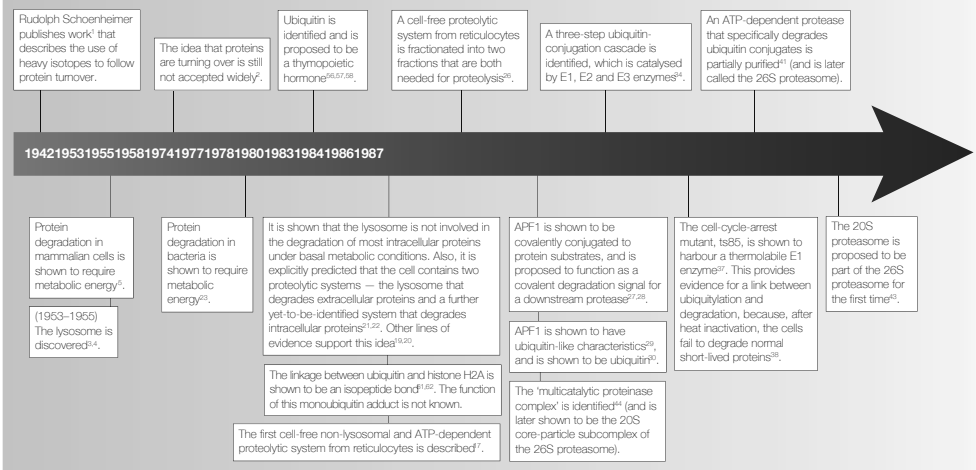
signalling system and the proteasome — will also be dicussed. The discovery of the ubiquitin–proteasome system resulted in another important development — the realization that regulated proteolysis is involved in controlling a broad array of cellular processes such as the cell cycle and cell division, apoptosis, transcription, antigen presentation, signal transduction, receptor-mediated endocytosis, protein quality control and the modulation of diverse metabolic pathways. Intracellular proteolysis was therefore transformed from a neglected process and research area into an important field in modern biology (see the TIMELINE for the history and key discoveries of the field of intracellular proteolysis).

Mechanisms of intracellular proteolysis

The discovery of the lysosome (see, for example, REFS 3,4; see also FIG. 1 and BOX 1) was a turning point in the studies on protein degradation. Several independent experiments had substantiated the idea that cellular proteins are in a constant state of synthesis and degradation (see, for example, REF. 5), so the parallel discovery of an organelle that contains a broad array of secluded proteases with different specificities provided, for the first time, a machinery that could potentially mediate intracellular proteolysis. However, over a period of more than two decades, between the mid-1950s and the late 1970s, accumulating lines of independent experimental evidence indicated that the degradation of at least certain classes of cellular protein under particular physiological conditions must be non-lysosomal.

First, an important discovery in this respect was the unravelling of the basic functional mechanism of the lysosome — microautophagy. During this process, which occurs under basal metabolic conditions, portions of the cytoplasm that contain the entire cohort of cellular proteins are segregated within a membrane-bound compartment. This compartment then fuses with a primary nascent lysosome, which results in the digestion of its protein contents. Under

Timeline | **Important discoveries and milestones in the five-decade history of intracellular protein degradation***



*The timeline shows, in my opinion, the most important discoveries in the five-decade history of intracellular protein degradation. It stops after describing the two main, basic discoveries in the ubiquitin field — ubiquitin conjugation as a degradation signal, and the proteasome. Later important developments in the field have been described in numerous review articles (see, for example, REF. 69). Furthermore, for an interesting historical perspective of the ubiquitin system that stresses the importance of this system in health and disease, see REF. 70. APF1, ATP-dependent proteolysis factor-1; E1, ubiquitin-activating enzyme; E2, ubiquitin carrier protein (ubiquitin-conjugating enzyme); E3, ubiquitin-protein ligase.

more extreme conditions — for example, starvation — mitochondria, endoplasmic-reticulum membranes, glycogen bodies and other cytoplasmic entities can be engulfed in a process that is known as macroautophagy (see, for example, REF. 6). It was conceptually difficult to reconcile this mode of non-selective degradation with the emerging concept that different proteins are degraded with distinct half-lives — particularly as protein half-lives can vary from minutes to days and can be markedly affected by changing pathophysiological conditions, such as nutrient or hormone availability (for reviews, see REFS 7,8). Interestingly, later evidence[9] indicated that lysosomal degradation might actually be specific and be mediated by the recognition of a defined motif in the target protein (KFERQ), although the existence of a similar sequence in ~30% of cellular proteins made it unlikely that such a mechanism could be substrate specific. However, it could function as part of a general mechanism that mediates substrate transport across the lysosomal membrane, although this would not be the only mechanism, as substrate entry into lysosomes is also mediated by other mechanisms, such as vesicle-membrane fusion and the formation of multivesicular bodies (MVBs; BOX 1).

Second, the discovery that specific and general inhibitors of lysosomal proteases have different effects on different populations of proteins made it clear that different proteolytic machineries function in the cell: the discovery that the degradation of endocytosed/extracellular proteins was significantly inhibited, whereas only a limited effect was observed on the degradation of long-lived proteins and almost no effect could be detected on the degradation of short-lived and abnormal/mutated proteins made it clear that different proteins are targeted by different proteolytic machineries.

Finally, the thermodynamically paradoxical observation that the degradation of cellular proteins requires metabolic energy and, more importantly, the emerging evidence that the proteolytic machinery might require energy in a direct manner were in contrast with the known mode of action of lysosomal proteases — that is, that under the appropriate acidic conditions and similar to all known proteases, they degrade proteins exergonically.

The hypothesis that the degradation of intracellular proteins is mediated by the lysosome was nevertheless logical. In general, proteolysis seemed to result from the direct interaction of substrates with proteases, and because it was clear that active proteases cannot be free in the cytosol, the most sound assumption was that intracellular protein degradation is lysosomal. Nobody could have predicted that a new mode of post-translational modification — polyubiquitylation — is needed for substrates to be specifically recognized by a giant protease that is about half the size of a ribosome. At the time, the lysosomal membrane — rather than the requirement for such a modification — seemed to provide the essential barrier between the protease (or proteases) and its substrates. It was just necessary to explain how proteins enter the lysosome and are degraded in a selective manner. According to one model, it was proposed that different proteins have different sensitivities to lysosomal proteases, and that their half-lives *in vivo* correlate with their sensitivity to the action of lysosomal proteases *in vitro*[10]. To explain an extremely long half-life for a protein that is nevertheless sensitive to lysosomal proteases, or alterations in the stability of a single protein under various physiological states, it was proposed that, although all cellular proteins are engulfed by the lysosome, only the short-lived proteins are degraded, whereas the long-lived proteins exit back into the cytosol[11]. According to a different model, selectivity is determined by the binding affinity of the different proteins for the lysosomal membrane and their subsequent entry into the lysosome, a process that controls their degradation rate[12]. The requirement for energy was described as

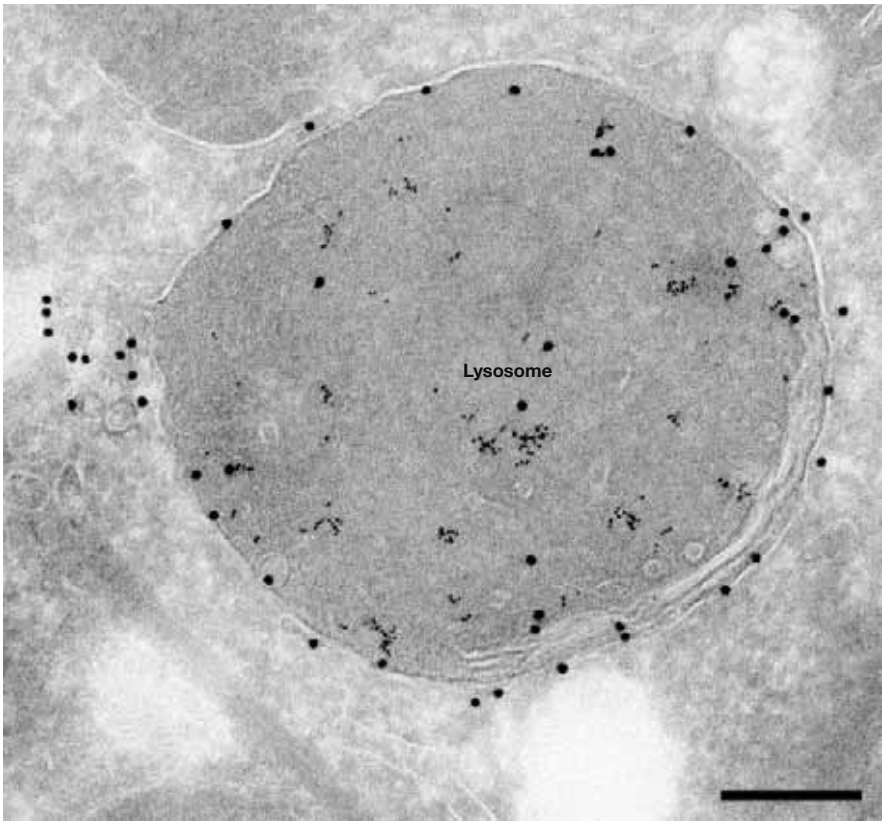


Figure 1 | **The lysosome.** Ultrathin cryosections of a human hepatoma G2 cell that had been loaded for three hours with bovine serum albumin (BSA)–gold (5-nm particles) and immunolabelled for the late endosomal/lysosomal marker CD63 (10-nm particles). The marker mostly decorates the lysosomal membrane, whereas the BSA resides in the lumen of the organelle. Bar, 200 nm. This figure was provided courtesy of Viola Oorschot and Judith Klumperman, Department of Cell Biology, Utrecht University Medical Center, Utrecht, The Netherlands.

indirect, and necessary, for example, for protein transport across the lysosomal membrane[13] or for the activity of the H^+ pump, which is required for the maintenance of the acidic intralysosomal pH that is necessary for the optimal activity of the proteases[14]. "Just as extracellular digestion is successfully carried out by the concerted action of enzymes with limited individual capacities, so, we believe, is intracellular digestion", summarized Christian de Duve[15], the discoverer of the lysosome.

Progress in identifying the putative non-lysosomal proteolytic system (or systems) was hampered by the lack of a cell-free preparation that could faithfully replicate the cellular proteolytic events — that is, a preparation that could degrade proteins in a specific, energy-requiring, yet non-lysosomal manner. An important breakthrough came from Rabinovitz and Fisher, who found that rabbit reticulocytes efficiently degrade abnormal haemoglobin that contains amino-acid analogues[16]. As reticulocytes are immature, terminally differentiating red blood cells and do not contain lysosomes, it was postulated that the degradation of haemoglobin is mediated by a non-lysosomal machinery. Etlinger and Goldberg[17] were the first to isolate a cell-free proteolytic preparation from reticulocytes.

The crude extract selectively degraded abnormal haemoglobin, required ATP hydrolysis and functioned optimally at a neutral pH, which strongly indicated that the proteolytic activity was non-lysosomal. A similar system was isolated and characterized shortly afterwards by Hershko, Ciechanover and colleagues[18], who later resolved, characterized and purified its components — an acheivement that resulted in the discovery of the ubiquitin signalling system (see below).

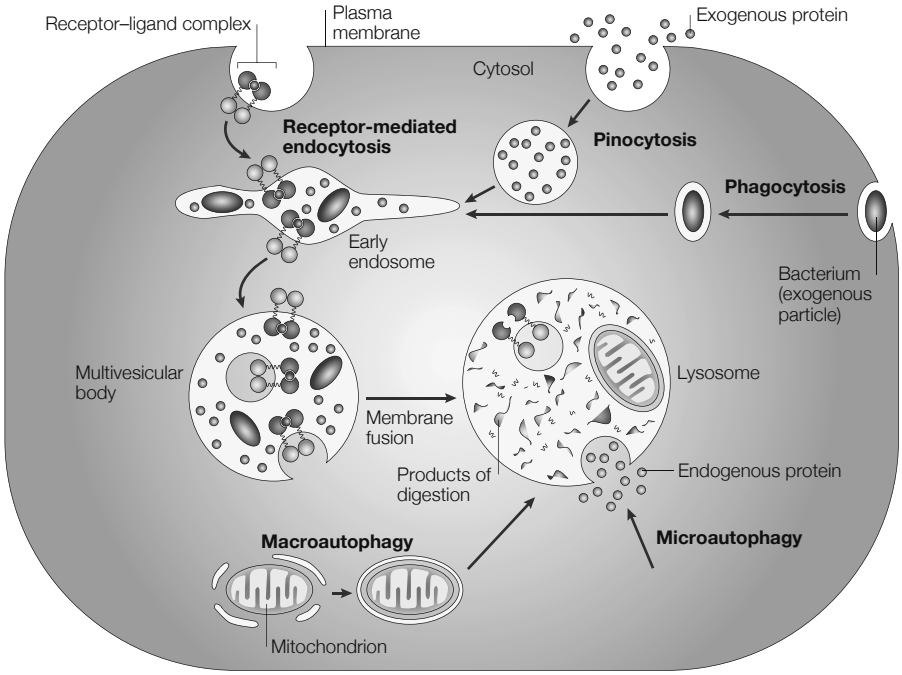
The lysosome and cellular proteolysis

As mentioned above, the functional mechanism (or mechanisms) of the lysosome could not be reconciled with several key emerging characteristics of intracellular protein degradation, such as the heterogeneous stability of individual proteins, the effect of nutrients and hormones on their degradation, the differential effect of selective inhibitors on the degradation of different classes of protein, and the dependence of intracellular proteolysis on metabolic energy.

The evolvement of methods to monitor protein kinetics in cells together with the development of specific and general lysosomal inhibitors resulted in the identification of different classes of cellular proteins (long- and short-lived), and the discovery of the differential effects of the inhibitors on these classes of proteins (see, for example, REFS 19,20). For example, Poole and his colleagues metabolically labelled endogenous proteins in living macrophages with ^{3}H-leucine and then 'fed' them with dead macrophages that had been previously labelled with ^{14}C-leucine. In this way, they were able to monitor, within one cell, the digestion of the same macrophage proteins that were presented to the cell from two different sources — from within the cell (^{3}H-labelled proteins) and from the extracellular milieu (^{14}C-labelled proteins). They followed the effect of lysosomotropic agents on the degradation of these two protein populations — specifically, they studied the effect of the weak bases chloroquine and ammonium chloride, which enter the lysosome and neutralize the H^+ ions, and the acid ionophore X537A, which dissipates the H^+ gradient across the lysosomal membrane. Treatment with these agents increases the intralysosomal pH, which results in the inhibition of the lysosomal proteases that function optimally at an acidic pH. They found that these drugs specifically inhibited the degradation of extracellular proteins, but not that of intracellular proteins[21]. Poole summarized these experiments by explicitly predicting the existence of a non-lysosomal proteolytic system that degrades intracellular proteins: "The exogenous proteins will be broken down in the lysosomes, while the endogenous proteins will be broken down wherever it is that endogenous proteins are broken down during protein turnover."[22]

The metabolic energy requirement for the degradation of both eukaryotic[5] and prokaryotic[23] proteins was difficult to understand. Proteolysis is an exergonic process and the thermodynamically paradoxical energy requirement for intracellular proteolysis made researchers believe that the energy could not be consumed directly by proteases or the proteolytic process *per se*, and therefore must be used indirectly. As Simpson summarized his findings[5]: "…the fact that protein hydrolysis as catalyzed by the familiar proteases and peptidases occurs exergonically, together with the consideration that autolysis in excised organs or tissue minces continues for weeks, long after phosphorylation or oxidation ceased, renders improbable the hypothesis of the direct energy dependence of the reactions leading to protein breakdown." With the discovery of lysosomes in eukaryotic cells, it could be argued, for example, that energy is required for the transport of substrates into the lysosome or for the maintenance of the low intralysosomal pH (see above). The observation by Hershko and Tomkins that the activity of tyrosine aminotransferase was stabilized following the depletion of ATP[24] indicated that energy might be required at an early stage of the proteolytic process, possibly before proteolysis occurs. However, it did not rule out, for

Box 1 | **The lysosome**



The lysosome (FIG. 1) **was first recognized biochemically in rat liver as a vacuolar structure that contains various hydrolytic enzymes, which function optimally at an acidic pH. It is surrounded by a membrane that endows the enclosed enzymes with a latency that is required to protect the cellular contents from their action[3,4,11,14]. The definition of the lysosome has been broadened over the years (see figure). This is because it has been recognized that the digestive process is dynamic and involves numerous stages of lysosomal maturation, together with the digestion of both exogenous proteins and particles, as well as the digestion of endogenous proteins and cellular organelles. Exogenous proteins are targeted to the lysosome through receptor-mediated endocytosis and pinocytosis, and exogenous particles are targeted by phagocytosis; these three processes are known as heterophagy. Endogenous proteins and cellular organelles are targeted by microautophagy and macroautophagy, respectively. The lysosomal/vacuolar system, as we now recognize it, is a heterogeneous, discontinuous digestive system that also includes structures that are mostly devoid of hydrolases — for example, early endosomes that contain endocytosed receptor–ligand complexes and pinocytosed/phagocytosed extracellular contents. At the other extreme, it includes the residual bodies — the end products of the completed digestive processes of heterophagy and autophagy. In between these extremes, there are: primary/nascent lysosomes that have not yet been engaged in any proteolytic process; early autophagic vacuoles that might contain intracellular organelles; intermediate/late endosomes and pinocytic/phagocytic vacuoles (heterophagic vacuoles) that contain extracellular contents/particles; and multivesicular bodies, which are the transition vacuoles between endosomes/heterophagic vacuoles and the digestive lysosomes.**

The figure shows the digestive processes that are mediated by the lysosome: specific receptor-mediated endocytosis; pinocytosis (the nonspecific engulfment of extracellular fluid); phagocytosis (the engulfment of extracellular particles); and autophagy (the engulfment of intracellular proteins (microautophagy) and organelles (macroautophagy)).

example, a possible role for the lysosome in the process, or a role for energy in another, non-proteolytic process that might lead to the inactivation of the enzyme. In bacteria, which lack lysosomes, the former argument could not have been proposed, but other indirect effects of ATP hydrolysis could have affected proteolysis in *E. coli*, such as the maintenance of the 'energized membrane state'. According to this model, proteins can become susceptible to proteolysis by changing their conformation, for example, following their association with cellular membranes that maintain a local, energy-dependent gradient of a certain ion. However, such an effect was ruled out[25], and it seemed that, at least in bacteria, energy is required directly for the proteolytic process, although the proteolytic machinery in prokaryotes had not been identified at that time. The metabolic energy requirement for protein degradation in both prokaryotes and eukaryotes indicated that energy is required directly for the proteolytic process, most probably for the regulation of it, and that a similar principle/mechanism must have been preserved during the evolution of the two kingdoms. The description of the cell-free proteolytic system in reticulocytes[17,18], which also lack lysosomes, further strengthened the idea that energy is probably directly required for the proteolytic process in eukaryotes as well, although, here too, the underlying mechanisms remained enigmatic at the time. However, the description of the cell-free system enabled the underlying mechanism (or mechanisms) to be unravelled.

The ubiquitin–proteasome system

The cell-free proteolytic system from reticulocytes[17,18] turned out to be an extremely important source for the purification and characterization of the enzymes that are involved in the ubiquitin–proteasome system (FIG. 2). Ciechanover and Hershko first found that fractionation of the crude reticulocyte cell extract on an anion-exchange resin yielded two fractions, I and II, which were both required to reconstitute the energy-dependent proteolytic activity that is found in the crude extract[26]. This was an important observation and a valuable lesson for the future dissection of the system, as it indicated that the system is not composed of a single 'classic' protease that evolved to acquire energy dependence, but that it has at least two components (although single proteases that require energy — the mammalian 26S proteasome (see below) and the prokaryotic *Lon* gene product — were discovered later). Learning from this discovery, the researchers reconstituted the activity using the resolved fractions whenever they encountered a loss of activity during further purification steps. This biochemical 'complementation' approach resulted in the discovery of further enzymes of the system, which are all required in the reaction mixture to catalyse the multistep proteolysis of a target substrate. The active component from fraction I was characterized and found to be a small, ~8.5-kDa heat-stable protein[26]. A plausible hypothesis was, for example, that the active component in fraction I could be an activator for a protease in fraction II. Extremely important findings that paved the way for future developments in the field were that several moieties of this heat-stable protein — which had been designated ATP-dependent proteolysis factor-1 (APF1) — are covalently conjugated to the target substrate when it is incubated in the presence of fraction II, and that this modification requires ATP[27,28].

The discovery that APF1 is covalently conjugated to protein substrates and stimulates their proteolysis in the presence of ATP and crude fraction II led to the proposal, in 1980, of a model in which protein-substrate modification by several moieties of APF1 targets it for degradation by a downstream, as-yet-unidentified protease that cannot recognize the unmodified substrate. In this model, reusable APF1 is released following protein-substrate degradation[28]. Amino-acid analysis of APF1, along with its known molecular mass and other general characteristics raised the suspicion that APF1 is ubiquitin[29], a known protein of previously unknown function (BOX 2). Indeed, Wilkinson and colleagues showed unequivocally that APF1 is indeed

ubiquitin[30]. This discovery, and the discovery that the mode of APF1 attachment to the substrate[31] is similar to the one that links ubiquitin to histone H2A (BOX 2), resolved the enigma of the energy requirement for intracellular proteolysis (see, however, below), and paved the way to understanding the complex mechanism of isopeptide-bond formation. This process turned out to be similar, in principle, to the mechanism of peptide-bond formation that is catalysed by tRNA synthetase following amino-acid activation during protein synthesis or during the non-ribosomal synthesis of short peptides[32]. Using the unravelled mechanism of ubiquitin activation and immobilized ubiquitin as a 'covalent' affinity bait, the three enzymes that are involved in the cascade reaction of ubiquitin conjugation were purified by Hershko, Ciechanover and colleagues. These enzymes are: enzyme-1 (E1), the ubiquitin-activating enzyme; E2, the ubiquitin carrier protein (ubiquitin-conjugating enzyme); and E3, the ubiquitin-protein ligase[33,34] (FIG. 2a). The discovery of an E3 enzyme, which is the specific substrate-binding component of the system, indicated a possible solution to the problem of specificity and the varying stabilities of different proteins — they might be specifically recognized and targeted by different ligases.

The ubiquitin-tagging hypothesis quickly received substantial support. For example, Chin and colleagues injected labelled ubiquitin and haemoglobin into HeLa cells and then denatured the injected haemoglobin by oxidizing it with phenylhydrazine. They found that ubiquitin conjugation to globin is markedly enhanced by the denaturation of haemoglobin, and that the concentration of globin–ubiquitin conjugates was proportional to the rate of haemoglobin degradation[35]. Hershko and colleagues observed a similar correlation for abnormal, short-lived proteins that contained amino-acid analogues[36]. A previously isolated mammalian cell-cycle-arrest mutant, which loses the ubiquitin–histone-H2A conjugate at the permissive temperature (BOX 2), was found by Finley, Ciechanover and Varshavsky to harbour a thermolabile E1 (REF. 37). Following heat inactivation, the cells fail to degrade normal short-lived proteins[38]. Although the cells did not provide direct evidence for substrate ubiquitylation as a destruction signal, this work nevertheless provided the strongest direct linkage between ubiquitin conjugation and protein degradation. In addition, because the work was carried out using a cell-cycle-arrest mutant, these observations enabled the researchers to predict the possible involvement of the ubiquitin system in controlling cell division — a hypothesis that later turned out to be correct.

At this point, the only missing link was the identification of the downstream protease that would specifically recognize ubiquitylated substrates. Tanaka and colleagues identified a second ATP-requiring step in the reticulocyte proteolytic system, which occurred after ubiquitin conjugation[39], and Hershko and colleagues showed that the energy is required for conjugate degradation[40]. An important advance in the field was a discovery by Hough and colleagues, who partially purified and characterized a high-molecular-mass alkaline protease that degraded ubiquitin conjugates of lysozyme, but not untagged lysozyme, in an ATP-dependent manner[41]. This protease, which later became known as the 26S proteasome (BOX 3), fitted all the necessary criteria for being the specific proteolytic arm of the ubiquitin system. This idea was confirmed, and the protease was further characterized by Waxman and colleagues, who found that it is an unusually large, ~1.5-MDa enzyme that is unlike any other known protease[42]. A further advance in the field was the finding[43] that a smaller, neutral, multisubunit 20S protease complex, which was discovered together with the larger 26S complex, is similar to a 'multi-

Box 2 | **Ubiquitin**

Ubiquitin is a small (76 residue), heat-stable and highly evolutionarily conserved protein. It was first purified during the isolation of thymopoietin[56] and was subsequently found to be ubiquitously expressed in cells from all kingdoms of life, including prokaryotes[57]. Interestingly, it was initially found to have lymphocyte-differentiating properties, a characteristic that was attributed to the stimulation of adenylate cyclase[58]. It was therefore named UBIP (ubiquitous 'immunopietic' polypeptide[57]). However, later studies showed that ubiquitin is not involved in the immune response[59], and that it was a contaminating endotoxin in the preparation that probably stimulated the adenylate cyclase and the T-cell-differentiating activity. Furthermore, the sequencing of several eubacterial and archaebacterial genomes, together with functional studies in these organisms, showed that ubiquitin is actually restricted only to eukaryotes. The identification of ubiquitin in bacteria[57] was probably due to contamination of the bacterial extract with yeast ubiquitin, which was derived from the yeast extract in which the bacteria were grown. Importantly, although the name ubiquitin is a misnomer because this protein is not as ubiquitous as was previously thought, it has remained the name of the protein for historical reasons.

An important breakthrough in the field of ubiquitin research was the discovery that a single ubiquitin moiety can be covalently conjugated to histones, particularly to histones H2A and H2B. The ubiquitin conjugate of H2A (which was designated protein A24) was characterized by Goldknopf and Busch[60,61] and by Hunt and Dayhoff[62], who found that the two proteins are linked through an isopeptide bond between the C-terminal glycine of ubiquitin (Gly76) and the ε-NH_2 group of an internal lysine (Lys119) of the histone. It should be noted that a bifurcated isopeptide bond between two different proteins that is generated post-translationally and is probably dynamic (that is, synthesized and hydrolysed) had not been described before. The isopeptide bond in this histone–ubiquitin conjugate is identical to the bond that was proposed for the linkage between ubiquitin and its target proteolytic substrates[31], and between the ubiquitin moieties in the polyubiquitin chain[63,64]. The polyubiquitin chain is synthesized on the substrate and functions as a recognition signal for proteolysis by the 26S proteasome (BOX 3). In this polyubiquitin chain, the linkage is between Gly76 of one ubiquitin moiety and the internal Lys48 of the previously conjugated ubiquitin moiety. The role of the monoubiquitin modification of histones in the regulation of transcription (for a recent review, see, for example, REF. 65) is unlike that of polyubiquitylation in proteolysis (FIG. 2; BOX 3; please also refer to the main text for further details).

catalytic proteinase complex' (MCP) that was described earlier by Wilk and Orlowski in bovine pituitary gland[44]. This 20S protease is ATP-independent and has several distinct catalytic activities, for example, it cleaves on the C-terminal side of hydrophobic, basic and acidic residues. Hough and colleagues raised the possibility — although they did not show it experimentally — that this 20S protease can be a part of the larger 26S protease that degrades the ubiquitin conjugates[43]. Later studies showed that, indeed, the 20S complex is the core catalytic particle of the larger 26S complex[45,46]. However, direct evidence that the active, double-'mushroom'-shaped 26S protease is generated through the assembly of two distinct subcomplexes — the catalytic 20S cylinder-like MCP and a further 19S ball-shaped subcomplex (that was predicted to have a regulatory role) — was provided by Hoffman and colleagues[47], who mixed the two purified particles to generate the active 26S enzyme (BOX 3).

Concluding remarks

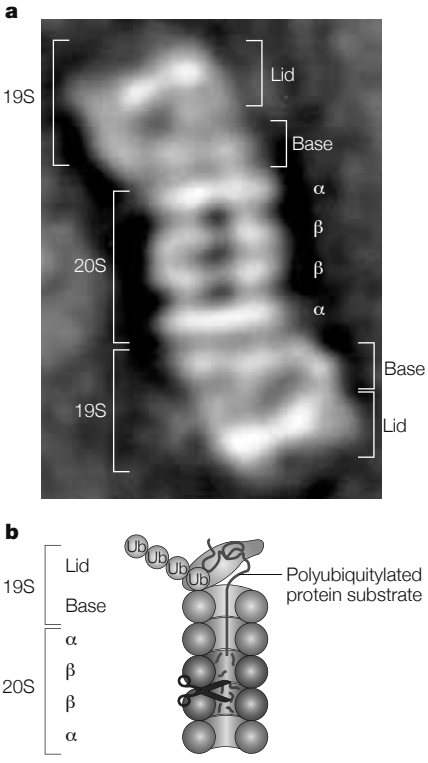
The emergence of proteolysis as a centrally important regulatory mechanism is a remarkable example of the evolution of a new biological concept and the accompanying battle to change paradigms. The journey between the early 1940s and early 1990s began with fierce discussions regarding whether proteins

are stable, as had been thought for a long time, or were turning over. The discovery of the dynamic state of proteins was followed by the discovery of the lysosome, which was believed — between the mid-1950s and mid-1970s — to be the organelle in which intracellular proteins are degraded. Independent lines of experimental evidence gradually eroded the 'lysosomal hypothesis' and led to it being substituted by a new hypothesis in which most intracellular proteins are degraded — under basal metabolic conditions — by a non-lysosomal machinery. This resulted in the discovery of the ubiquitin system in the late 1970s and early 1980s.

With the identification of the reactions and enzymes that are involved in the ubiquitin–proteasome cascade (FIG. 2a), a new era in the protein-degradation field began in the late 1980s and early 1990s. Studies began to show that the system is involved in targeting key regulatory proteins — such as light-regulated proteins in plants, transcription factors, cell-cycle regulators, tumour suppressors and promoters (see, for example, REFS 48–52). These studies were followed by numerous investigations into the mechanisms that underlie the degradation of specific proteins, with each having its own unique mode of recognition and regulation. The recent unravelling of the human genome highlighted the existence of hundreds of distinct E3 enzymes, which confirms the complexity, high specificity and selectivity of the system.

Two further important advances in the field were the discovery of non-proteolytic functions for ubiquitin, such as in the activation of transcription, and the discovery of ubiquitin-like proteins (FIG. 2). Some of the latter proteins function through the covalent modification of their targets, and are also involved in numerous non-proteolytic functions such as directing proteins to their subcellular destination (FIG. 2d) and protecting other proteins from ubiquitylation. Others have different functions in the ubiquitin signalling system that do not involve the covalent modification of target proteins. Further interesting discoveries include the finding that the ubiquitin-like protein Apg12 is essential for the function of the vacuole in autophagy in *Saccharomyces cerevisiae*[53] (later studies produced similar findings in mammals), and that ubiquitylation functions as a signal for sorting into endosomes[54] and the MVB pathway[55] (BOX 1). The latter sorting pathways are complex and involve the modification of both the cargo substrates and the components of the vesicular system. These discoveries closed an exciting historical cycle, linking together what were thought to be two distinct systems — lysosomal digestion and modification by ubiquitin and ubiquitin-like proteins. All these studies have led to the emerging realization that this mode of covalent conjugation has a key role in regulating — through both proteolytic and non-proteolytic mechanisms — a broad array of cellular processes. These include: the cell cycle and cell division; cell growth and differentiation; the activation and silencing of transcription; apoptosis; immune and inflammatory responses; signal transduction; receptor-mediated endocytosis and the sorting of proteins in the cell; various metabolic pathways; and protein quality control. As there are numerous substrates that need to be targeted and processes that have to be regulated, it has not been surprising to discover that aberrations in the system are implicated in the pathogenesis of many diseases, including several malignancies and neurodegenerative disorders. It seems that one important goal of the era that we are now entering will be to discover new drugs that target specific processes — for example, drugs that inhibit the aberrant E3(MDM2)-mediated targeting of the p53 tumour suppressor, which is observed in many malignancies.

Box 3 | **The 26 proteasome**



The proteasome is a large, 26S, multicatalytic protease that degrades polyubiquitylated proteins to produce small peptides (see figure). It is composed of two subcomplexes — a 20S core particle (CP) that carries the catalytic activity, and a 19S regulatory particle (RP). The 20S CP is a barrel-shaped structure that is composed of four stacked rings, two identical outer α-rings and two identical inner β-rings. The eukaryotic α- and β-rings are each composed of seven distinct subunits, which gives the 20S complex the general structure of $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$. The catalytic sites are localized to some of the β-subunits. One or both ends of the 20S barrel can be capped by a 19S RP that is composed of 17 distinct subunits — 9 in a 'base' subcomplex, and 8 in a 'lid' subcomplex. One important function of the 19S RP is to recognize polyubiquitylated proteins. Several ubiquitin-binding subunits of the 19S RP have been identified, but their biological roles and mode of action have not been discerned. A second function of the 19S RP is to open an orifice in the α-ring, which allows the substrate to enter the proteolytic chamber. In addition, as a folded protein cannot fit through the narrow proteasomal channel, it is thought that the 19S particle unfolds substrates and inserts them into the 20S CP. Both the channel-opening and the substrate-unfolding functions require metabolic energy and, indeed, the base of the 19S RP contains six different ATPase subunits. Following substrate degradation, short peptides that have been derived from the substrate are released, as is reusable ubiquitin. For a recent review on the proteasome, see REF. 66**.**

Part a of the figure shows an electron-microscopy image of a 26S proteasome from *Saccharomyces cerevisiae*, and part b shows a schematic representation of the structure and function of the 26S proteasome. Ub, ubiquitin. Part a was reproduced with permission from REF. 67 **© (1998) Elsevier. Part b was modified with permission from *Nature Reviews Molecular Cell Biology*** REF. 66 **© (2004) Macmillan Magazines Ltd.**

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Competing interests statement
The author declares no competing financial interests.

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A CONVERSATION WITH AARON CIECHANOVER

For decades, the attention of the scientific community was focused on the central dogma of biology — the decoding of the genetic information embedded in DNA. Little research was dedicated to how proteins are degraded and removed from cells. Enter onto the scene a young graduate student, Aaron Ciechanover, who with his mentor Avram Hershko, uncovered the complex and elegant ubiquitin proteolytic system. For his discovery, Ciechanover (Figure 1) shared in the 2004 Nobel Prize in Chemistry with Hershko and Irwin Rose. The complete interview, with more stories about being a member of the Pontifical Academy of Sciences, the magic of "A-ha" moments, and the conflict between religion and Darwinism, can be seen on the *JCI* website, http://www.jci.org/kiosk/cgm.

JCI: What was your childhood like?

Ciechanover: I was born in Israel in 1947, the year Israel became independent, to parents that had emigrated as children from Poland, escaping the rising anti-Semitism there. I grew up in a modest Jewish conservative home. My mother was an English teacher and my father a lawyer.

I remember that our home was cluttered with books. We had wall-to-wall, floor-to-ceiling libraries filled with Jewish and law books, but not so many on science, as I was the only one interested in science. My parents taught me to read and write early on. It was a scholarly environment, but very free. I have a brother who is 14 years older than I am, and I was lucky to have this age difference, because when my parents died during my childhood, he and my aunt kind of adopted me.

JCI: What kindled your interest in science?

Ciechanover: I cannot pinpoint it, by maybe it was the close vicinity to nature. I used to walk on the slopes of the Carmel Mountain just behind our home, collecting and drawing flowers, plants, lizards, skeletons — so it was an interest in what I would call today 'taxonomic' biology. When I was 11-years-old, my brother went abroad and I asked him to bring me a microscope — I still have it. My first tiny, small microscope! I started to do experiments: peeling onions and putting the thin layers under the microscope to see the cells, immersing them in pure and salted water to expand and shrink the cells. I remember piercing myself with a needle and smearing blood on a cover glass.

JCI: What led you to medical school?

Ciechanover: I was interested in the complexity of the human body and disease mechanisms.

Figure 1
Aaron Ciechanover on May 9, 2013. Image credit: Alena Soboleva.

I joined a program called the "Academic Reserve" where the Israeli military postpones the mandatory national service for students who study professions that are also in need for the Army, as medicine and engineering for example. The first years at the Hebrew University in Jerusalem were exciting because we studied basic sciences such as biochemis try, microbiology, and pathology.

But, when we approached the clinic, I started to feel restless — it was not what I wanted to do — and I decided to take a year off and carry out research in biochemistry; I fell in love with this discipline. I then completed my medical studies and my military service as a combat physician, including serving in the 1973 war. After being discharged, I landed safely in biochemistry.

JCI: It was after this that you first came to Avram Hershko's laboratory?

Ciechanover: Medical students have to submit a small research thesis in order to graduate. This I did with Avram Hershko, who had just returned from his postdoctoral training as a young assistant professor. We kept our ties during my military combat physician service after which I decided to do my PhD with him.

JCI: How did you come across the idea to study protein degradation?

Ciechanover: Avram had started to work on the subject when he was a fellow at UCSF. He had come across earlier — apparently thermodynamically paradoxical data — that protein degradation in both bacteria and mammalian cells require metabolic energy. Our dietary proteins provide us with energy, so it did not make sense to invest energy to degrade them to their low-energy amino acids building blocks. Yet, energy is required for proteolysis via the lysosome that was discovered by Christian de Duve, as the maintenance of its low acidic milieu requires pumping of hydrogen ions. There were, however, signs in the literature that it was not the lysosome that degrades most of intracellular proteins. We hung on those findings and glued them together, initiating a search for a non-lysosomal proteolytic system.

We started by establishing a cell-free system from reticulocytes that degrades a model protein. Shortly after, we realized we had a novel finding — the activity we discovered was not resolved as expected, as a single protease, but rather as two complementing inter-dependent activities. At that point in the summer of 1977, less than a year after I started my studies, Avram went on sabbatical to Philadelphia to work with Irwin [Ernie] Rose, and I stayed behind in Israel.

JCI: How did you hit on using red cells extracts?

Ciechanover: The reticulocyte is a great cell, because it doesn't have lysosomes; along its differentiation to the mature red blood cell, it extensively degrades all its machineries and proteins, leaving behind mostly hemoglobin. The problem was that hemoglobin comprises ~85% of all the cellular proteins, and it was difficult getting rid of it. One of our two complementing activities was resolving along with hemoglobin, but we could not isolate the culprit whatever we did. As a last resort, and while Avram was away, along with a colleague of mine, Michael Fry, we decided to literally boil the "red" extract. The hemoglobin precipitated like mud and the yellowish supernatant had all the activity. While we could not believe it was a protein, it was sensitive to proteases, had a high molecular weight, and was precipitable with ammonium sulfate. The two findings — the two complementing activities and the heat resistance of one of them — were critically important as they set our future research direction. These embarrassingly simple experiments were published in *BBRC* after they were rejected from *JBC*, teaching us an important lesson, particularly these days — it does not matter where you publish but what you publish.

JCI: Rose provided crucial input, given that he was coming at the problem from a different direction.

Ciechanover: During the summer of '79, on another visit to the Fox Chase Cancer Center, Ernie helped us to solve an important problem. Beforehand we had purified the active heat-stable polypeptide and realized that when incubated in the presence of ATP and the other active fraction, it generated high molecular weight complex with some other protein(s). We thought that the complex could result from a gentle associa-

tion — for example, a cryptic protease that is activated by our protein. With Ernie's advice, we found that ATP helps to catalyze a stable peptide bond between our protein and endogenous substrates in the crude extract, and hypothesized that this conjugation signals the tagged proteins for degradation. We called our first purified protein APF-1 (ATP-dependent proteolysis Factor-1). Other people at Fox Chase highlighted to us a protein with a similar molecular weight — ubiquitin — that is conjugated to histone H2A in an isopeptide bond. The similarity to APF-1 was striking, and along with Keith Wilkinson and Art Haas at Fox Chase, we found that APF-1 was indeed ubiquitin. This was an important discovery, as not only it did unravel the nature of the chemical bond between APF-1 and its target substrate, but it also explained the mechanism of action of ATP, solving the energy requirement mystery, and enabled the discovery of the conjugating machinery that is made of three enzymes: E1, E2, and E3, that act in concert.

JCI: What motivated you to do your post-doc with Harvey Lodish at MIT?

Ciechanover: Following graduation, though I could stay as a faculty member, I wanted to become independent. I wrote to Harvey who worked on the cleavage of the Poliovirus polyprotein.

Arriving at the laboratory, Harvey suggested I could work on receptor-mediated endocytosis. This was good advice: coming from pure biochemistry, I not only delved into a new field — that of cell biology and the problem of routing of proteins in cells — but, along with Alan Schwartz and Alice Dautry-Varsat, we discovered the cycle of the transferrin receptor and iron delivery into cells. It was an important experience, but then, I gradually slid back to work on ubiquitin.

JCI: Meaning that during that time you were also interacting with Alexander Varshavsky, who drew you back into ubiquitin research.

Ciechanover: Alex had discovered in the literature a temperature-sensitive cell cycle arrest mutant in which ubiquitinated histone H2A disappeared at the high temperature. We discussed and thought either the cells lost their ability to ubiquitinate the histone or gained the ability to rapidly deubiquitinate it. It made more sense that they lost their ability to ubiquitinate, as mutations typically result in a loss of function. Along with Daniel Finley, a graduate student in Alex's laboratory, we ended up discovering a mutation in E1 — the ubiquitin-activating enzyme, the first enzyme in the ubiquitination cascade. Needless to say, the cells were defective also in degradation of short-lived proteins. We were lucky, because if it had been a mutation in the histone E3, it would have taken a long time to discover it. Since it was a cell cycle arrest mutant, we speculated that the ubiquitin system is involved also in cell cycle regulation, which later turned out to be correct. The description of the E1 mutation was another corroboration of our earlier findings that ubiquitination signals proteins for degradation.

I went on to study the requirement for tRNA in the proteolytic process, which I discovered when I was still with Avram, but never pursued. This turned out later to be part of the N-end rule, discovered and stud ied independently by Alex. Basically, in the second half of my post-doctoral fellowship, I became a 'freelancer' in Harvey's laborato ry. He did not know much about ubiquitin, but was gracious to learn it and help me. I owe a lot to the independence that Harvey gave me and to the openness I enjoyed in his laboratory. I remember fondly his advice when I hesitated about returning to Israel. He was all for it, where he knew my family will be most comfortable. He argued that even under less favorable conditions it is mostly the scientist's quality and drive that would be detrimental to his/her success.

JCI: You decided to go back to the same institute as Hershko in Israel.

Ciechanover: To the same institute, yes, but I was completely independent. I brought my own projects and started an independent career, completely away from Avram, though being close and having the ability to discuss problems clearly helped.

JCI: Does ubiquitin still motivate you, 35 years later?

Ciechanover: More than ever! We haven't even begun to understand the complexity of the system. It has almost 2,000 components, approximately 7% of the human genome. The ubiquitin system plays a major role in clearing defective/misfolded proteins. Besides quality control, it removes in a programmed manner important cellular proteins like cell cycle regulators and transcription factors. Importantly, there are drugs already on the market to treat aberrations in the system and now more diseases — inflammatory disorders, neurodegenerative diseases and malignancies — are being tied to defects in the system.

JCI: You've said you did your science because of scientific curiosity, not to win prizes. But surely, sharing in the Nobel Prize with Hershko and Rose must have been fairly sweet.

Ciechanover: You celebrate for one day, and then you celebrate the second day, and the third day you have to decide what you are going to do with yourself. I decided to do two things: first, to continue my research; being in the laboratory is so exciting now, with smart students and fellows, and sophisticated technologies. Second, to leverage my 'status' to highlight two major issues. One is education of children in Israel and worldwide. I talk to them at eye level and they see, wow — it is possible to make a major achievement and still remain a regular human being. We need children falling in love with science at an early age.

The recognition also enabled me to trace my Jewish heritage. I try to build close relations with Jewish communities, mostly small and remote ones. For example, there is a tiny Jewish community in Greece left after the extermination during the holocaust, or a tiny one in Paraguay made mostly of holocaust survivors. I speak in different community activities — sermons in synagogues, for example — and it is fascinating, as I feel I blow wind in their sails. I feel an amazing sense of belonging.

JCI: If you were not a scientist, what do you think you would have done?

Ciechanover: The natural track would have taken me to surgery, and I believe to cardiac or neurosurgery. There is something mystical about these two organs where emotion and reason reside. But the fact that the discovery of the ubiquitin system led to the unraveling of related diseases and development of drugs is kind of a cycle closure for me.

Ushma S. Neill

□ □ □

THE UBIQUITIN–PROTEASOME PATHWAY: ON PROTEIN DEATH AND CELL LIFE

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• ArticleFigures & DataTransparent Process
• E1E2E3PathogenesisProteasomeProtein DegradationUbiquitin

Introduction

The discovery of the ubiquitin pathway and its many substrates and functions has revolutionized our concept of intracellular protein degradation. From an unregulated, non-specific terminal scavenger process, it has become clear that proteolysis of cellular proteins is a highly complex, temporally controlled and tightly regulated process which plays important roles in a broad array of basic cellular processes. It is carried out by a complex cascade of enzymes and displays a high degree of specificity towards its numerous substrates. Among these are cell cycle and growth regulators, components of signal transduction pathways, enzymes of house keeping and cell-specific metabolic pathways, and mutated or post-translationally damaged proteins. The system is also involved in processing major histocompatibility complex (MHC) class I antigens. For many years it has been thought that activity of the system is limited to the cytosol and probably to the nucleus. However, recent experimental evidence has demonstrated that membrane-anchored and even secretory pathway-compartmentalized proteins are also targeted by the system. These proteins must be first translocated in a retrograde manner into the cytosol, as components of the pathway have not been identified in the endoplasmic reticulum (ER) lumen. With the multiple cellular targets, it is not surprising that the system is involved in the regulation of many basic cellular processes such as cell cycle and division, differentiation and development, the response to stress and extracellular modulators, morphogenesis of neuronal networks, modulation of cell surface receptors, ion channels and the secretory pathway, DNA repair, regulation of the immune and inflammatory responses, biogenesis of organelles and apoptosis. One would also predict that aberrations in such a complex system may be implicated in the pathogenesis of many diseases, both inherited and acquired. Recent evidence shows that this is indeed the case.

Degradation of a protein by the ubiquitin system involves two distinct and successive steps: (i) covalent attachment of multiple ubiquitin molecules to the target protein (Figure 1A); and (ii) degradation of the tagged protein by the 26S proteasome (Figure 1B) or, in certain cases, by the lysosomes/vacuole. Conjugation of ubiquitin to the substrate proceeds via a three-step mechanism. Initially, ubiquitin is activated in its C-terminal Gly by the ubiquitin-activating enzyme, E1. Following activation, one of several E2 enzymes (ubiquitin-carrier proteins or ubiquitin-conjugating enzymes, UBCs) transfers ubiquitin from E1 to a member of the ubiquitin-protein ligase family, E3, to which the substrate protein is specifically bound. This enzyme catalyzes the last step in the conjugation process, covalent attachment of ubiquitin to the substrate. The first moiety is transferred to an -NH2 group of a Lys residue of the protein substrate to generate an isopeptide bond. The first moiety can be also conjugated in a linear manner to the N-terminal residue of the substrate (Breitschopf *et al*., 1998). In successive reactions, a polyubiquitin chain is synthesized by transfer of additional ubiquitin moieties to Lys48 of the previously conjugated molecule. The chain serves, most probably, as a recognition marker for the protease. The structure of the system appears to be hierarchical (Figure 2): a single E1 activates ubiquitin required for all modifications. It can transfer ubiquitin to several species of E2 enzymes, and each E2 acts with either one or several E3s. Only a few E3s have been identified so far, but it appears that these enzymes belong to a large and rapidly growing family of proteins. A major, as yet unresolved problem involves the mechanisms that underlie the high specificity and selectivity of the system. Why are certain proteins extremely stable while others are exceedingly short-lived? Why are certain proteins degraded at a particular time point in the cell cycle or only following specific extracellular stimuli, while they are stable under all other physiological conditions? It appears that specificity is determined by two distinct groups of proteins. Within the ubiquitin system, substrates are recognized by the different E3s. Some proteins are recognized via primary signals and bind directly to E3s. However, many proteins must undergo post-translational modification such as phosphorylation, or associate with ancillary proteins such as molecular chaperones prior to recognition by the appropriate ligase (for modes of substrate recognition, see Figure 3). Thus, the modifying enzymes and ancillary proteins also play an important role in the recognition process. As for the E3s, except for a few cases, it is not likely that each substrate is targeted by a single ligase; rather, it is conceivable that a single E3 recognizes a subset of similar, but clearly not identical, structural motifs.

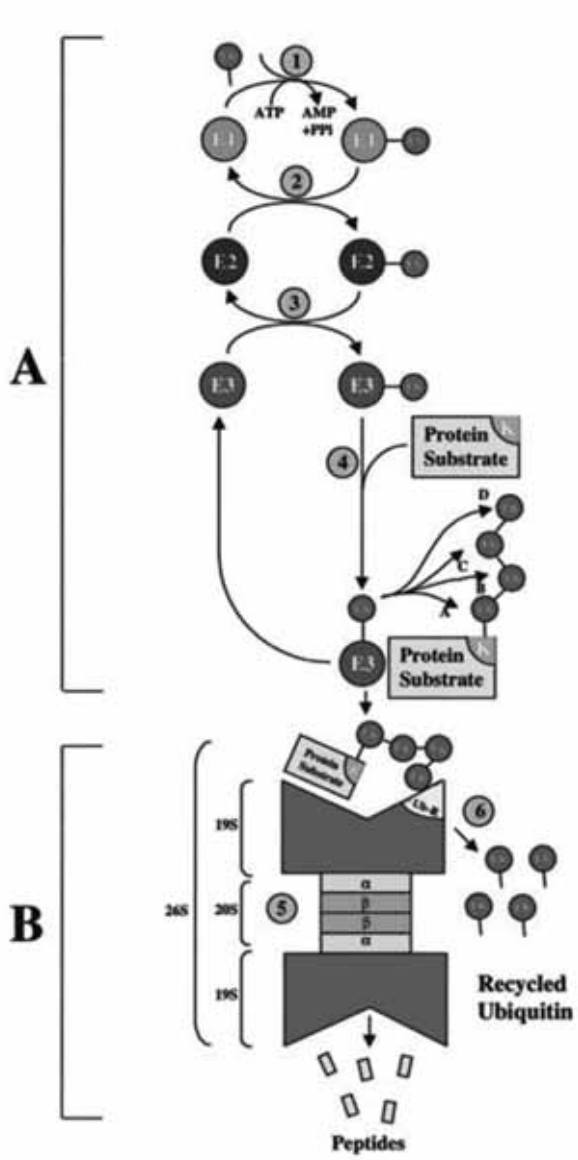

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Figure 1.

The ubiquitin–proteasome pathway. (A) conjugation of ubiquitin to the target molecule. (B) Degradation of the tagged substrate by the 26S proteasome. (1) Activation of ubiquitin by E1. (2) Transfer of activated ubiquitin from E1 to a member of the E2 family. (3) Transfer of activated ubiquitin from E2 to a substrate-specific E3. (4) Formation of a substrate–E3 complex and biosynthesis of a substrate-anchored polyubiquitin chain. (5) Binding of the polyubiquitinated substrate to the ubiquitin receptor subunit in the 19S complex of the 26S proteasome and degradation of the substrate to short peptides by the 20S complex. (6) Recycling of ubiquitin via the action of isopeptidases.

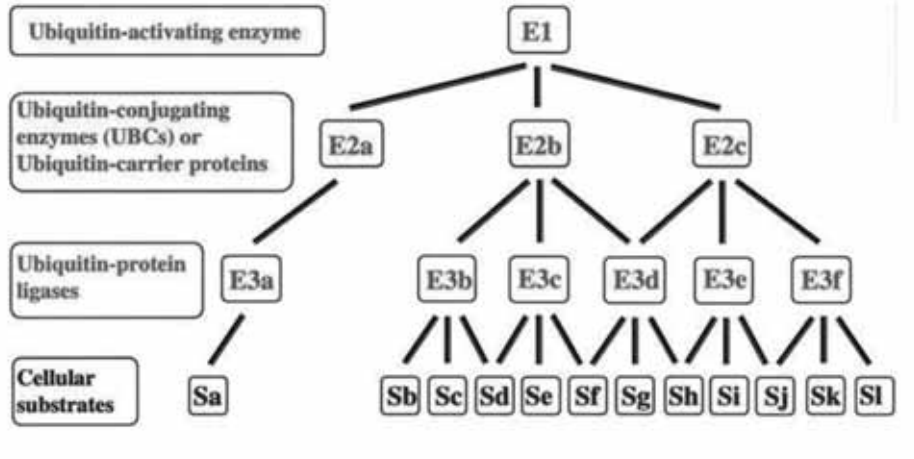

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Figure 2.

Hierarchical structure of the ubiquitin-conjugating machinery. A single E1 catalyzes activation of ubiquitin and transfers it to several E2 enzymes. In most cases, an E2 transfers ubiquitin to several E3s, while in a few cases the E2 is E3-specific. E3s can be substrate-specific or can recognize several substrates via similar, but not identical motifs. Certain substrates can be targeted by several E3s, probably via distinct recognition motifs.

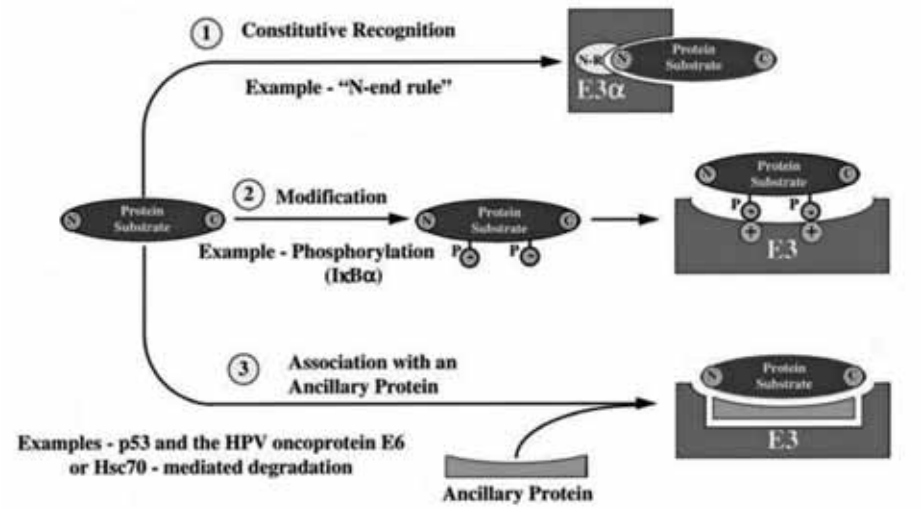

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Figure 3.

Modes of recognition of protein substrates by different E3s. An E3 can recognize a substrate constitutively via a primary motif such as the N-terminal residue (N-end rule). Many proteins are recognized following post-translational modification (e.g. phosphorylation) or association with an ancillary protein (e.g. Hsc or HPV-E6). N-R, N-terminal receptor.

The exponential increase of information on the ubiquitin system has made it impossible to describe all the important advances in the field in a single review, however comprehensive. Many recent review articles and monographs have described different aspects of the pathway (see, for example, Coux *et al*., 1996; Hochstrasser, 1996; Baumeister *et al*., 1998; Hershko and Ciechanover, 1998; Peters *et al*., 1998). Here, I shall summarize for the novice reader the enzymes and mechanisms involved in ubiquitin-mediated proteolysis and describe some recent advances in the pathophysiology of the system.

The ubiquitin system cascade
Ubiquitin-conjugating machinery

E1. This enzyme generates a high-energy thiolester intermediate with ubiquitin that involves an internal Cys residue.

E2s, *UBCs*. The activated ubiquitin is transferred from E1 to a Cys residue of an E2 enzyme, thereby generating yet another thiolester intermediate. The genome of *Saccharomyces cerevisiae* encodes for 13 E2s and E2-like proteins, and many more have been described in mammals. Some E2s are involved in specific cellular processes while the role of others is still obscure. However, they all act via their function as UBCs: all are inactivated by mutation of the active Cys residue. The yeast UBC2/RAD6 is involved in degradation of 'N-end rule' substrates and also in DNA repair. The mechanism that underlies this activity is still obscure. UBC3/CDC34 is required for G1–S transition, probably via degradation of certain cell-cycle regulators, while UBC4 and UBC5 are involved in the degradation of many short-lived, normal and abnormal proteins. E2-C acts along with the cyclosome/anaphase promoting complex (APC) in the degradation of some cell-cycle regulators. *Drosophila* UBCD1 is involved, probably via degradation of some telomere-associated proteins, in proper detachment of telomeres during mitosis and meiosis. The *Drosophila bendless* gene encodes an E2 enzyme required for the formation of synaptic networks. HRB6B, one of the two mouse homologs of the yeast UBC2/RAD6, is involved in degradation of histones occurring during spermatogenesis (see below). Disruption of *UBCM4*, a mouse homolog of yeast UBC4/UBC5, causes embryonic lethality which is probably due to impairment in the development of the placenta (reviewed in Hershko and Ciechanover, 1998). Other E2s are membrane-associated and may be involved in degradation of abnormal or virus-targeted ER proteins (see below). One membrane-associated E2 contains a baculovirus inhibitor of apoptosis repeat, suggesting that these enzymes are structurally and functionally more diverse, and play a role in more than one process (Hauser *et al*., 1998).

Due to the specific effects of certain E2s on defined processes, it has been proposed that they can interact directly with the substrate protein. While such interactions have been described using protein–protein interaction screening methods, their physiological significance is not clear. Most probably, the specific functions of E2s are due to their association with distinct E3s (reviewed in Hershko and Ciechanover, 1998).

E3s. An E3 enzyme is defined as a protein that binds the target substrates, either directly or indirectly, via ancillary proteins, and catalyzes transfer of ubiquitin from a thiolester intermediate on E2 or E3 to an amide linkage with the substrate or with a polyubiquitin chain already anchored to it. Since the target proteins bind to the ligases prior to conjugation, E3s are key players in determining the high specificity of the system. Despite their importance, the number of known E3s is few and the information concerning their mode of action is rarer. Lack of sequence homology among different E3s and the frequent association of these enzymes with multisubunit complexes in which the identity of the ligase subunit is not known, render their study difficult.

The four families of E3 enzymes that have been described so far are as follows. (i) The main N-end rule E3, E3α, and its yeast counterpart UBR1, contain two distinct sites that recognize either basic (Type I) or bulky-hydrophobic (Type II) N-terminal residues of their substrates. However, they also recognize non-N-end rule substrates such as N-α-acetylated proteins that bind via a yet uncharacterized 'body' site. E3β is a related enzyme that binds proteins with small uncharged N-termini. Although the N-end rule recognition mechanism is highly conserved, its cellular substrates and physiological roles are still obscure (reviewed in Varshavsky, 1996). (ii) A second group is the HECT (homologous to E6-AP C-terminus) domain family. One member of the family, E6-AP (E6-associated protein) is required, along with the human papillomavirus (HPV) E6 oncoprotein, for the ubiquitination and degradation of p53 (Scheffner *et al*., 1993). The enzyme recognizes p53 in a *trans* manner following formation of a ternary complex with E6 that recognizes both the ligase and the tumor suppressor. A large family of proteins that contain a HECT-domain has been identified in many eukaryotes (Huibregtse *et al*., 1995). The C-terminal domain that contains the ubiquitin-binding Cys residue is highly conserved, whereas the N-terminal region of the various HECT proteins is variable and is probably involved in specific substrate recognition. Members of the family are involved in the targeting of specific proteins. For example, yeast RSP5 conjugates the large subunit of RNA polymerase II (Huibregtse *et al*., 1997) and also the FUR4 uracil permease which is targeted to the vacuole following ubiquitination (Galan *et al*., 1996). NEDD4 targets the kidney epithelial sodium channel (ENaC; Staub *et al*., 1997; see below). (iii) A third type of ligase is the ~1500 kDa cyclosome (Sudakin *et al*., 1995) or anaphase promoting complex (APC; King *et al*., 1995). This complex has a ubiquitin ligase activity specific for cell-cycle regulators, such as mitotic cyclins, certain anaphase inhibitors and spindle-associated proteins, that contain a nine amino acid motif designated the 'destruction box' (see below) and are degraded during mitosis. The

complex is inactive during interphase. At the end of mitosis it is activated by phosphorylation mediated by the cyclin-B/cyclin-dependent kinase (CDK)1 complex MPF (M-phase promoting factor; Lahav-Baratz *et al*., 1995). The *Xenopus* complex has eight subunits, three of which are homologous to *S.cerevisiae* CDC16, CDC23 and CDC27, which are required for exit from mitosis and for the degradation of B-type cyclins. A fourth subunit is homologous to *Aspergillus* BimE which is essential for completion of mitosis. The subunit of the cyclosome involved in its ubiquitin ligase function has not been identified. (iv) A different type of multi-subunit ubiquitin ligase is involved in the degradation of certain other cell-cycle regulators, such as the SIC1 CDK inhibitor or certain G1 cyclins. Here, phosphorylation of the substrate converts it to a form susceptible to the action of the ligase complex. Several such complexes, designated Skp1-cullin-F-box protein ligase complexes (SCFs), have been described that share some common subunits, but also contain distinct subunits specific for certain substrates. Thus, the degradation of the CDK inhibitor SIC1, a process essential for G1→S transition in yeast, requires its phosphorylation by a G1 cyclin-activated kinase as well as the products of *CDC34*, *CDC53*, *CDC4* and *SKP1* genes. CDC34 is an E2, but the role of the other proteins is not known. CDC34, CDC53 and CDC4 generate a complex that is responsible for ubiquitination of phosphorylated SIC1 (Feldman *et al*., 1997; Skowyra *et al*., 1997). Ubiquitination and degradation of the yeast G1 cyclin CLN2, also requires its phosphorylation and the action of CDC34, CDC53, GRR1 and SKP1, but not of CDC4. Both CDC4 and GRR1 contain a motif called the F-box that is present in a variety of proteins that bind to SKP1. It was proposed that SKP1 is a component of SCF complexes that binds to specific 'adaptor' proteins such as CDC4 and GRR1, which in turn bind to specific protein substrates such as phosphorylated SIC1 and CLN2 (reviewed in Hershko and Ciechanover, 1998). Components of the SCF complexes are highly conserved during evolution and have been identified in organisms ranging from *Caenorhabditis elegans* to human. They are designated cullins, and their existence suggests that similar complexes may be involved in the degradation of a variety of regulators in higher organisms.

Structural motifs that target proteins for ubiquitination

The numerous substrates of the pathway are recognized by the different ligases via specific motifs. These can be either primary, or secondary, post-translational modifications. Primary motifs do not necessarily lead to constitutive degradation of the proteins that contain them. They can be hidden and exposed only following misfolding or dissociation of subunits. For example, masking

of a degradation signal by heterodimerization blocks the proteolysis of the MATα2·MATa1 heterodimeric yeast transcription factor (Johnson *et al*., 1998). Binding to its specific DNA promoter blocks the degradation of MyoD (Abu Hatoum *et al*., 1998). Certain substrates will not be recognized by their ligases unless they associate with an ancillary protein or molecular chaperone that act as *trans* recognition elements.

The best studied primary signal is the N-terminal residue (N-end rule; Varshavsky, 1996). Association with ancillary proteins such as viral oncoproteins or molecular chaperones has been shown to accelerate the degradation of certain substrates (reviewed in Hershko and Ciechanover, 1998). As for secondary motifs, recent evidence indicates that many proteins are targeted by phosphorylation. The yeast G1 cyclin CLN3 (Yaglom *et al*., 1995) and the GCN4 transcriptional activator (Kornitzer *et al*., 1994) are degraded following phosphorylation at a PEST sequence. The mammalian G1 cyclin D1 is targeted for degradation following phosphorylation on a Thr residue that does not reside within a PEST [Pro(P), Glu(E), Ser(S), Thr(T)] sequence (Diehl *et al*., 1997). Phosphorylation of Ser32 and Ser36 targets IκBα (Chen *et al*., 1995). Here, the phosphorylation site constitutes the ligase-binding site (Yaron *et al*., 1997). Degradation of β-catenin is also mediated by phosphorylation at Ser37 which resides in a region similar to the targeting domain of IκBα (Rubinfeld *et al*., 1997; see below). Phosphorylation at Ser3 of c-Mos (Nishizawa *et al*., 1992) or multiple phosphorylations of c-Jun (Musti *et al*., 1997) suppress their ubiquitination and degradation. Ligand binding to Ste2p, the G protein-coupled membrane receptor of the α factor, leads to phosphorylation of Ser residues that reside on a well defined internalization signal, SINNDAKSS. Phosphorylation signals ubiquitination which is required for internalization of the ligand–receptor complex (Hicke *et al*., 1998).

An important degradation signal, the 'destruction box', was discovered in mitotic cyclins and certain other cell-cycle regulators. It is a nine-amino-acid motif, usually located ~40–50 amino acid residues from the N-terminus. It has the following general structure: R1(A/T)2(A)3L4(G)5X6(I/V)7(G/T)8(N)9. Amino acid residues shown in brackets occur in most known destruction sequences. R1 and L4 are indispensable. Cyclosome-mediated ubiquitination of destruction box-containing proteins is an example of a limited set of proteins that perform related functions, share a common targeting signal and are recognized by a common E3. The mechanistic role of the destruction box is not known. It does not serve as a phosphorylation or ubiquitination site but may serve as a 'docking' domain for the E3 subunit of the cyclosome.

In most cases, the Lys residues that serve as ubiquitination sites are not specific or part of the recognition motif. In the case of IκBα, however, Lys21 and 22 are indispensable (Scherer *et al*., 1995), although they are not part of the E3-binding site (Yaron *et al*., 1997).

Conjugation of ubiquitin to cell-surface membrane proteins, such as the growth hormone receptor (Strous *et al*., 1996), leads to their targeting to the lysosome. The mechanism(s) and signals that underlie this unique trafficking are not known. In the case of certain membrane proteins, formation of the poly-ubiquitin chain can proceed via Lys63 (Galan and Haguenauer-Tsapis, 1997), while in others monoubiquitination appears to be sufficient for targeting (Terrel *et al*., 1998).

Ubiquitin conjugates degrading enzymes

The 20S and 26S proteasomes. The 26S proteasome is composed of the 20S core catalytic complex flanked on both sides by the 19S regulatory complexes. With one known exception (ornithine decarboxylase which is proteolyzed following association with its inhibitor antizyme but without prior ubiquitination) the 26S complex recognizes specifically ubiquitin-tagged proteins.

An important advance in studies of the 26S complex has been the resolution of the crystal structure of the yeast 20S proteasome at 2.4 Å (Groll *et al*., 1997). The complex is arranged as a stack of four rings, two α and two β, organized in the general structure of αββα. Both α and β rings are composed of seven distinct subunits. Thus, the general structure of the complex is α1–7β1–7β1–7α1–7. The three catalytic sites: the trypsin-, chymotrypsin- and post-glutamyl peptidyl hydrolytic-like sites, reside on some of the β subunits, and are generated topologically by obliquely adjacent pairs of identical β subunits residing in different β rings. The crystal structure has also shown that the catalytically inactive α chains play an essential role in stabilizing the two-ring structure of the β chains. They also play a role in the binding of the 19S 'cap' regulatory complexes.

An important, as yet unresolved, problem involves the entry of substrates and exit of proteolysis products from the proteasome. In the *Thermoplasma* proteasome, there are two entry pores at the ends of the cylinder. These pores do not exist in the yeast 20S proteasome: the N-terminal domains of the α subunits protrude towards each other and fill the space. Entry from the ends may

be possible only following substantial ATP-dependent rearrangement that may occur following association with the 19S complex. The yeast complex displays narrow side orifices at the interface between the α and β rings. These openings lead directly to the active sites. They can potentially rearrange to generate entry apertures for unfolded/extended substrates.

Substrate recognition by the 26S proteasome is probably mediated by the interaction of specific subunits of the 19S complex with the polyubiquitin chain. Polyubiquitin-binding subunits have been described in human (S5a), yeast (RPN10; MCB1) and plants (MBP1). Surprisingly, Δ*mcb1* yeast mutant does not display any growth defect and degrades normally the vast majority of ubiquitinated proteins. The mutant also displays a slight sensitivity to stress (van Nocker *et al*., 1996). It is possible that ubiquitinated proteins are recognized by an additional, at present undefined proteasomal subunit.

An additional complex that associates with the 20S proteasome is PA28 (REG or 11S; Song *et al*., 1997). Unlike assembly of the 19S–20S–19S, complex formation with PA28 is ATP-independent, the PA28–20S–PA28 complex digests only peptides but not ubiquitin-conjugated intact proteins. The activator is a ring-shaped hexamer composed of alternating α and β subunits. Both subunits are induced by IFN-γ, suggesting a role for the particle in antigen processing. Indeed, overexpression of PA28α in cell lines that also express viral protein antigens results in an enhanced presentation of peptides derived from these proteins (Groettrup *et al*., 1996). Since the PA28–20S–PA28 proteasome cannot digest intact proteins, it must act downstream to the 26S proteasome. It can act in trimming large peptides that were generated by the 26S complex to the precise epitopes recognized by the class I MHC complex and T-cell receptors. The existence of a single, asymmetrical 19S–20S–PA28 proteasome has been reported (Hendil *et al*., 1998). Such a complex has the potential to carry out, in a consecutive manner, the two-step proteolytic processes, initial proteolysis to large peptides and final trimming to the antigenic peptides.

An important development involves the discovery of proteasome inhibitors which have become powerful research tools in probing the structure and function of the proteasome and the ubiquitin pathway. The first inhibitors were derivatives of the calpain inhibitors I [N-Acetyl-Leu-Leu-Norleucinal (ALLN)] and II [N-Acetyl-Leu-Leu-Methioninal (ALLM)]. These inhibitors block degradation of the bulk of cellular proteins, short- and long-lived alike (Rock *et al*., 1994), suggesting that the vast majority of cellular proteins are targeted by the system. While these inhibitors are quite specific, they also inhibit calpains. In

contrast, the *Streptomyces* metabolite lactacystin appears to be highly specific (Fenteany *et al*., 1995).

De-ubiquitinating enzymes. An important step in the ubiquitin pathway involves the release of ubiquitin from its various adducts. Release of ubiquitin plays an essential role in two processes, the first of which is protein degradation. During degradation, it is important to release ubiquitin from Lys residues of end proteolytic products, to disassemble polyubiquitin chains and to 'proofread' mistakenly ubiquitinated proteins. The second process is ubiquitin biosynthesis. Ubiquitin is synthesized in a variety of functionally distinct forms. One of them is a linear, head-to-tail polyubiquitin precursor. Release of the free molecules involves specific enzymatic cleavage between the fused residues. The last ubiquitin moiety in many of these precursors is encoded with an extra C-terminal residue that has to be removed in order to expose the active C-terminal Gly. In a different precursor, ubiquitin is synthesized as an N-terminal fused extension of two ribosomal proteins and serves as a covalent 'chaperone' that targets them to the ribosome. Following their incorporation into the ribosomal complex, ubiquitin is cleaved.

In general, the recycling enzymes are thiol proteases that recognize the C-terminal domain/residue of ubiquitin (reviewed in Hochstrasser, 1996; Wilkinson, 1997). They are divided into two classes: ubiquitin C-terminal hydrolases (UCHs) and ubiquitin-specific proteases (UBPs; isopeptidases). UCHs are ~25 kDa enzymes that are involved in co-translational processing of pro-ubiquitin gene products and in the release of ubiquitin from adducts with small molecules, such as amines and thiol groups. UBPs are ~100 kDa enzymes that catalyze release of ubiquitin from conjugates with cellular proteins or from free polyubiquitin chains. A large number of UBPs are encoded by the yeast genome and higher eukaryotes, suggesting that some of them may have specific functions, such as recognition of distinct tagged substrates. In accordance with the broad spectrum of their functions, they also differ in their characteristics. Some are free, while others are subunits or associated with the 19S complex. Some require ATP for their activity while others act in an energy-independent manner. Their mechanisms of action also differ, as some are sensitive to ubiquitin aldehyde, while others are not. De-ubiquitinating enzymes can either accelerate proteolysis or inhibit it. By removing ubiquitin moieties from mistakenly tagged proteins, they inhibit proteolysis. Stimulation of proteolysis can be mediated by release of free ubiquitin from biosynthetic precursors and terminal proteolytic products and restoring cellular ubiquitin pool, or by release of ubiquitin from polyubiquitin chains that bind to the 26 proteasome and inhibit it,

or by 'editing' polyubiquitin chains and 'fitting' them better for recognition by the 26S proteasome.

Recent experimental evidence indicates that some of these enzymes play an essential role in specific processes and must therefore target specific substrates. The *Drosophila melanogaster FAT FACETS* (*FAF*) gene affects eye development (Huang *et al*., 1995). Mutant *FAF* flies have more than eight photoreceptors in each of the compound eye units. The protein is probably involved in generating the inhibitory signal sent by the photoreceptor cells to undifferentiated surrounding cells, to stop differentiation and migration to the facet unit. Due to the fact that inactivation of *FAF* can be suppressed by another mutation in a proteasome subunit, it appears that the enzyme stabilizes some unidentified protein(s). A specific serotonin-inducible UCH has been implicated in activation of cAMP-dependent protein kinase A (PKA) in *Aplysia* via stimulation of the degradation of the inhibitory regulatory subunit of the enzyme (Hegde *et al*., 1997). Degradation is initiated by cAMP that leads to dissociation of the holoenzyme and release of free R subunits. PKA-dependent phosphorylation of a variety of proteins in sensory neurons is responsible for a broad array of morphological changes in the synapse that produce the continuous presynaptic facilitation necessary for long-term behavioral sensitization. UBP3 has been implicated in gene silencing (Moazed and Johnson, 1996). Actively transcribed genes can be silenced following positioning near heterochromatic regions. SIR4 is one *trans*-acting factor that is required for the establishment/maintenance of silencing. One identified SIR4-interacting protein is UBP3, an inhibitor of silencing that acts by either stabilizing an inhibitor or by removing a positive regulator.

Ubiquitin-like proteins

The high evolutionary conservation of ubiquitin enabled the discovery of many ubiquitin-related proteins. Some, such as Parkin, which is implicated in the pathogenesis of certain forms of Parkinson's disease (Kitada *et al*., 1998), are larger than ubiquitin and possess ubiquitin-like domains that display only slight homology to ubiquitin; they lack the C-terminal Gly and cannot be conjugated. Their physiological significance has remained obscure. A second group contains small proteins with a higher degree of homology to ubiquitin that are involved in post-translational, single or multiple modification of target proteins that serves non-proteolytic purposes (reviewed in Hochstrasser, 1998).

UCRP is an interferon-inducible 15 kDa protein that resembles two tandem repeats of ubiquitin and may be involved in targeting proteins to the cytoskeleton (Loeb and Haas, 1994). Small ubiquitin-related modifier-1 (SUMO-1) is an 11.5 kDa polypeptide involved in targeting RanGAP1 to the nuclear pore complex (NPC) protein RanBP2 (Mahajan *et al*., 1997). RanBP2 is a GTPase required for the transport of proteins and ribonucleoproteins across the NPC. Its guanosine 5′-triphosphate/diphosphate (GTP/GDP) cycle is regulated by RanGAP1. Localization of RanGAP1 to the NPC is dependent on its single, stable, covalent modification by SUMO-1. The SUMO-1–RanGAP1 conjugate generates a complex with RanBP2 that is essential for the function of RanBP2. SUMO-1 modification of IκBα stabilizes the protein and inhibits NF-κB activation (Desterro *et al*., 1998). Here, SUMO-1 acts antagonistically to ubiquitin by generating a degradation-resistant protein. SUMO-1 is identical to Sentrin involved in protecting cells against anti-FAS/TNFα-induced apoptosis and, like ubiquitin, can generate multiply modified conjugates with cellular proteins (Kamitani *et al*., 1997a). NEDD8 is a mammalian protein that is developmentally downregulated and is expressed in high levels in post-mitotic cells characterized by high protein turnover rate, such as skeletal and heart muscle (Kamitani *et al*., 1997b). RUB1 is a yeast ubiquitin-like protein that was found to modify CDC53/Cullin (Liakopoulos *et al*., 1998), a common subunit of the SCF ubiquitin ligase complex (see above). While the modification of CDC53 does not affect its stability, it may influence the activity of SCF or its specificity towards its different substrates. Agp12 is another yeast ubiquitin-like protein. Its single, Agp7 (E1)- and Agp10 (E2)-mediated conjugation to Agp5 is essential for autophagy (Mizushima *et al*., 1998). Conjugation of the ubiquitin-like proteins raises several questions related to the chemical nature of the adduct, the identity of the conjugating enzyme(s) and the specificity of substrate targeting. The C-terminal domain of SUMO-1 is processed proteolytically at residue 97 (TGG97-H98STV) to generate a free -G96G97-COOH that, like the C-terminal Gly76 of ubiquitin, is essential for conjugation. Similarly, RUB1, SMT3 and NEDD8 are also processed to yield a free C-terminal -Gly-Gly. Activation of SMT3 requires at least three proteins: AOS1, UBA2 and UBC9. AOS1 and UBA2 are homologous to the N-terminal and C-terminal domains of E1, respectively, and are probably heterodimerizing to generate an active E1 (Johnson *et al*., 1997). UBC9 can serve as the E2 in the modification reaction (Schwarz *et al*., 1998). Conjugation of RUB1 requires ULA1/UBA3 that serve as a heterodimeric E1, and UBC12 as an E2 (Liakopoulos *et al*., 1998). While conjugation of the known ubiquitin-related proteins does not require E3, it is not clear that this is the case for all of these modifications. The requirement for E3s probably depends on the breadth of spectrum of substrates, and the functions of each of the modifying proteins.

Involvement of the ubiquitin system in the pathogenesis of diseases

Considering the broad range of substrates and processes in which the ubiquitin pathway is involved, it is not surprising that aberrations in the system have been implicated in the pathogenesis of several diseases, both inherited and acquired. The pathological states can be divided into two groups: (i) those that result from loss of function, a mutation in an enzyme or substrate that leads to stabilization of certain proteins; and (ii) those that result from a gain of function, resulting in accelerated degradation.

Malignancies

It has been noted that the level of p53 is extremely low in uterine cervical carcinomas caused by high-risk strains of HPV. It has been shown that the suppressor is targeted for degradation by E6-AP following formation of a ternary complex with E6-16 or 18, members of the high-risk family of HPV E6 oncoproteins. E6s derived from low-risk strains do not associate with E6-AP and do not destabilize p53 (Scheffner *et al*., 1993; see above). The strong correlation between sensitivity of different genetic polymorphic isotypes of p53 to E6-mediated degradation and the prevalence of cervical carcinoma in women, further corroborates the direct linkage between targeting of p53 and malignant transformation. p53-Arg72 is significantly more susceptible to E6 targeting than p53-Pro72. Accordingly, individuals homozygous for the Arg72 allele are 7-fold more susceptible to HPV-associated tumors than heterozygotes (Storey *et al*., 1998). Removal of the suppressor by the oncoprotein appears to be a major mechanism utilized by the virus to transform cells. In another case it was shown that c-Jun, but not its transforming counterpart v-Jun, can be ubiquitinated and rapidly degraded. It has been shown that the δ domain of c-Jun, a 27 amino acid sequence that is missing in the retrovirus-derived molecule, destabilizes the protein (Treier *et al*., 1994). This domain is not ubiquitinated but may serve as an anchoring site for the specific E3. The lack of the δ domain from v-Jun, a protein that is otherwise highly homologous to c-Jun, provides a mechanistic explanation for its stability, and possibly for its transforming activity. This is also an example of the complex mechanisms evolved by viruses to ensure continuity of replication and infection. An interesting correlation was found between low levels of p27, the G1 CDK inhibitor whose degradation is essential for G1→S transition, and aggressive colorectal (Loda *et al*., 1997) and breast carcinomas (Catzvaelos *et al*., 1997). This low level is due to specific activation of the ubiquitin system, as the p27 found in these tumors is the wild type. The strong correlation between the low level of p27 and the

aggressiveness of the tumor makes p27 a powerful prognostic tool for survival. Another interesting example involves β-catenin, which plays a major role in signal transduction and differentiation of the colorectal epithelium, and possibly in the multi-step development of the highly prevalent colorectal tumors. In the absence of signaling, glycogen synthase kinase-3 (GSK-3) is active and, via phosphorylation of a specific Ser residue, targets β-catenin for degradation (Aberle *et al*., 1997; Rubinfeld *et al*., 1997). Stimulation promotes dephosphorylation, stabilization and subsequent activation of β-catenin via complex formation with otherwise inactive subunits of transcription regulators such as lymphocyte enhancer factor (LEF) and T-cell factor (TCF). In the cell, β-catenin generates a complex with other proteins, including the tumor suppressor adenomatous polyposis coli (APC). The complex may be analogous to the ligase complexes cyclosome/APC and SCF (see above); here too, the identity of the ligase subunit is unknown.

Genetic diseases

Cystic fibrosis (CF). The CF gene encodes the CF transmembrane regulator (CFTR), which is a chloride ion channel. Only a small fraction of the wild-type protein matures to the cell surface; most of the protein is degraded from the ER by the ubiquitin system (Ward *et al*., 1995). The most frequent mutation in CFTR is ΔF508. Despite normal ion channel function, CFTRΔF508 does not reach the cell surface at all and is retained in the ER, from which it is degraded. It is possible that the rapid and efficient degradation results in complete lack of cell surface expression of the ΔF508 protein, and contributes to the pathogenesis of the disease.

Angelman's syndrome. This is a rare inherited disorder characterized by mental retardation, seizures, frequent out-of-context laughter and abnormal gait. The syndrome is an example of genomic imprinting and the deleted chromosomal segment is always maternal in origin. The affected protein is the E3 enzyme E6-AP (Kishino *et al*., 1997). While the target substrate of E6-AP has not been identified, elucidation of the defect clearly demonstrates an important role for the ubiquitin system in human brain development. It also shows that E6-AP has a native cellular substrate(s) targeted in the absence of E6.

Liddle syndrome. This is an hereditary form of hypertension that results from deletion of a proline rich (PY) region in the β and γ subunits of the heterotrimeric (αβγ) amiloride-sensitive ENaC. The HECT domain E3 NEDD4 binds

to the PY motif of ENaC via its WW domain. ENaC is short-lived *in vivo*, and its α and γ chains were shown to ubiquitinated (Staub *et al*., 1997). Mutations affecting recognition of the channel result in its stabilization, excessive reabsorption of sodium and water, and the subsequent development of hypertension.

Immune and inflammatory responses

Two interesting examples involve an interaction between the ubiquitin pathway and viruses, where the viruses exploit the system to escape immune surveillance. The Epstein–Barr nuclear antigen 1 (EBNA-1) protein persists in healthy carriers for life and is the only viral protein regularly detected in all EBV-associated malignancies. Unlike EBNAs-2, 3 and 4, which are strong immunogens, EBNA-1 cannot elicit a cytotoxic T lymphocyte (CTL) response. The persistence of EBNA-1 contributes, most probably, to some of the virus-related pathologies. A long C-terminal Gly-Ala repeat was found to inhibit degradation of EBNA-1 by the ubiquitin system (Levitskaya *et al*., 1997). Thus, the GA repeat constitutes a *cis*-acting element that inhibits processing and subsequent presentation of the resulting epitopes. A second example involves the human cytomegalovirus (CMV) that encodes two ER resident proteins, US2 and US11. These proteins target MHC class I heavy-chain molecules for degradation. The MHC molecules are normally synthesized on ER-bound ribosomes and transported to the ER. In cells expressing US2 or US11, the MHC molecules are transported in a retrograde manner back to the cytoplasm, deglycosylated and degraded by the proteasome following ubiquitination (Wiertz *et al*., 1996). The viral products bind to the MHC molecules and escort them to the translocation machinery, where they are transported back into the cytoplasm. The virus-mediated destruction of the MHC molecules does not allow presentation of viral antigenic peptides, thus enabling the virus to evade the immune system.

Neurodegenerative diseases

Ubiquitin immunohistochemistry has revealed enrichment in conjugates in senile plaques, lysosomes, endosomes, and a variety of inclusion bodies and degenerative fibers in many neurodegenerative diseases such as Alzheimer's (AD), Parkinson's and Lewy body diseases, amyotrophic lateral sclerosis (ALS) and Creutzfeld–Jakob disease (CJD) (reviewed in Mayer *et al*., 1996). However, from these morphological studies it is impossible to conclude what

pathogenetic role the ubiquitin system plays in these pathologies. While there can be a cell-specific defect in one of the enzymes of the system, it is more likely that an alteration in one of the protein substrates, either inherited or acquired, renders it resistant to proteolysis. Accumulation of the substrate(s) and/or of the resulting conjugates in aggregates and inclusion bodies may be toxic to the cell. Lack of animal models for most of these diseases and their long periods of development make any mechanistic approach to the problem difficult.

An interesting case involves the proteasome-mediated degradation of the cleaved, C-terminal fragment of presenilin 2 (PS2; Kim *et al*., 1997). PS2 is a transmembrane protein that is probably involved in trafficking\processing of proteins between different cellular compartments. It is implicated in the transport of the amyloid precursor protein (APP) and its processing to amyloid β42. Mutations in PS2 and in its homologous protein, PS1, are responsible for the majority (>50%) of cases of early onset AD. One mutation, N141I, is prevalent in the Volga-German type of familial AD. For normal functioning, PS2 is first cleaved and the C-terminal domain is degraded. The N-terminal domain probably constitutes the active form of the molecule. Proteasome inhibitors lead to accumulation of polyubiquitinated PS2, and also to accumulation of the C-terminal fragment. Introduction of the Volga-German mutation to wild-type presenilin leads to a dramatic decrease in the rate of processing of PS2, similar to that observed in proteasome inhibitor-treated cells. Thus, it appears that a defect in the processing (and possible subsequent activation) of PS2 may play a role in the pathogenesis of this form of AD. In a different example, a frameshift mutation in the ubiquitin-B gene was identified in a patient with the more prevalent nonfamilial late-onset form of AD (van Leeuwen *et al*., 1998). While it is clear that the mutation plays an important role in the pathogenesis of the disease, it is possible that a primary, so far unidentified event leads to formation of abnormal protein(s), and the lack of a functional ubiquitin system leads to their accumulation and the resulting pathology.

In Huntington disease and spinocerebellar ataxias, the affected genes, *HUNTINGTIN* and *ATAXINS*, encode proteins with various lengths of CAG/polyglutamine repeats. Recent studies have shown that these proteins aggregate in ubiquitin- and proteasome-positive intranuclear inclusion bodies (Davies *et al*., 1997; Cummings *et al*., 1998). It is possible that these abnormal proteins cannot be removed by the system, and their aggregation and precipitation play a role in cell toxicity and subsequent pathologies.

Ubiquitin and muscle wasting

Skeletal muscle wasting, which occurs in various pathological states such as fasting, starvation, sepsis and denervation, results from accelerated ubiquitin-mediated proteolysis (reviewed in Mitch and Goldberg, 1996). The extracellular stimuli and signaling pathways that activate the ubiquitin system in response to the different pathological states are still obscure.

Diseases associated with animal models

Two interesting pathological states have been described in mouse models which may also have implications for human diseases. Inactivation of HR6B, an E2 involved in DNA repair and in targeting of the N-end rule pathway and other protein substrates, leads to the single defect of male sterility due to defects in spermatogenesis. The target substrate proteins may be histones, as their degradation is critical for postmeiotic chromatin remodeling which occurs during spermatogenesis (Roest *et al*., 1996). Another interesting case is that of the *Itch* locus which encodes a novel E3 enzyme. Defects in the locus result in a variety of syndromes that affect the immune system. Some animals develop inflammatory disease of the large intestine. Others develop a fatal disease characterized by pulmonary interstitial inflammation, alveolar proteinosis, inflammation of the stomach and skin glands that results in severe itching and scarring, and hyperplasia of the lymphoid and hematopoietic cells (Perry *et al*., 1998). The target protein(s) of the Itch E3 is not known.

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□ □ □

EXCMO. SR. DR. JOSEP MARIA GIL VERNET VILA

Discurso de presentación

Dr. Pedro Clarós
Académico de Número de la Real Academia Europea de Doctores.

Excelentísimo Señor Presidente de la Real Academia Europea de Doctores.
Excelentísimos y Magníficos Sres. Rectores.
Excelentísimas y Excelentísimos Académicos.
Excelentísimas Autoridades.
Profesor Dr. José Maria Gil-Vernet.
Señoras y Señores.

Quiero dar las gracias al Presidente de la Real Academia Europea de Doctores, por permitirme hacer el discurso de presentación para la recepción pública del Excelentísimo Doctor José María Gil-Vernet, una figura relevante de la medicina española, como Académico de Honor en esta Corporación.

Mis palabras van a constar de dos apartados preceptivos: la exposición de su curriculum y algunas consideraciones sobre la repercusión de su trabajo en la medicina mundial.

CURRICULUM VITAE

Sócrates afirmaba que **"una vida examinada era la única que merecía la pena ser vivida",** pues para trabajar bien, con excelencia, hay que saber lo que se quiere, cómo se debe de hacer, programarse para ello, establecer prioridades y, al mismo tiempo, estar impregnado de un sentido moral, el llamado **"Exemplus de los clásicos".** Pues bien Señores, todo eso está en el currículo de nuestro nuevo Académico de Honor. Verdaderamente él tiene la Autoritas y Potestas de los clásicos, adornada con todos los adelantos de nuestra civilización.

Para mí es muy difícil, tremendamente difícil, sintetizar toda una trayectoria científica de una gran extensión y calidad, como la del Profesor Gil-Vernet. No sé si voy a ser capaz de mencionar sus méritos más importantes. Espero que él perdone que, forzosamente, haya dejado numerosos aspectos, no ya por comentar, sino, ni siquiera citar**.**

Aspectos personales

Nació en Barcelona el 9 de diciembre de 1922, en el seno de una familia de saga de médicos catalanes. Se casó con Olga Sedó y tuvieron tres hijos, José María y Alfredo (excelentes médicos Urólogos) y Carlos (Economista).

Es un excelente deportista y fue en dos ocasiones campeón de España de tiro al plato, en 1946 y 1947. Su afición continúa aún hoy a sus 94 años, junto con la pesca deportiva.

La relación de su familia con las nuestras, Clarós-Doménech, Doménech-Clarós y Clarós es de una gran amistad y profesionalidad durante años, por lo que les ruego me disculpen si hablo con gran entusiasmo y afecto, pero, les aseguro que no me podrán negar que lo hago con justicia y tino hacia sus múltiples méritos.

Títulos Académicos

Licenciado en Medicina y Cirugía por la Universidad de Barcelona en 1947, y Doctor en Medicina y Cirugía por la Universidad de Madrid, con la Tesis Doctoral **"Implantación de uréteres en el intestino"** en 1951, con la calificación de sobresaliente "cum laude".

En 1956 se especializó en Urología en la Cátedra de Urología de su padre, el prestigioso Profesor Dr. Don Salvador Gil-Vernet, y en 1972 fue nombrado Catedrático Extraordinario de Urología de la Universidad de Barcelona. En 1973, Director de la Escuela Profesional de Urología de la Universidad de Barcelona. Profesor Emérito de la Universidad de Barcelona en 1988. Y de 1994 a 2001 Profesor Docente de la Universidad Autónoma de Barcelona.

Nuestro Académico ha recorrido todas las etapas universitarias, desde Profesor ayudante de clases prácticas, Profesor Adjunto, Profesor Agregado, Profesor Titular y Catedrático de la Universidad de Barcelona y por último culminó su carrera Universitaria como Vicerrector de la Universidad de Barcelona de 1973 a 1980. En este peregrinar en cargos universitarios, ha impartido docencia en casi todo el mundo.

Actividades Docentes.

El buen docente no es el que sabe mucho, sino el que es capaz de trasmitir ese saber, rebasando los límites de la propia Universidad y volcándose en todos los ambientes.

Si Julio César decía que su vida estaba marcada por su voluntad, es evidente que el Prof. Gil-Vernet ha ejercido e incluso ejercitado de una forma férrea la de su propia vida. Me atrevería a decir que él puede con todo, pues su constancia en el trabajo le ha llevado a conseguir todo lo que se ha propuesto en la vida.

Es **Visiting Profesor** por las Universidades de: Columbia (New York) 1961-1967, Johannesburgo 1965, Salysbury (Rodesia) 1965, Rochester 1966, Missouri 1966, Medical Center (California) 1967, Medical Center (New York) 1967, Sao Paulo 1968, Bucarest 1969, Berlín 1973, Chicago Country Hospital 1978, Moscou 1978, Londres Medical School 1979, Bruselas 1985, Nigata (Japon) 1986, Paris 1991, entre otras. Ha realizado 308 demostraciones quirúrgicas en Hospitales Universitarios extranjeros.

Director de la Unidad de Trasplante Renal 1964, Premio Nacional de Cirugía 1967. Presidente Nacional de la Especialidad de Urología, 1972, y un sinfín más.

Ha dirigido 24 Tesis Doctorales en sus años de actividad docente.

Como curiosidad añadiré que el Ex-Presidente de la Casa Blanca, Lyndon B. Johnson, escribió, en el año 1962, siendo Vice-Presidente, y en el año 1965, como Presidente de los Estados Unidos, cartas al Profesor Gil-Vernet, que tengo en mis manos, pidiéndole que aceptara a médicos americanos para formarles en cirugía urológica.

Actividades Profesionales

En su larga y fértil trayectoria practicó en España el primer trasplante renal con éxito, en Barcelona, el 23 de julio de 1965, junto con el internista Dr. Antonio Caralps, venciendo todas las dificultades de aquellos momentos, que él conoce perfectamente.

Realizó en 1983 el primer trasplante de páncreas en España, así mismo en 1978, en Barcelona, fue el autor del primer trasplante de testículo en el mundo. Como pionero en la cirugía de los trasplantes de órganos sólidos, enseñó a sus discípulos y al mundo entero como hacerlo y además con éxito.

Ha realizado más de 90 films científicos, recibiendo diferentes premios internacionales, entre ellos, el primer Oscar científico "The Golden Eagle" de EEUU. Ha dictado 367 comunicaciones-ponencias en Congresos Nacionales e Internacionales, escrito 182 publicaciones, 21 capítulos en libros extranjeros y 2 tratados de Urología. Fue el director de 10 Cursos internacionales de Urología, 2 de Trasplantes Renales. Las sesiones operatorias se retransmitieron por televisión en directo y en color en doble pantalla panorámica desde el Hospital Clínico al Palacio de Congresos de Montjuic, donde acudieron más de 2000 médicos extranjeros y españoles.

La habilidad quirúrgica del Prof. Gil-Vernet fue reconocida en todos los ambientes. Se decía que sus dedos llegaban allí donde no alcanzaban los demás cirujanos.

Actividades de Investigación

En sus trabajos de investigación, contó con las becas concedidas por la Fundación March de 1974-1976, beca del Fondo de Investigación Sanitaria, Beca del Ministerio de Educación y Ciencia, Beca del Fondo de Investigación Sanitaria de la Seguridad Social, y por último Beca del Ministerio de Educación y Ciencia (Comisión Asesora de Investigación Científica y Técnica).

Como resultado de sus investigaciones científicas iniciadas en el año 1952 y hasta 1998, ha descrito 52 nuevas intervenciones quirúrgicas que han sido reconocidas y aplicadas universalmente. Todas sus investigaciones han sido realizadas en España.

Yo creo que se necesita tiempo, serenidad y perspectiva, circunstancias que conducen a la inteligencia, al poso y al sedimento. Pero también se necesita tener la mente amplia y clara, el espíritu abierto y ser capaz de salir de las propias fronteras, no sólo geográficas, sino con capacidad de captación. El nuevo Académico de Honor ha salido al exterior para trasmitir y enseñar.

Uno de los proverbios de Publio Terencio dice **"Quot homines, tot sententiae"** (tantos hombres, tantas opiniones). Este dicho, que es válido para todos los intelectuales, es la base del debate científico y, por supuesto, la esencia de las Academias. Yo, por ello, estoy seguro que su participación y colaboración con la nuestra será muy fructífera.

Principales Publicaciones Originales.

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-Uretroplastie perineoescrotale. Technique II .Enc. Med. Chir. Paris. 41320-4.1.07, 1967

-Human renal homotransplantations. New surgical technique. Urol. Int., 23: 201-223, 1968

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-New surgical technique for balanic hypospadias correction. J. Urol., 112:673, 1974.

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-New approach to the splenic vessels. J. Urol., 119: 313-315, 1978

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-Advances in intraoperative renal radiography 3-Dimensional radiography of the kidney. J. urol., 125:614-619. 1981.

-Renal Autotrasplantation. Eur. Urol., 8. 61-73, 1982.

-New surgical approach to complicated renal anomalies. J. Urol., 128.10-17, 1982.

-A new technique for surgical correction of vesicoureteral reflux. J. Ural. 131:456-458, 1984

-Whole organ and pancreaticoureterostomy in clinical pancreas transplant. Transplant Proceedings. 17: 2019-2022, 1985

-Orthotopic renal Transplant. New surgical technique in 139 consecutive cases. J. Uro1.142: 248-252, 1989.

-New surgical approach for treatment of vesico-vaginal fistula. J. Urol. 141:513-516,1989

-Cirugía renal en hipotermia. En: Tratado de Urología. Jimenez Cruz, J.F. y Rioja Sanz, L., Ed. Prous, J.R. Barcelona 1993

-Prostate cancer. Anatomical and surgical considerations. British J. Urol., 78.161-168, 1996

-A new biaxial epilated scrotal flap for reconstructive urethral surgery. (Technique III). J. Urol. 158: 412, 1997

-Prótesis hidráulica para regular la tensión del "sling" en el tratamiento de la incontinencia urinaria en la mujer. Patent number. U.S.A., 6,117,067. Arch. Esp. Urol., 8.871-874, 1999

-Total anterior urethral reconstruction with the "BAES flap" in a spinal cord-injured patient. Spinal Cord, 39,290, 2001

Instituciones a las que pertenece

El Prof. Gil-Vernet ha representado y representa la esencia del buen hacer y la dedicación intelectual. Es por lo que ha sido nombrado Académico de Honor de prestigiosas Academias del mundo entero como:

Miembro de honor de la Sociedad Española de Cirujanos, 1967. Profesor Honorario de la Universidad de Córdoba (Argentina) 1972. Miembro de Honor de La Sociedad Francesa de Trasplantes. Académico de la Real Academia de Medicina de Cataluña, 1977. Académico de Número de la Real Academia Nacional de Medicina (sillón número 14). Miembro de Honor de New York American Academy of Urology. Miembro de Honor de la Academia de Medicina de la URSS, 1978. Miembro de 26 Asociaciones Extranjeras de Urología. Miembro de Honor de la Sociedad de Cirugía Laparoscópica 2002, entre otras muchas y en el día de hoy por la Real Academia Europea de Doctores.

Otros Méritos

Como en los apartados anteriores, podría citar toda otra larga serie de ellos, pero no quiero alargar demasiado el tiempo de mi exposición. Por ello, simplemente diré que es Miembro de Honor de muchas Sociedades Médicas nacionales e internacionales.

Gran Cruz de la Orden Civil de Sanidad, por su Majestad el Rey Juan Carlos I de España. Doctor Honoris Causa por la Universidad Rey Juan Carlos (Móstoles) Madrid. (2008)

Múltiples condecoraciones, entre ellas la de Comendattore dell´Ordine al Mérito, por el Presidente de la Republica Italiana en 1974, Medalla de Oro de la Sociedad Catalana de trasplantes, Honorary President of America Urological Association, Concesión por la Société Internationale d' Urologie del premio "**Distinguished Career Award**" (Shanghai, China, November 2009).

El último de ellos ha sido, **The European Association of Urology Award**, de "Innovación en Urología" dado en Munich en marzo 2016, en reconocimiento a la importancia de sus innovaciones quirúrgicas en el tratamiento de las enfermedades urológicas.

Propuesto para el Premio Príncipe de Asturias en 1999 y para el Premio Nobel de Medicina. Todo lo expuesto es parte de los méritos que nos han decidido proponerle y aceptarle en esta Real Corporación, en el día de hoy, como Académico de Honor, junto a otros distinguidos miembros de la Comunidad Europea. Objetivos de nuestra Real Academia, que tiene un amplio espíritu de integración internacional de los valores personales más relevantes.

Ahora voy a hacer un breve comentario sobre:

"El Trasplante de órganos. Su repercusión en cirugía urológica."

El trasplante de órganos nació en la mitad del siglo pasado, en un ambiente de gran escepticismo y conflictividad, pero que hoy continúa hacia un futuro aún más esplendoroso, si cabe, y cada día se torna presente salvando muchas vidas.

Los resultados del Dr. Murray en Boston en 1954 con el primer trasplante mundial de riñón, entre dos hermanos mellizos, los del Dr. Hamburger en el Hospital Necker de Paris, llevaron al equipo de Barcelona del Dr. Gil-Vernet a profundizar en esta materia.

Pero aquellos esfuerzos constituyeron el banco de pruebas tanto quirúrgico, médico o inmunológico, que encaminó a la medicina en España al trasplante de otros órganos humanos como el de corazón, por el Dr. José María Caralps, el de hígado, por el Dr. Margarit, y el de pulmón, por el Dr. Julio Astudillo (1990).

La estrategia quirúrgica que se aplicó en aquel entonces ha sido la base de los tratamientos complejos que hoy se están haciendo de rutina y que ya no entenderíamos que no existieran. La vida ha cambiado completamente en medio siglo. Los enfermos condenados a morir por fallos en sus órganos vitales hoy tienen una gran oportunidad de vivir, después de ser sentenciados a muerte por la enfermedad. Y los muertos sanos, fallecidos por causas accidentales, pueden tener el orgullo de donar sus órganos y así dar vida a sus semejantes. La esperanza a salvar la vida y la generosidad de los que la dan se mezclan y combinan en una bella y bonita cooperación entre los seres humanos.

Una de las causas de mi admiración por el recipiendario es el gran respeto y cariño que le tenía y sigue teniendo a su padre Don Salvador. En sus comentarios y en sus escritos no falta la constante cita de lo que, su maestro, le enseñó. Este reconocimiento a su padre, le engrandece y dignifica como persona.

Así mismo al agradecimiento a sus colaboradores como el Dr. Antonio Caralps, eminente internista y nefrólogo, y el inmunólogo Dr. José Vives, se unen los de todo su equipo médico que ha estado a la cabeza del ranking internacional de los trasplantes.

Y acabo, Excelentísimo Señor Presidente, diciendo que: **El destino de cada hombre y de la misma historia es semejante al de Moisés, que aunque no llegó a ver la tierra prometida, no por eso dejó de caminar, no dejó de buscar, pues sabía lo que quería.**

Creo que la vida de nuestro nuevo Académico de Honor es una fiel copia de una frase que leí del Premio Nobel de literatura, el portugués José de Sousa Saramago, hace ya mucho tiempo que decía **"aunque posiblemente nunca encuentre nada, mientras pueda, seguiré buscando".**

Señores Académicos, el Nuevo Académico de Honor ha buscado y ha encontrado el rigor científico en la investigación y la docencia. Ahora La Real Academia Europea de Doctores le ha encontrado a él. Enhorabuena a los dos.

Permítanme hacer un breve resumen en inglés para los invitados extranjeros.

SUMMARY IN ENGLISH

Out of respect for the New Academics who will be joining us today as Honorary Members of the Royal European Academy of Doctors, I wish to summarize in English Prof. Gil-Vernet's career.

Mr. José María Gil-Vernet, is a Medical Doctor, PhD and Professor at the University of Barcelona. He is the first surgeon in Spain to transplant, with success, a kidney in 1965. Later on, in 1983 he was the first to transplant a pancreas organ, and in 1978, a testicle. These advanced surgeries changed the prognosis of a lot of patients. Nowadays , these procedures seem common and easy, but to reach this point, a lot of investigations and experimental studies have been necessary, and therefore he has a lot of merits.

He is a scientist who understood than surgery was based on Anatomy and when applying this concept, as well as his great expertise as a surgeon, attained his success.

In his intensive research it is worth highlighting the studies and work on intestinal grafts in urology, and new surgical approach techniques in the retroperitoneal zone, to reach the splenic vessels, among others. He has discovered and described more that 45 surgical techniques.

His most important international publications have been published in the Journal of Urology, as well as a large number of books on Urology.

Among his many awards it is important to mention those as Honorary Professor of many Academies and Societies around the world.

Now, at the age of 94 and with a clear mind, he is receiving this recognition from the Royal European Academy of Doctors as a token of appreciation for his scientific studies from all the members of this Royal Institution.

Welcome Professor José María Gil-Vernet to our Royal European Academy of Doctors in a ceremony that we organize to receive other important Academics.

With my compliments.

□ □ □

Discurso de ingreso

Profesor Dr. José María Gil-Vernet
Catedrático Emérito de Urología. Universidad de Barcelona.

Excelentísimo Sr. Presidente de la Real Academia Europea de Doctores.
Excelentísimos y Magníficos Rectores.
Excelentísimas e Ilustrísimas Autoridades.
Excelentísimos Académicos
Señoras y Señores.

Es para mí un placer recibir de esta Insigne Real Academia la distinción que me otorga de **Académico de Honor**, a la cual quiero corresponder con mi compromiso de colaborar con ella y ser un defensor de su principio de difusión de la cultura, las artes y la ciencia.

Como médico y Profesor de cirugía he tenido la suerte de poder investigar y aportar mi grano de arena a la ciencia médica. Ahora, con la conciencia tranquila de haber contribuido y aplicado mis conocimientos, me queda el placer de ver, a mis 94 años, que no ha sido inútil este esfuerzo. Pertenezco a una generación de médicos que tuvimos la gran suerte de entusiasmarnos y creer en lo que hacíamos.

Mi vida está marcada por la de mi padre a la que no podía ni debía sustraerme y también por el mundo que desde muy joven me ha rodeado.

El fenómeno biológico de diferenciación celular se ha comparado al de ser médico. La diferenciación es debido a dos factores: el código genético y el ambiente. Igual que en el embrión, tres cosas son necesarias para el desarrollo del cirujano. La primera es una habilidad intrínseca y la capacidad de sufrir las maduraciones para alcanzar un alto grado de diferenciación. La segunda, es el ambiente adecuado para cada nivel de desarrollo. Por consiguiente, además de la vocación genética es necesario no apartarse del ambiente Universitario y vivir en comunidad con un Hospital acorde a la maduración deseada. La tercera, la más importante, es tener el conocimiento anatómico preciso de la región u órgano donde se interviene, es la base para operar con seguridad y éxito. Por lo que mi discurso versará sobre la Anatomía en el desarrollo de las técnicas quirúrgicas modernas.

La Anatomía en el desarrollo de las técnicas quirúrgicas modernas.

Se ha repetido una y mil veces que para el cirujano la Anatomía es el sustrato esencial de la técnica quirúrgica.

Nada más cierto. Desde la distancia temporal se puede comprender que la Anatomía, siendo como es una rama básica en las ciencias médicas, y más aún para el conocimiento quirúrgico, es hoy, si así puede afirmarse, una materia que fue completada, a lo largo de los siglos XVII y XIX, en sus vertientes descriptiva y topográfica. Cuando parecía que todo estaba escrito, se inicia en el siglo XX el desarrollo de la **Anatomía funcional**, con la proyección en el campo de las ciencias morfológicas de los problemas que planteaba la fisiología, la clínica y la cirugía.

En la actualidad, la creciente importancia del conocimiento anatómico radica precisamente en su vertiente clínica. Gracias a la aplicación de nuestros conocimientos anatómicos en la práctica quirúrgica, ha sido posible el desarrollo y creación de nuevas técnicas operatorias más eficaces a la vez que menos agresivas y traumáticas, como por ejemplo la incisión de lumbotomía vertical posterior para acceder al riñón y al uréter, en la que no se lesionan estructuras musculares ni nerviosas y sin posibilidad de eventraciones. Para la cirugía de la litiasis renal y ureteral significó un avance importante.

De una cirugía más segura, como fue nuestro hallazgo anatómico de una vía de abordaje lumbar retroperitoneal a los vasos del bazo, facilitando las anastomosis vasculares esplenorrenales arteriales y venosas en el tratamiento de la hipertensión arterial renovascular y de la hipertensión portal en los cirróticos, sin los peligros de la vía transperitoneal clásica, lastrada de importante morbilidad y mortalidad. Con esta vía también logramos el transplante renal ortotópico que soluciona el problema de los retransplantes renales, y en el 1973 desarrollamos una nueva cirugía que hizo posible la extracción de un órgano enfermo, su traslado a un banco de trabajo, su reparación y posteriormente devuelto al paciente. Es la cirugía extracorpórea o cirugía "ex vivo".

Y en otro aspecto hemos llegado a una cirugía restauradora. El conocimiento de los estudios realizados por Salvador Gil Vernet sobre la anatomía y fisiología de la unión uretero-vesical, y del esfínter ureteral prevesical, nos permitió desarrollar una técnica para el antirreflujo ureteral que es la más simple, la más anatómica, mínimamente agresiva, carente de morbilidad, universalmente admitida y de la que se han beneficiado miles de niños en todo el mundo.

Investigaciones anátomo-quirúrgicas de las fístulas vesico-vaginales complejas nos permitió descubrir en 1989 una nueva operación, consiguiendo la curación en un 100 % de los casos. Afortunadamente queda para la Historia la conocida frase del gran ginecólogo Dieffenbac, al describir estas fístulas como **"La mayor desgracia que puede suceder a una mujer, condenada a vivir con ella y sin la esperanza de morir de ella".**

Incluso hemos alcanzado una cirugía creadora de un orden funcional nuevo. Para ello, en 1953, conjuntamente con el Dr. Rafael Gosálbez, realizamos los estudios necesarios en el plano anatómico, biológico y fisiológico, consiguiendo identificar al colon sigmoide como el segmento intestinal más idóneo para ampliar o sustituir la vejiga urinaria. En 1957, los trabajos experimentales de Weimberg, propuestos por la universidad de Nueva York, confirmaron nuestras investigaciones. Asimismo preconizamos la utilización del segmento intestinal ileocólico para la sustitución parcial o total de la vejiga y el uréter simultáneamente en el tratamiento de patologías diversas, en particular en el cáncer vesical, con lo que el paciente recupera la micción por sus vías naturales y que transcurrido más de medio siglo por esta técnica mantienen su validez y eficacia.

Como asimismo fueron imprescindibles los estudios previos de disección anatómica a fin de descubrir una vía de abordaje al seno renal, lo que cambió totalmente los conceptos de la cirugía tradicional en el campo de la litiasis, facilitando la extracción completa de los grandes cálculos del riñón sin lesionar el parénquima ni sus vasos.

Al conseguir la exposición del "sinus renalis" desarrollamos la "cirugía intrarrenal" que abarca la cirugía de los infundíbulos caliciales y la cirugía reparadora de los aneurismas de las ramas de división de la arteria renal, e incluso de las arterias interlobares de unos 2mm de diámetro.

Nuevas precisiones anatómicas en la disposición de la estructura muscular de la pelvis renal nos llevaron a sustituir la pielotomía vertical clásica, generadora de estenosis y fístulas, por la pielotomía transversa, y también describimos la pielotomía ampliada a los cálices, lo que permitió la extracción total de los grandes cálculos coraliformes sin lesionar el parénquima renal.

Fueron asimismo imprescindibles los estudios de microdisección de las colaterales de la arteria perineal superficial a nivel del septum escrotal y su tinción con la técnica de Spalteholz. Estos hallazgos en la anatomía quirúrgica orientada al estudio de la vascularización de la piel del escroto, nos facilitó la descripción de colgajos cutáneos con garantías de excelente vascularización, lo que, a su vez, nos permitió la reconstrucción o sustitución total de la uretra anterior incluso de la uretra posterior en el hombre, que sin duda es el problema más importante que tiene planteado hoy la cirugía urológica en las roturas traumáticas de la uretra membranosa.

La anatomía sistemática tradicional era una ciencia implicada sólo en la descripción y en la ordenación de las partes anatómicas. A partir del siglo XVIII evoluciona hacia una anatomía topográfica y funcional, indispensable para el desarrollo de la Cirugía.

Creo que esto es básico para interpretar la anatomía moderna, la anatomía aplicada a la cirugía que, en definitiva, constituye la anatomía quirúrgica. Su conocimiento exhaustivo y de determinados puntos anatómicos precisos, que no habían tenido relevancia en la Anatomía clásica, ha dado carácter y dimensión científica a la ciurgía urológica y, por supuesto, que se reconozca la investigación quirúrgica como método científico para el progreso de la ciencia médica.

Mi padre, en su monumental legado nos dejó escrito que la cirugía científica exige, para merecer este calificativo, estar cimentada en dos bases fundamentales: el conocimiento perfecto de la anatomía normal y de la patológica.

Sabios consejos de mi padre, que forman parte también de su recuerdo, de su presencia en mi vida y en mi obra.

Para terminar, quiero reiterar mi testimonio de gratitud a esta Real Academia y a los Académicos que la componen, que desde hoy son mis compañeros, a los que les digo que me tendrán siempre a su disposición y eternamente agradecido en lo que me quede de vida.

Señores, ¡qué hermosa es la escueta palabra GRACIAS cuando con ella se expresa un profundo e imborrable agradecimiento!

Gracias, muchas gracias.

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Trabajos aportados por el nuevo Académico de Honor

A NEW TECHNIQUE FOR SURGICAL CORRECTION OF VESICOURETERAL REFLUX

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ABSTRACT

For more than 2 decades a major problem in pediatric urology has been the medical and surgical management of vesicoureteral reflux. Many effective surgical procedures have been advocated and used. A new surgical technique, particularly effective in patients with megatrigone, has been used in 38 children and adults with excellent results. The technique involves a single stitch that imbricates the trigone, effectively lengthening the intramural segment of terminal ureter.

For about 25 years the medical and surgical management of vesicoureteral reflux has been a topic of primary interest, particularly in pediatric urology. Numerous surgical techniques have been used, the majority of which are successful in eliminating reflux. The more widely applied techniques include the Politano-Leadbetter method,[1] the procedure advocated by Gregoir and Van Regemorter,[2] and Lich and associates[3] and, more recently, the operation described by Cohen.[4] In all of these procedures the juxtavesical ureter must be dissected free and thoroughly mobilized. A new technique that is simple and rapidly accomplished without mobilization of the distal ureter is described.

The evolution of this surgical technique is based upon several considerations. It is recognized that lateral ectopia and megatrigone frequently are associated with vesicoureteral reflux.[5,6] In the presence of megatrigone there is an increased distance between the ureteral orifices and the vesical outlet. The anatomical investigations of Gil Vernet indicate that the intrinsic muscular fibers of the transmural ureter may provide sphincteric action in preventing reflux.[7-9] These observations, confirmed by Ruano and associates,[10] suggest that preservation of the investing musculature of the terminal ureter may be important in reflux prevention.

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TECHNIQUE

The principles of the operative technique are shown schematically in figure 1. The terminal ureters, including the intrinsic musculature, are advanced across the trigone. Traction sutures demonstrate the mobility of the ureters for this maneuver. At completion of the procedure the ureteral orifices lie near the midline, the length of intramural submucosal ureter has been increased, and the intrinsic and extrinsic musculature of the terminal ureters has been preserved.

The procedure involves a transverse Pfannenstiel incision with a transverse cystotomy, exposing the base of the bladder (figs. 2 to 4). Indwelling ureteral catheters are placed for the duration of the procedure and traction sutures are taken at each ureteral orifice to effect subsequent approximation of the ureters. A transverse incision is made through the mucosa across the superior aspect of the trigone between the ureteral orifices. The mucosa is then elevated from the underlying musculature of the trigone and base of the bladder. A single nonabsorbable (3-zero polypropylene or other nylon) mattress suture then is taken at the base of each ureter, including the periureteral sheath of Waldeyer and the intrinsic ureteral musculature, avoiding perforation of the ureteral mucosa. When this stitch is secured the ureters are advanced and approximated near the midline, increasing the intramural length of each distal ureter. The single nonabsorbable suture is then buried by placing 2 additional sutures of absorbable material (2-zero polyglycolic acid) through the bladder mucosa and musculature. Finally, the mucosa is approximated vertically with multiple interrupted fine absorbable sutures, ureteral catheters are removed, a Foley catheter is positioned, and the bladder and suprapubic incisions are closed in routine fashion.

DISCUSSION

This technique has been used indiscriminately in cases of unilateral and bilateral reflux, in almost all instances for cor-

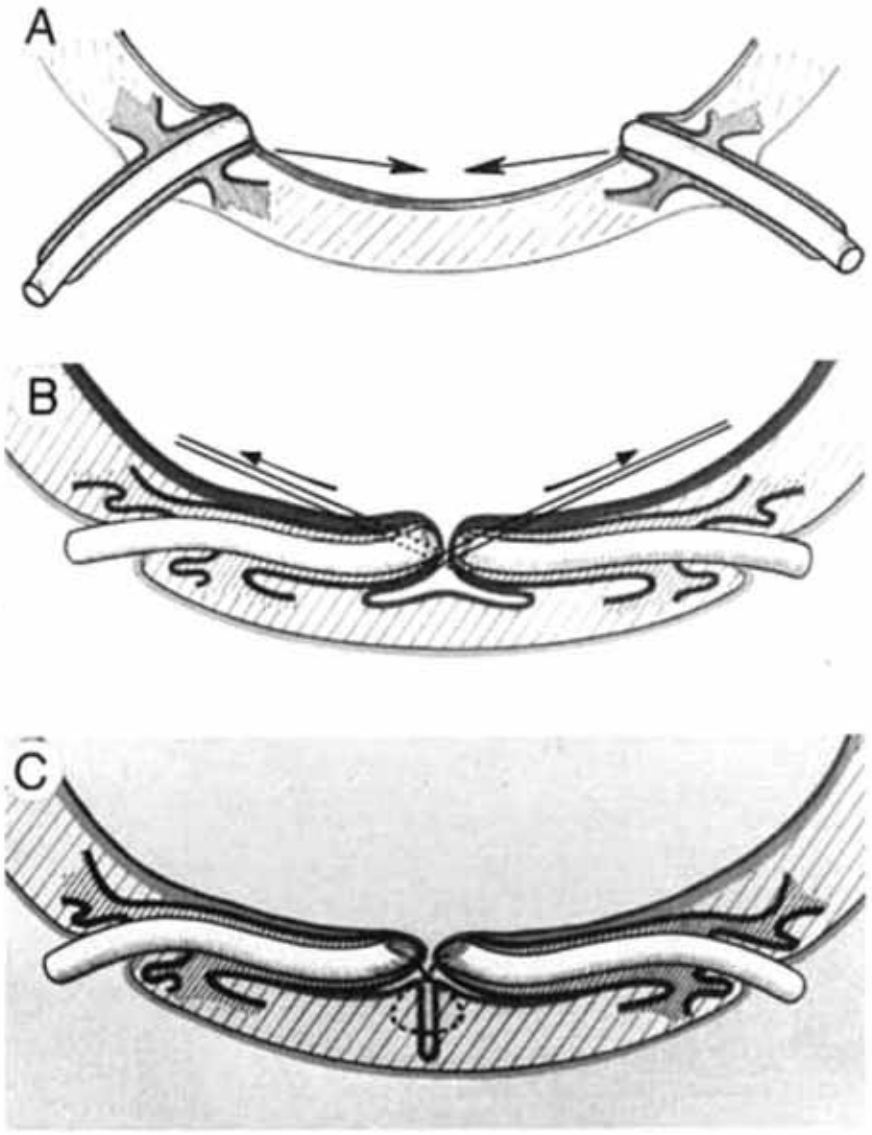

FIG. 1. Schematic representation of technique. *A*, principle involves advancing ureters across trigone. *B*, traction sutures are used to demonstrate desired result. *C*, on completion ureteral orifices are in close proximity near midline and submucosal length of ureter has been increased, preserving intrinsic and extrinsic periureteral musculature.

SURGICAL CORRECTION OF VESICOURETERAL REFLUX

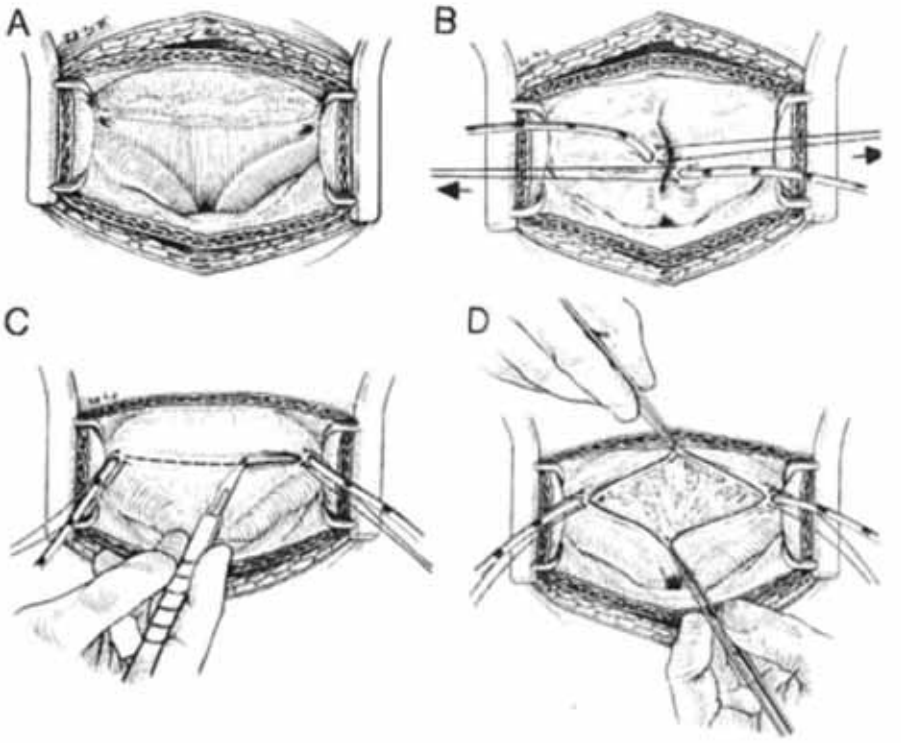

FIG. 2. *A*, transverse cystotomy exposes trigone. *B*, with indwelling ureteral catheters, traction sutures approximate orifices. *C*, mucosal incision is across trigone. *D*, mucosa is elevated from underlying muscle fibers.

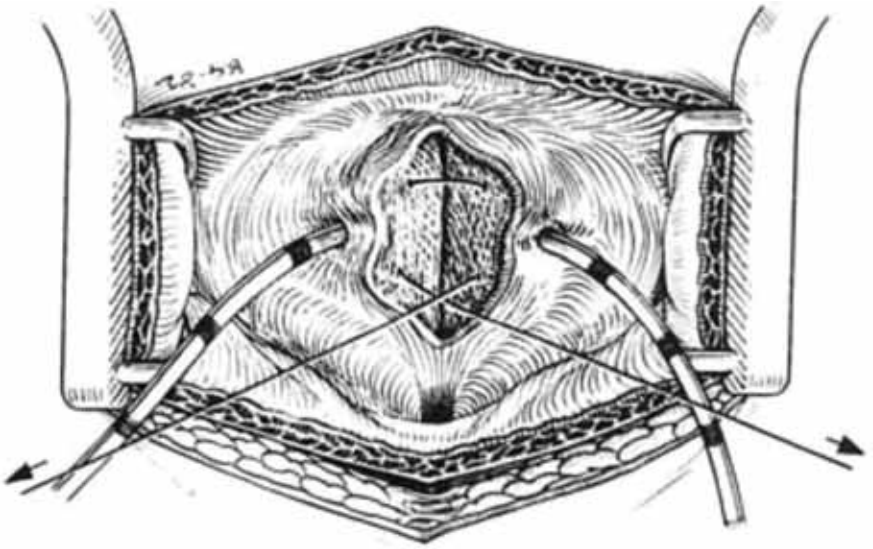

FIG. 3. Trigone is imbricated by securing single suture

rection of primary (congenital) vesicoureteral reflux. Essentially, all grades of reflux, including grades III and IV, have been corrected by this technique. The method has not yet been used in instances of neuropathic bladder associated with reflux but it may be speculated that it would be equally efficacious in such instances.

From July 1981 to July 1983, 20 adults and 18 children were operated upon for correction of reflux by this technique. Followup studies at 3 to 6 months included cystoscopy, voiding cystourethrograms and excretory urography. Success was judged by absence of reflux, absence of obstruction and satisfactory clinical course. Among the 38 cases 36 were successful and 2 were failures. The 2 failures were early cases in which the imbricating mattress suture in the trigone was of absorbable material and apparently disrupted, allowing the ureters to retract to the original position. In both cases reoperation using a nonabsorbable nylon mattress suture was readily accomplished and was successful.

The advantages of this technique include simplicity and rapidity of accomplishment, as well as the development of a satisfactory length of submucosal ureter with preservation of the intrinsic musculature of the distal ureter. The procedure

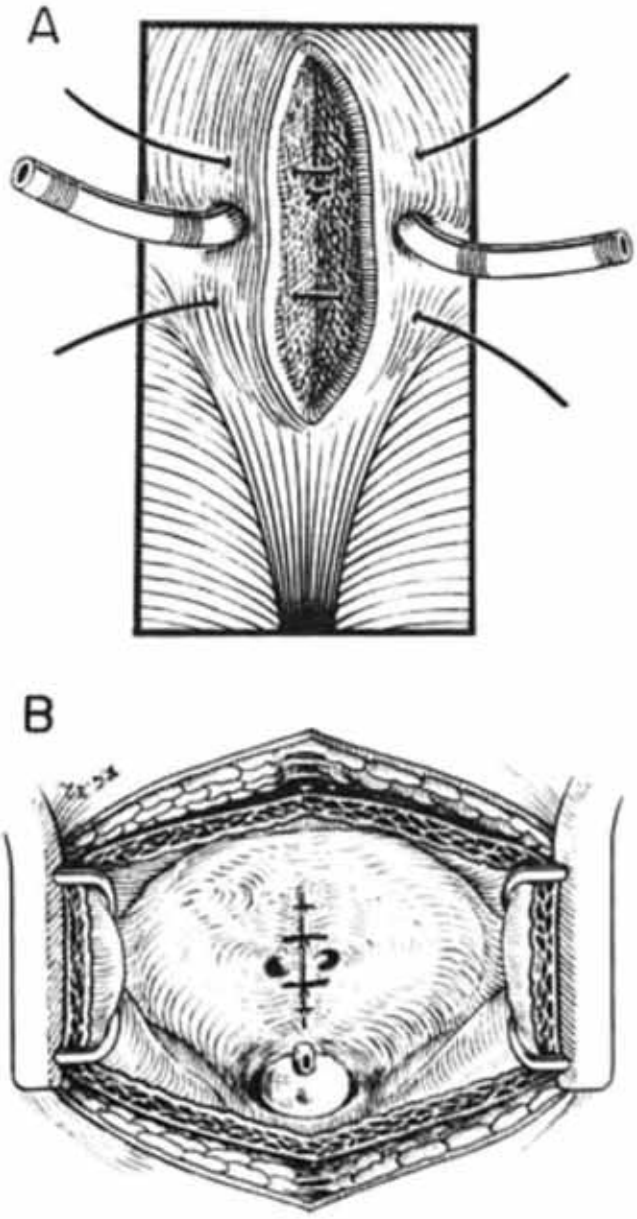

FIG. 4. *A*, knot of nylon suture is buried beneath absorbable sutures. *B*, after closure of mucosa, ureters lie near midline at completion of operation with removal of ureteral catheters and placement of Foley catheter.

can be recommended as a logical and physiological method for correction of vesicoureteral reflux.

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EDITORIAL COMMENT

The author is an outstanding surgeon and has made another valuable contribution. Through the medium of a surgical motion picture I have had an opportunity to see him accomplish this procedure and it is, indeed, simple and rapidly accomplished. He reports total success in the first 38 patients, although 2 required reoperation using the same method. This is remarkably good, although virtually all of the widely used surgical methods for correction of reflux now provide success, as measured by correction of reflux without causing obstruction, in 95 per cent or more of all cases.

The author embraces the concepts of intrinsic ureteral muscular function proposed by his father, Prof. Salvatore Gil-Vernet, and this is in part reminiscent of the early observations of Hutch.[1] The technique itself further reminds us of the concept of intravesical intussusception encompassed in the antireflux procedure proposed by Williams and associates.[2] In addition, advancement across the trigone is an essential part of the distal tunnel ureteral reimplantation method of Glenn and Anderson.[3]

One might express concern that there will ultimately be failures in patients operated on by this technique, either initially or later due to lateral drift of the ureters. Furthermore, not all ureters so advanced may have sufficient transmural length to prevent reflux despite satisfactory technical accomplishment of the procedure. Finally, one might worry about the nonabsorbable suture migrating to the mucosal surface to provide a focus for infection or a nidus for stone formation. Despite these considerations, this interesting technique might well be used by other surgeons for further evaluation.

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DESCRIPCIÓN DE UNA NUEVA VÍA DE ABORDAJE A LOS VASOS ESPLÉNICOS PARA LAS ANASTOMOSIS ESPLENORRENALES ARTERIALES Y VENOSAS*

Dres. J. M.ª Gil-Vernet, A. Caralps Y D. Ruano

RESUMEN

Descripción de una nueva vía de abordaje a los vasos esplénicos para las anastomosis esplenorrenales arteriales y venosas.

Para la realización de las anastomosis esplenorrenales arteriales o venosas, los cirujanos vienen utilizando sistemáticamente la vía transperitoneal gravada por un alto porcentaje de mortalidad y morbilidad. En base a consideraciones anatomoquirúrgicas, se ha encontrado una vía de abordaje retroperitoneal al hilio del bazo a través de la región lumbar, realizándose en 1972 la primera anastomosis arterial esplenorrenal por esta vía, y en 1974 la primera anastomosis esplenorrenal venosa por hipertensión portal, también por esta vía, que se ha mostrado como la menos agresiva al evitar la lesión del páncreas, la más quirúrgica y directa para llegar a los vasos esplénicos lo que facilita una mejor exposición y más fácil realización de las anastomosis. Al ser retroperitoneal, se evita en el cirrótico la pérdida o la infección del líquido ascítico, así como los accidentes hemorrágicos intraoperatorios por la gran circulación de suplencia. No ha habido mortalidad en las 13 anastomosis esplenorrenales arteriales y venosas que hemos efectuado por esta vía.

SUMMARY

Description of a new approach to the splenic vessels for arterial and venous spleno-renal anastomoses.

To perform arterial or venous spleno-renal anastomoses, surgeons have so far systematically used the transperitoneal way which is burdened by a high mortality and morbility percentage. On the basis of anatomo-surgical considerations, a retroperitoneal approach has been found reaching the hilus of the spleen via the lumbar region; the first arterial spleno-renal anastomosis by this way was performed in 1972 and the first venous spleno-renal anastomosis due to portal hypertension also by this way was performed in 1974, the latter proving to be the least aggressive by avoiding damaging the pancreas, the most surgical and direct for reaching the splenic vessels thereby enabling a better exposure and an easier performing of the anastomoses. By being retroperitoneal, the loss or infection of the ascitic liquid in the cirrhotic patient is prevented as well as the intraoperative haemorrhagic accidents caused by the extent of replacement circulation. There has been no mortality in the 13 arterial and venous spleno-renal anastomoses which we have performed by this way.

Los vasos del bazo interesan al cirujano. La arteria esplénica para la revascularización del riñón en la hipertensión arterial de causa renovascular, y la vena esplénica para derivar la sangre del sistema porta al sistema cava en la hipertensión portal.

En ambas situaciones, el cirujano, para llegar a los vasos esplénicos utiliza sistemáticamente la vía transperitoneal, bien a través de una incisión abdominal pura, bien a través de una vía mixta toraco-abdominal. La gran mortalidad y frecuencia de complicaciones en las anastomosis esplenorrenales, tanto venosas como arteriales, son dependientes, en gran parte del traumatismo quirúrgico de la vía transperitoneal que requiere la movilización de vísceras y el despegamiento y la sección de mesos.

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En los pacientes con hipertensión portal, con el uso de la vía transperitoneal, no sólo se pierde líquido ascítico, sino que además, en el campo operatorio se encuentra una enorme circulación de suplencia, particularmente en los epiplones gastroesplénico, gastrocólico y en el suelo de la transcavidad, lo que origina accidentes hemorrágicos y plantea dificultades en la hemostasia. Pero el mayor peligro de la vía convencional está en la posibilidad de lesión del páncreas en el curso de la disección de los vasos esplénicos y renales, puesto que no debe olvidarse que la cola del páncreas, constituye un obstáculo serio para el abordaje del riñón izquierdo por esta vía. También la vía transperitoneal plantea dificultades técnicas en la realización de las anastomosis, por la profundidad del campo operatorio particularmente en pacientes obesos.

Interesados desde hace años en la cirugía de la hipertensión arterial vasculorrenal, y ante los graves problemas que planteaban las anastomosis arteriales esplenorrenales utilizando la vía transperitoneal, orientamos nuestras investigaciones a la búsqueda de una nueva vía más directa, menos traumática y más quirúrgica, a través de un abordaje para nosotros muy conocido y habitual como es la región lumbar.

Consideraciones anatomoquirúrgicas nos permitieron encontrar una vía de abordaje retroperitoneal al hilio del bazo, a través de la región lumbar, realizando en 1972 la primera anastomosis arterial esplenorrenal retroperitoneal con éxito, y en 1974 llevamos a cabo la primera anastomosis esplenorrenal venosa por hipertensión portal también con éxito.

La experiencia nos ha demostrado que la nueva vía que presentamos facilita extraordinariamente la disección simultánea de los vasos esplénicos y renales proporciona un mejor campo de exposición para la realización de las anastomosis tanto arteriales como venosas, es mucho menos agresiva para el paciente y evita las complicaciones propias de la vía transperitoneal, en particular las lesiones pancreáticas, ya que la disección de los vasos esplénicos se inicia a nivel del hilio esplénico.

BASES ANATOMICAS

El pedículo del bazo está alojado en el epiplón pancreático-esplénico, que constituye la celda de los vasos esplénicos. Este epiplón, está constituido por dos hojillas: la ventral, formada por el peritoneo, es la hojilla derecha del mesogastrio posterior. La dorsal es la hojilla izquierda no soldada del mesogastrio posterior. Por dentro existe una fascia de coalescencia (de Treitz), que adosa la cara posterior del páncreas al peritoneo parietal posterior (fig. 1). La sección de éste y de la fascia de Treitz, permite poner al descubierto los vasos esplénicos, que se pueden seguir fácilmente tanto proximal como distalmente al hilio del bazo y siempre por el retroperitoneo.

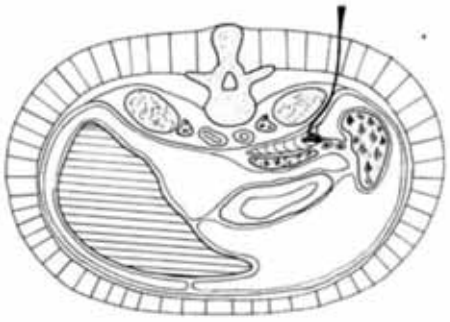

FIG. 1.—*Corte esquemático horizontal pasando por el polo superior de los riñones. La flecha señala la vía de abordaje retroperitoneal al hilio del bazo.*

TECNICA

El enfermo se sitúa en posición dorsolateral combada. A través de una lumbotomía clásica (postero-lateral), se reseca la 12.ª costilla (figura 2), se abre la celda renal y el riñón y la suprarrenal son reclinados hacia la pared

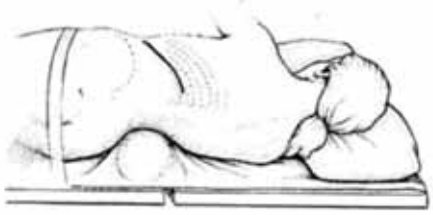

FIG. 2.—*Incisión de lumbotomía clásica con resección de la 12.ª costilla.*

FIG. 3.—*Reclinados el riñón y la suprarrenal, aparece el relieve de la arteria esplénica por encima de la cola del páncreas. En el caso de hipertensión portal es la vena esplénica la que hace relieve o se transparenta.*

FIG. 4.—*Incisión del peritoneo parietal posterior y de la fascia de Treitz.*

posterior del espacio lumbar. Después de esta maniobra, en los individuos delgados, inmediatamente por encima de la cola del páncreas, se transparenta o hace relieve la arteria esplénica (fig. 3) y es muy fácil identificarla con la vista a través del peritoneo parietal posterior. En los individuos obesos se localiza fácilmente por palpación. Es precisamente a nivel del relieve de la arteria esplénica, que se incide sucesivamente el peritoneo parietal posterior y la fascia de Treitz (fig. 4). Aparece entonces la arteria esplénica y se la sigue en el espesor del epiplón pancreático-esplénico hasta el hilio del bazo, disecándose su bifurcación o sus ramas si se estima necesario (fig. 5).

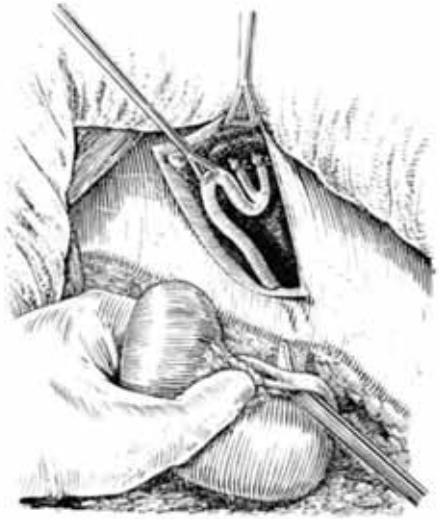

FIG. 5.—*Se diseca el segmento paraesplénico de la arteria, o bien el segmento paraporta de la vena esplénica.*

Normalmente, a este nivel, la vena esplénica se encuentra inmediatamente por detrás y en el mismo canal que la arteria, pero cuando existe una hipertensión portal, la vena adquiere el tamaño del pulgar, se sitúa entonces al lado de la arteria, se transparenta a través de la hoja dorsal del epiplón pancreático-esplénico (fig. 6) y hace mayor relieve que la

FIG. 6.—*En la hipertensión portal es la vena esplénica la que hace relieve o se transparenta.*

arteria, por lo que su identificación es sumamente fácil y previa ligadura de cuatro o seis pequeñas venas pancreáticas, se le puede disecar en toda su circunferencia hasta por detrás del páncreas (fig. 7), lo que proporciona una suficiente longitud de la misma en el caso de practicar una anastomosis términolateral de la vena esplénica a la renal (fig. 8).

Hemos observado que en los casos de esplenomegalia, la distancia entre los vasos esplénicos y los renales es muy corta, prácticamente están en contacto debido a que el

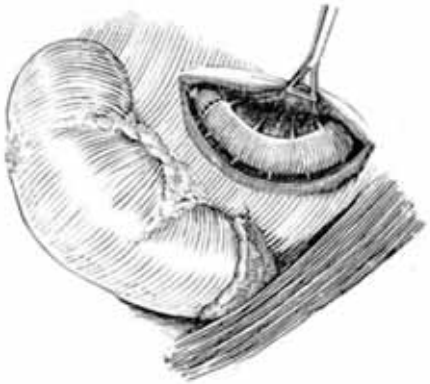

FIG. 7.—*Incisión del peritoneo parietal posterior y de la fascia de Treitz y disección del segmento de la vena esplénica.*

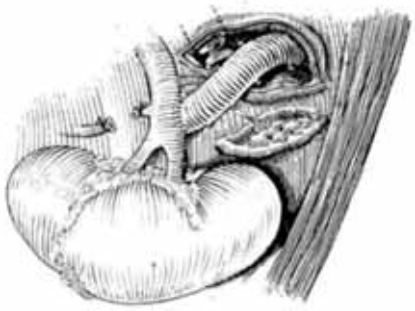

FIG. 8.—*Anastomosis venosa esplenorrenal términolateral.*

agrandamiento del bazo origina el descenso lumbar de su hilio que llega a contactar con el riñón, desplazándolo y provocando su ptosis.

Este descenso y alargamiento del pedículo esplénico, facilita la anastomosis venosa. En los cuatro casos de anastomosis esplenorrenal por hipertensión portal, no hemos observado circulación de suplencia en el campo operatorio. No ha habido hemorragias ni problemas en la hemostasia.

ESTADISTICA

En el tratamiento de la hipertensión arterial por estenosis de la arteria renal izquierda, hemos llevado a cabo ocho anastomosis esplenorrenales a través de la vía de abordaje retroperitoneal descrita: en cinco casos se hizo una anastomosis término-terminal (fig. 9) del tronco de la arteria esplénica con la renal y en otros tres se hicieron anastomosis términoterminales de las ramas de la esplénica con las ramas de división de la renal (fig. 10).

En 6 casos, la intervención se indicó por estenosis de la arteria renal izquierda aparentemente responsable de una hipertensión arterial. Después de un tiempo de evolución comprendido entre dos y cuatro años, los seis enfermos están normotensos. En otros dos casos, la intervención se indicó por estenosis de la arteria renal izquierda en enfermos con insuficiencia renal crónica muy avanzada, en un intento por mejorar la función renal. Uno de los enfermos no presentó mejoría de la fun-

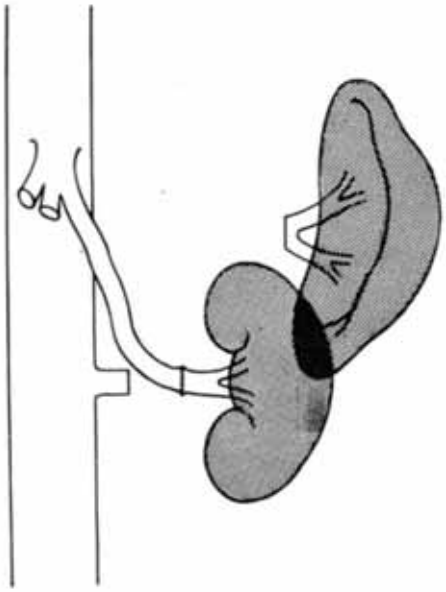

FIG. 9.

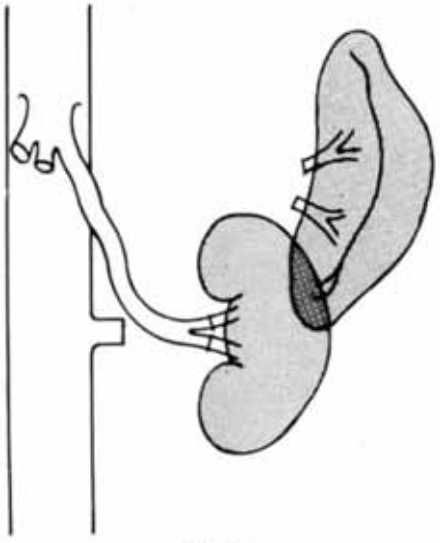

FIG. 10.

ción por lo que fue incluido en un programa de hemodiálisis periódica. En la enferma restante, que tenía una hipertensión arterial rebelde, el filtrado glomerular permanece estable alrededor de 6 ml. por min., y la presión arterial responde actualmente a pequeñas dosis de antihipertensores. En ningún caso ha habido morbilidad ni mortalidad operatoria.

En el tratamiento de la hipertensión portal, hemos utilizado la vía retroperitoneal para la realización de la anastomosis espleno-renal venosa en cuatro enfermos de edades comprendidas entre los treinta y los cincuenta años de edad, afectos de cirrosis hepática, que habían presentado por lo menos un episodio de hemorragia digestiva por varices. En el primero de ellos se hizo una anastomosis término-lateral de la vena renal a la esplénica (fig. 11). En el segundo enfermo, se hizo una anastomosis latero-lateral (fig. 12) y en los dos últimos,

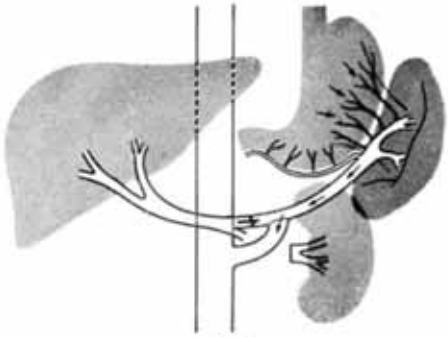

FIG. 11.

las anastomosis fueron tipo Warren, es decir, en término lateral de la vena esplénica a la vena renal (fig. 13) con la variante de que para preservar la función del riñón isquémico, utilizamos un método de hipotermia mediante perfusión intraarterial renal "in situ".

Todos los enfermos, excepto uno de ellos que estaba en fase de hemorragia al ser operado, estaban bien compensados en el momento de la intervención. El tiempo máximo de evolución postoperatoria ha sido de dos años y medio. En todos los casos, se comprobó la desaparición de las varices esofágicas y no repitieron las hemorragias digestivas. El segundo

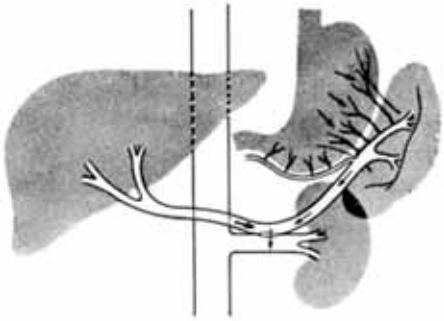

FIG. 12.

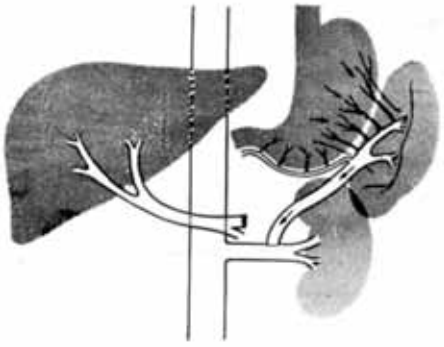

FIG. 13.

enfermo al que se le hizo una anastomosis latero-lateral, comenzó a presentar signos de encefalopatía a los dos meses de la intervención, falleciendo tres meses más tarde.

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COMENTARIO

Los resultados que hemos obtenido después de cuatro años de experiencia mediante la vía retroperitoneal que describimos, han demostrado que es muy superior a la transperitoneal por ser menos agresiva, más quirúrgica y más directa para llegar a los vasos esplénicos 1, 2, 3. Su mayor ventaja es que no hay peligro de lesión pancreática, a la vez que permite una mejor y simultánea exposición de los vasos renales y esplénicos, facilitando la correcta y minuciosa ejecución de las anastomosis.

La disección de los vasos a nivel del hilio esplénico, permite utilizarlos en toda su longitud, lo que evita problemas de tensión a nivel de las anastomosis.

No requiere la abertura del peritoneo, lo que evita la pérdida de líquido ascítico o su infección con el consiguiente peligro en los cirróticos.

El tiempo de intervención es más corto que por vía transperitoneal.

La esplenomegalia no sólo no ha constituido ningún obstáculo para las anastomosis, sino que las facilita debido a que la distancia existente entre los vasos esplénicos y los renales es más corta.

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EXTRACORPOREAL RENAL SURGERY

Work Bench Surgery

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ABSTRACT — In cases in which renal repair through conventional in situ surgery is not possible, we have proceeded to remove the organ outside of the human body and placed it on a work bench where ex situ repair is aided by microsurgery, x-ray films, and image amplifiers. In most cases the damaged kidney has recovered its function and a grave problem has been solved. Extracorporeal surgery means a new tactical solution to extreme situations.

Advances made in renal transplantation such as the successful preservation of the ischemic kidney for several hours and improvement in the techniques of vascular anastomoses and reestablishment of continuity of the upper urinary tract, together with simultaneous developments in microsurgery, have given rise to bold surgery for severe damage to the kidney. The removal of the damaged kidney and its repair outside the human body, with subsequent reimplantation, is now a reality in those cases in which conventional surgery with reparation in situ is not technically possible. It is a transitory nephrectomy.

Our purpose was (1) to find a new surgical technique for extreme situations in which nephrectomy or permanent nephrostomy is not possible or desirable (such as in patients with a single kidney or when the other kidney is not normal); and (2) to make surgery possible in certain kidney diseases or diseases of the vessels of the kidney where surgery in situ would be impossible or dangerous. These are situations produced by peculiar anatomic conditions or by singular pathologic circumstances.

Indications for such a procedure include: repeated operated kidney, with cicatricial scarring of the pelvis or ureteropelvic junction and especially of the intrasinusoidal structures; dense fibrotic adhesions surrounding and involving the hilum and presenting serious vascular risks during dissection of the renal collecting system; ureterocalyceal anastomotic failures; certain recurrent staghorn calculi with urinary infection where conventional surgery cannot guarantee complete removal of calculi; severe tissue damage preventing plastic reconstructive operations that require the use of healthy tissues (ureter, pelvis, or intestine) and necessitate the removal of the kidney to another position in the pelvic cavity; failure of corrective operations for congenital malformations of the kidney with obstruction; intrasinusoidal renovascular pathologic conditions (stricture of small arterial branches, dyplasias, aneurysms, arteriovenous fistulas) which require the use of microsurgical techniques; such techniques are difficult to carry out in situ because of inadequacy of renal sinus capsule and also because of the risk of prolonged renal ischemia or

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operative hemorrhage; and vascular anomalies (polar arteries) in donor kidneys.

Rationale

Extracorporeal renal surgery is the offshoot of the enormous experience gained in kidney transplantation:

1. The transplanted kidney functions perfectly in a state of complete denervation.

2. Lymphatic drainage is restored through lymphatic-venous connections or through regeneration of the vessels. If not restored, it appears that lymphatic drainage is not essential for the correct functioning of the kidney.

3. With present methods of renal preservation (perfusion with Collins-3 solution and maintenance at 4° C.) the kidney is protected from the effects of ischemia during the time required for its repair without its function being altered and with immediate reestablishment of diuresis.

4. Our experience with the clinical results with isotransplants (1 case due to terminal uremia) and uni- or bilateral autotransplants (20 cases due to stricture of the renal artery, 2 cases due to extensive stricture of the lumbar ureter) show that secretory and excretory physiologic functions are completely normal in the transplanted kidney, mortality is not prohibitive (no mortality), and vascular complications (thrombosis or stenosis) not significant (no thrombosis or stenosis). Although extracorporeal renal surgery does not result in deleterious consequences to the kidney or the patient, it requires that the parenchyma be fairly thick and that the urologist be well trained in vascular surgery.

Surgical Techniques

The vascular configuration in the kidney as well as the iliac vessels must be previously known by means of aortography. After multiple retroperitoneal operations, the kidney may become closely adherent to the superficial layers and a different route of access such as a transperitoneal approach may be necessary. A lumbar approach may result in lacerations of the parenchyma and further reduce precariously an already lessened renal function.

Stages

First stage. Dissect the proximal trunk of the renal artery (renoaortic ostium) and the renal vein (the cava on the right side or its intersection with the aorta on the left side), and ligate the collateral vessels. This allows an eventual control of the hemorrhage, by means of Bulldog clamps, and ligature of the pedicle prior to the temporary nephrectomy. After this the kidney is freed by means of scissors.

The technique will differ if following ex situ repair the kidney is reimplanted in its place of origin, reanastomosed to its own vessels, as in cases with vascular indications, or if it is reimplanted in another area (ipsi- or contralateral iliac fossa) as in cases with urologic indications.

Second stage. In freeing the kidney, it is always preferable to include with the organ and surrounding tissues fragments from the superficial and deep-set parietal muscles rather than leave behind some renal parenchyma attached to the muscles. The kidney is removed along with the perirenal tissue or fibrous adhesions to which it may be attached.

Third stage. Immediately after removal the kidney is transferred to another surgical area where intra-arterial perfusion is carried out with Collins-3 solution. Once the perfusion is completed, the kidney is placed in a dish where it remains submerged in Collins-3 liquid to which magnesium sulfate has not been added. The temperature is kept at 4° C. and monitored by electronic telethermometer in the kidney and in the Collins' bath solution (Fig. 1).

To maintain a constant temperature of the renal bath, we first use a continuous pumping mechanism. However, simply adding and removing pieces of ice proved sufficient and adequate for maintaining the low temperature and viability of the organ (Fig. 2).

Preservation of renal function in the ischemic kidney involves other measures, such as the administration of hypertonic mannitol thirty minutes before opening the clamps and of furosemide at the moment of opening the clamps. Careful control of the blood volume and hydration of the patient is equally essential before, during, and after surgery. Supervision of the circulatory parameters, including the measuring of the central venous pressure are also essential.

Fourth stage. Ex situ repair is done at this stage. Motion of the kidney within the dish is prevented by supporting it with sponges. The organ is submerged, but the hilum remains on the surface, facing the surgeon. If surgery is expected to be lengthy, the kidney should be immersed completely 0.5 cm. from the surface during the procedure. Use of the operating binocular microscope allows more precise dissection and more

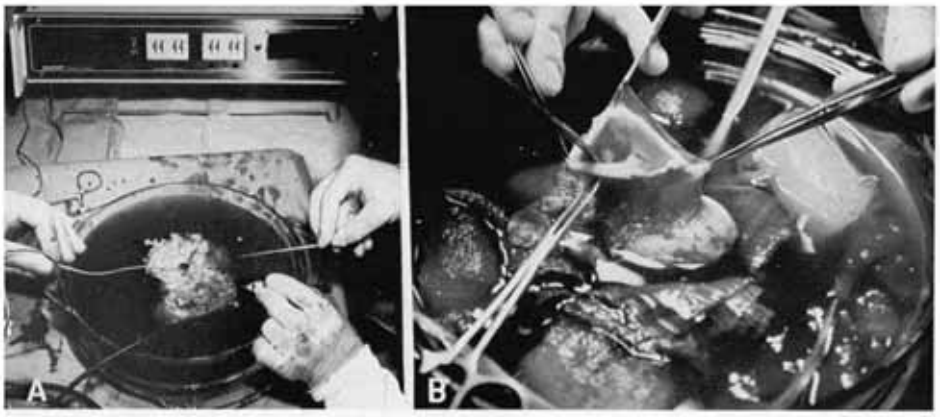

FIGURE 1. *(A) Pathologic kidney has been perfused and placed on dish with liquid kept at 4° C.; telethermometer allows constant control of temperature. (B) Extracorporeal operation: kidney is operated on in the dish and submerged in constant solution to keep it hypothermic. (C) Microsurgery is valuable because hilar structures are magnified.*

exact marking of the limits of the pathologic tissues, allows preservation of normal structures (branches of the renal artery), and allows surgery in the depth of the sinus (Fig. 3). In such conditions the identification of the different hilar structures, resections, anastomosis, and suture of the excretory tract or of the vessels is carried out easily (in a bloodless field) and with greater precision.

If the kidney also presents multiple calculi or staghorn calculus, it is placed directly on a radiographic chassis (Figs. 4 and 5). These films provide good and clear images, making it possible to detect the smallest calcification and to check that removal has been complete. The image intensifier is also useful for location of stones at various levels and their removal with minimum trauma (Fig. 6).

In renal transplantation it is not uncommon that both kidneys of a living donor have vascular anomalies (such as polar arteries); excision of a patch of the aorta is not necessary because once the kidney has been removed and perfused, the polar artery can be anastomosed to the trunk of the main artery, end to side, with the help of the operating microscope. Thus, regardless of the number of renal arteries, it is possible to use all kidneys for transplantation purposes.

Fifth stage. After completion of the ex situ operation, if the indication was for a vascular problem, the kidney may be reimplanted in its place of origin by reanastomosis of its vessels and renal pelvis. If stricture of the trunk is also present, it is preferable to transfer the kidney to another vascular area, except with the left kidney where the splenic artery can be used for a

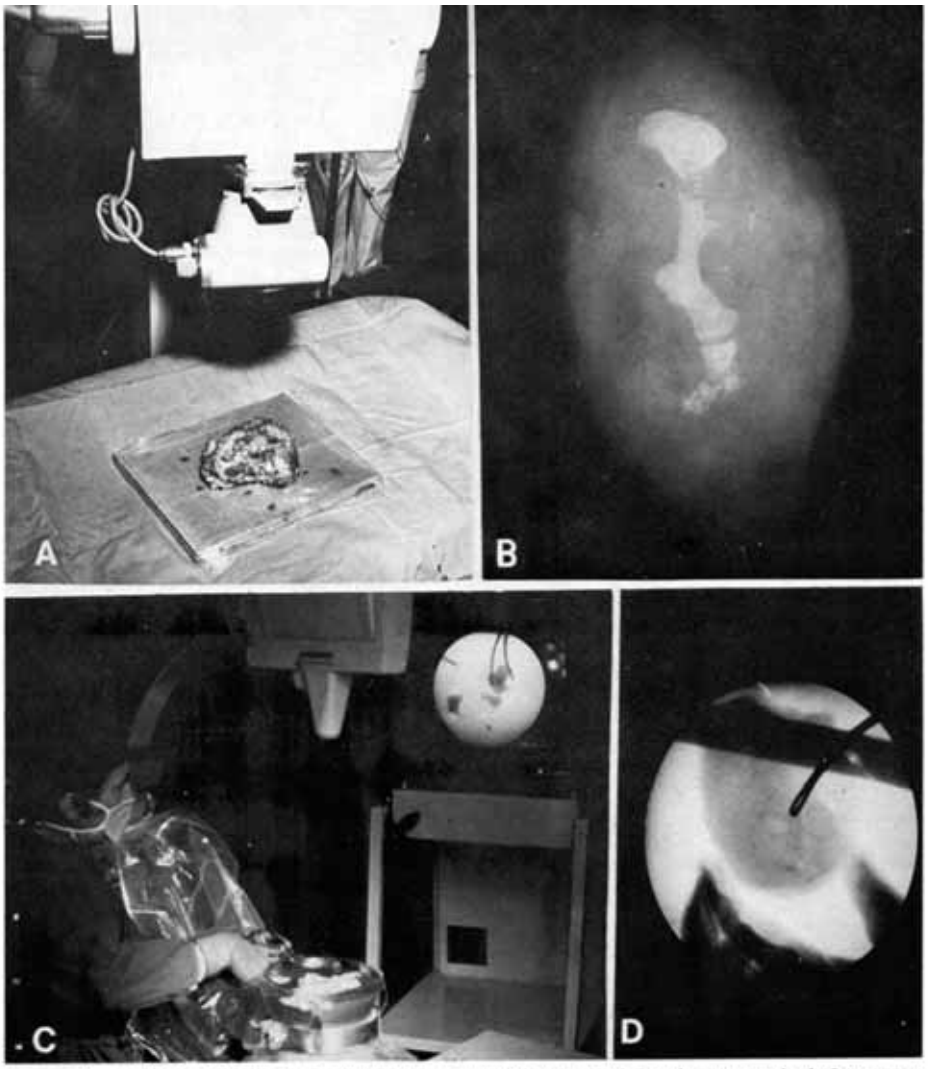

FIGURE 2. *(A) Kidney is brought to radiology department; (B) contact radiography: stone and calcifications are detailed; (C) image amplifier helps to locate and extract calculus with minimum trauma (dish containing kidney is radiotransparent); (D) television image: Randall's forceps is easily directed toward bottom of calyx to remove small stone.*

splenorenal anastomosis. If the indication was for a urologic problem, the kidney is transplanted to the iliac fossa where healthy iliac ureter can be used to reestablish urinary continuity. If reimplanted in the lumbar fossa, a pyelopyelic anastomosis would be necessary.

In extracorporeal surgery the ex situ stage is not difficult because it is carried out under conditions particularly favorable to the surgeon: lighting is perfect, structures are magnified, the organ is in his hands, ample time is available, and he is comfortably situated and separated from any tension-producing influence.

It is bloodless and atraumatic surgery. During surgery of the ex situ organ, the patient is under light anesthesia.

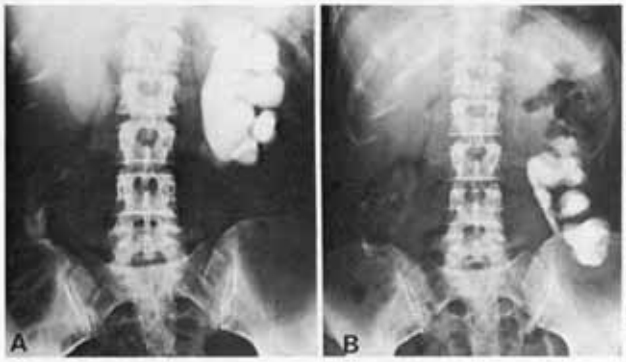

FIGURE 3. *Case 4. (A) Anomalous right kidney (lumboiliac ectopy); left kidney with large hydronephrosis complicated by extensive stricture of ureter caused by previous surgery. (B) Postoperative intravenous pyelogram: local condition of tissues made operation in situ impossible. Resection and intrasinusoidal pyeloplasty ex situ under microscope. Reimplanted kidney in lumboiliac area in inverted position.*

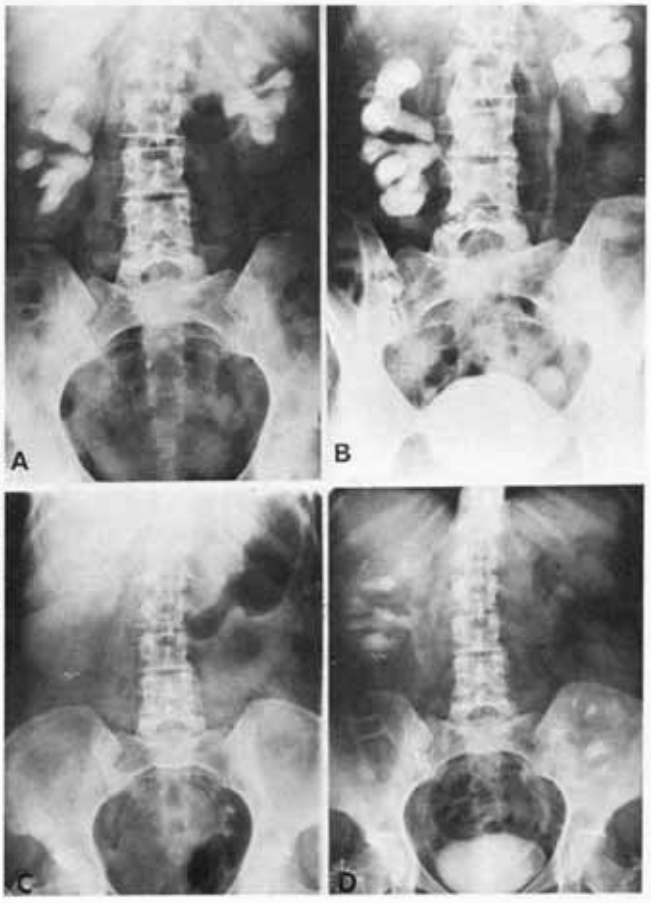

FIGURE 4. *Case 7. (A) Roentgenogram showing bilateral staghorn calculus; patient had two operations on left kidney. (B) Intravenous pyelogram showing extensive stricture of ureteropelvic junction on left side. (C) Postoperative roentgenogram revealing complete removal of stones on both sides. (D) Intravenous pyelogram, postextracorporeal surgery, showing left kidney in iliac fossa.*

Surgical Indications

Urologic

Urologic indications include: (1) strictures extending over the ureteropelvic junction and the pelvis, usually a sequela of surgery for lithiasis, when a correct technique is not followed; (2) recurrent staghorn calculi; (3) failures in plastic surgery for hydronephrosis, strictures following ureteral reimplantations and ureterocalyceal anastomoses where the restoration of the excretory tract according to conventional procedures is not possible; (4) congenital renal malposition or malrotation where it is possible to rectify the anomaly surgically and provide improved urinary drainage; (5) structural anomalies of the renal hilum that make normal conventional operations dangerous; and (6) multiple tumors in a single kidney in which selective antineoplastic chemotherapy may be used.

Vascular

The following are vascular indications: (1) vascular anomalies (26 per cent of cases) in donor kidneys in the form of polar arteries which cannot be ligated (partial nephrectomy is dangerous in

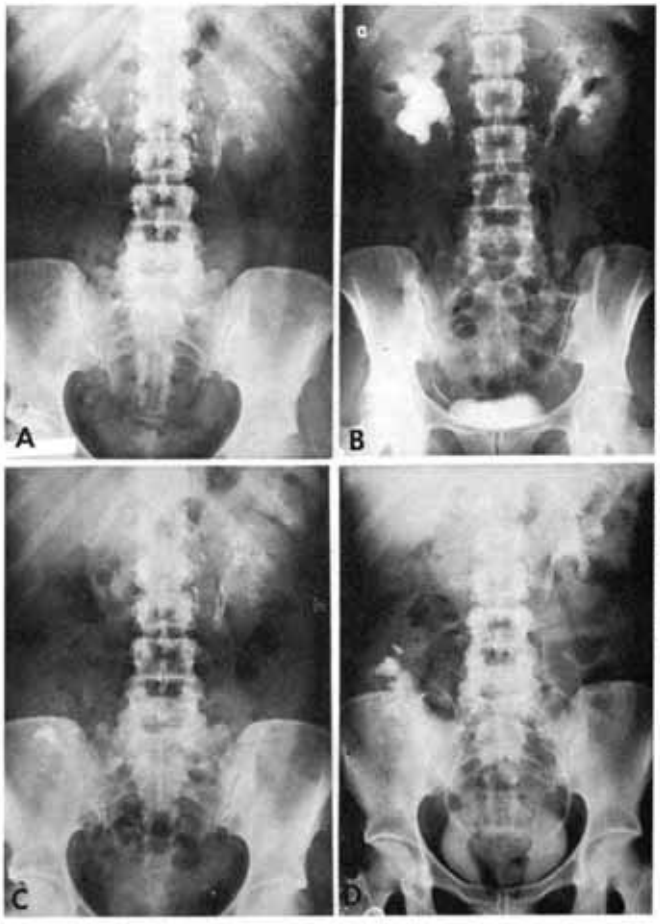

FIGURE 5. *Case 12. (A) Roentgenogram showing Thorotrast residue following bilateral pyelography. (B) Intravenous pyelogram: hydronephrosis from extensive fibrous stricture of ureteropelvic junction due to deposits of Thorotrast at adventitial layer. (C) Postoperative radiography: ex situ resection of lower portion of renal pelvis and lumbar ureter (right side) and partial removal of deposits of Thorotrast in the calyceal system using operating binocular microscope. (D) Postoperative intravenous pyelogram: transplanted kidney to iliac fossa.*

these cases); and (2) intrarenal aneurysms and arteriovenous communication and strictures of branches of the renal artery, especially when associated with stricture of the trunk. There is a single essential condition: that the kidney maintain sufficient function.

The indications for extracorporeal surgery are, for the moment, not very frequent but should not be ignored, particularly in the single kidney.

Results and statistics

Of a total of 12 cases of surgery, there were 10 successful results and 2 failures, with no mortality.

Case Abstracts

*Case 1**

This fifty-year-old man had congenital hydronephrosis on both sides. Conventional pyeloureteroplasty on the right side resulted in failure. There was no possibility for a new intervention in situ. Extracorporeal surgery in May, 1972, gave a good result.

Case 2

This eleven-year-old boy with permanent nephrostomy in a solitary kidney had a previous history of renoureteral trauma, severe pyelonephritis, and renal insufficiency. The purpose of the operation was to eliminate the permanent nephrostomy. Results of extracorporeal surgery in March, 1973, were considered to be unsatisfactory. In this case the mistake was failure to recognize that the injuries were nonreversible. This patient is awaiting allotransplantation.

Case 4

Previous surgery on the left kidney had been performed in this forty-nine-year-old woman. There was advanced hydronephrosis with hypoplasia of the lumbar ureter and of the ureteropelvic junction, and iliac ectopy of the contralateral kidney. A good result was obtained following extracorporeal surgery in May, 1973.

Case 7

This patient, a fifty-year-old woman, had bilateral staghorn calculi. The left kidney had multiple surgery followed by extensive stricture of the left ureteropelvic junction and lumbar ureter, and recurrence of the stone. There was impaired function of the right kidney. Extracorporeal surgery on the left kidney in June, 1973, gave a good result. Later, staghorn calculi of the right kidney were removed by conventional surgery (intrasinusoidal approach).

Case 8

A fifty-five-year-old man had bilateral staghorn calculi with multiple operations. In the left kidney there was extensive stricture of the ureteropelvic junction, a thinned parenchyma due to previous bivalve nephrotomy, and pyelonephritis. Extracorporeal surgery in June, 1973, was followed by total necrosis of the organ and required nephrectomy. The patient is living, with sufficient function of the remaining kidney. Two hypotheses that explain the cause of failure are: (1) malfunction of the temperature-regulating mechanism of the conservation liquid which was maintained under 0° during four and one half hours of the ex situ operation, and (2) by a deficient perfusion attributable to the effects of the previous bivalve nephrotomy.

Case 9

This woman, aged forty-five years, had a left renal staghorn calculus, repeated surgical procedures, intrasinusoidal in position, with scarred retraction of the pelvis, and hydronephrosis of the other kidney. A good result followed extracorporeal surgery in January, 1974.

Case 12

This forty-seven-year-old woman had a history of retrograde pyelography on both sides with Thorotrast (a contrast medium). This resulted in right hydronephrosis with fibrous stricture of the pelvis. Contralateral kidney was also affected, although without any morphologic alterations. Extracorporeal surgery performed in June, 1974, gave a good result.

Other cases

There were 5 cases (Cases 3, 5, 6, 10 and 11) of polar arteries in kidneys from live donors in which both organs revealed vascular anomalies. A successful surgical result was obtained in all.

Comment

Statistical results are satisfactory considering that these were very difficult cases, with repeated

*Case presented to the VI International Urology Course, July, 1972, Barcelona, Spain.

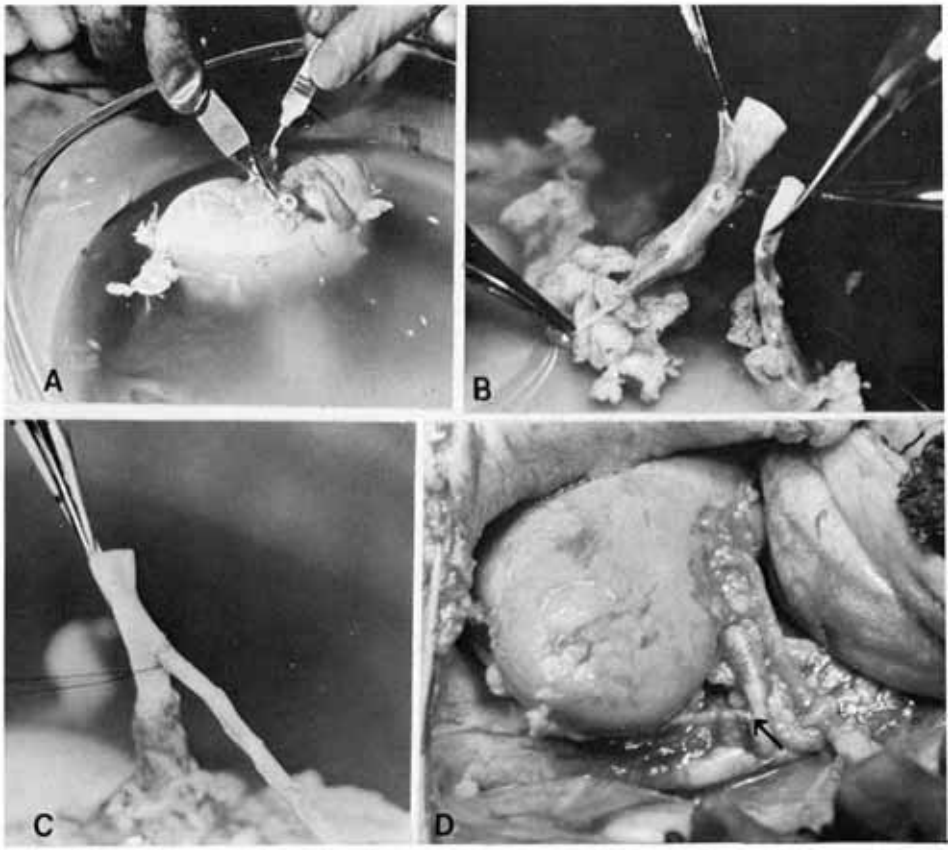

FIGURE 6. *(A) Vascular indication for extracorporeal surgery; renal homotransplant; polar artery of 1.5-mm. caliber. (B) Anastomosis end to side of polar artery to main arterial trunk. (C) Anastomosis is completed. (D) Homotransplant in iliac fossa; anastomosis of artery end to side with common iliac; arrow shows polar artery anastomosed to renal artery.*

surgical interventions, in which repair by conventional techniques was not possible and in which extracorporeal surgery was the only and maybe the last recourse.

The operation is justified since it avoids nephrectomy when the other kidney is not normal, avoids nephrostomy in a single kidney, and makes possible surgery which would be impracticable in situ.

The idea that nephrectomy is preferable if the other kidney is normal and, in the case of a single kidney, nephrostomy now ceases to be true.

Urology enters the stage of extracorporeal surgery with the successful repair of an organ outside the human body, and this opens to urology and surgery in other areas a new field of hopeful possibilities.

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NEW SURGICAL CONCEPTS IN REMOVING RENAL CALCULI

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Summary. After a critical study of the methods in use for the lithiasis surgery of the kidney; the great risk of some of them, the inefficacy and the danger—especially in the staghorn cases, the calicial stones or those contained in a pelvis of intrarenal type—are pointed out. The causes due to the operative lithogenic disease are analysed.

The submitted new surgery for the renal lithiasis is based upon the combined and simultaneous utilization of several principles—some known, such as the surgery of the kidney 'in situ', others modified, such as the posterior vertical lumbotomy replacing the classic and dangerous oblique lumbotomy, and finally some new ones, such as: the extracapsular approach to the renal sinus, the transverse intrasinusal pyelotomy incision and the selective calicotomy incision.

The exposure of the renal sinus by this new extracapsular approach is technically easy and offers visibility to all the intrarenal portion of the pelvis and to the major calices. It is completely bloodless.

For several reasons the transverse intrasinusal pyelotomy is far superior to the classic vertical pyelotomy.

The intrasinusal calicolithotomy is also a new term which must enter into the nomenclature and practice of urological surgery. The postoperative stage elapses with no leakage of urine so that drainage of the lumbar fossa is omitted thus avoiding complications. Patients leave the hospital by the seventh, and many by the fourth day after the operation.

This type of surgery has changed the prognosis for staghorn calculi and multiple calculi cases obtaining a total extraction with no injury to the renal parenchyma, and no trauma to the excretory tract. It is of great advantage to the patient who carries a simple pelvic calculus.

Following this kind of operation reinterventions do not offer inconveniences, but then, the sinusal space must be entered by way of another approach, the intracapsular one. The number of recurrences is notoriously lower than those observed with the classic techniques.

Our conclusions are based on an experience of 324 cases of no mortality, no complications and with excellent results.

Neue chirurgische Vorstellungen bei der Entfernung von Nierensteinen

Zusammenfassung. Nach einer kritischen Untersuchung der gebräuchlichen Methoden der Chirurgie der Nierensteine, wird auf das große Risiko einiger von ihnen, die Wirkungslosigkeit und die Gefahren – besonders bei Nierenbeckenausgußsteinen, Kelchsteinen oder bei intrarenal gelegenen Nierenbeckensteinen – hingewiesen. Die Gründe der operativen Steinbildungserkrankung werden analysiert.

Die vorgeschlagene neue Chirurgie der Nierensteine basiert auf der kombinierten und gleichzeitigen Anwendung mehrerer Prinzipien – einige bekannt, wie die Chirurgie der Niere «in situ», andere abgeändert, wie die hintere vertikale Lumbotomie anstelle der klassischen und gefährlichen schrägen Lumbotomie, schließlich einige neue, wie der extrakapsuläre Zugang an den Nierensinus, die transverse Pyelotomieschnittführung im Sinus und der selektive Calicotomieschnitt.

Die Darstellung des Nierensinus durch diesen neuen extrakapsulären Zugang ist technisch leicht und bietet Einsicht zum ganzen intrarenalen Anteil des Beckens und den größeren Kelchen. Er ist völlig blutfrei. Aus verschiedenen Gründen ist die transverse Pyelotomie im Sinus der klassischen vertikalen Pyelotomie überlegen.

Die Calicolithotomie im Sinus ist auch ein neuer Ausdruck der in die Nomenklatur und Praxis der urologischen Chirurgie Eingang finden muß. Das postoperative Stadium vergeht ohne Heraussickern von Urin, so daß die Drainage der Fossa lumbalis entfällt und so Komplikationen vermieden werden. Die Patienten verlassen das Krankenhaus am siebenten und viele am vierten Tag nach der Operation.

Diese Art der Chirurgie hat die Prognose bei Ausgußsteinen und multiplen Steinen verändert und durch völlige Extraktion ohne Verletzung des Nierenparenchyms und ohne Trauma des Ausscheidungstraktes. Sie ist von großem Vorteil für die Patienten, die einen einfachen Beckenstein tragen.

Nach einer derartigen Operation bringt ein nochmaliger Eingriff keine Ungelegenheiten, aber dann muß der sinusale Raum durch einen anderen Zugang, den intrakapsulären, eröffnet werden. Die Zahl der Rückfälle ist sicher niedriger als bei der klassischen Technik.

Unsere Folgerungen gründen sich auf die Erfahrungen bei 324 Fällen ohne Mortalität, ohne Komplikationen und mit hervorragenden Ergebnissen.

Nouvelles conceptions chirurgicales dans le traitement des calculs rénaux

Résumé. Après une étude critique des diverses méthodes chirurgicales du traitement de la lithiase urinaire dont les risques, l'inefficacité et les dangers sont exposés en détail spécialement en ce qui concerne les calculs coralliformes, les calculs caliciels ou les calculs du bassinet intrarénal, l'auteur expose les causes de la «maladie lithogénique chirurgicale».

Les nouveaux procédés chirurgicaux dans le traitement de la lithiase rénale sont basés sur la combinaison et l'utilisation simultanée de principes différents dont les uns sont connus comme la chirurgie du rein «in situ», les autres des techniques modifiées comme la lombotomie verticale postérieure qui remplace la lombotomie oblique classique et dangereuse, et enfin les techniques nouvelles comme la voie d'accès extracapsulaire jusqu'au sinus rénal, la pyélotomie transversale intrasinusale et l'incision sélective des calices.

La mise en évidence du sinus rénal par la nouvelle voie d'approche extracapsulaire est facile au point de vue technique. Elle permet une exploration de toute la portion intrarénale du bassinet et des calices principaux. L'intervention est exsangue.

Pour différentes raisons, la pyélotomie intrasinusale transverse est de loin supérieure à la pyélotomie verticale classique.

La calicolithotomie intrasinusale représente un nouveau terme qui doit prendre sa place dans la nomenclature et dans la pratique chirurgicale urologique. Il n'est pas nécessaire de faire un drainage de la fosse lombaire pour éviter des complications ultérieures. Les malades quittent l'hôpital en général le 7e jour, certains même le 4e jour après l'opération.

Le nouveau type d'intervention a modifié le pronostic des calculs coralliformes et des cas présentant des calculs multiples qui peuvent être extraits sans lésion du paren-

chyme rénale ou du système d'excrétion. Il est également avantageux pour un malade présentant un simple calcul pyélique.

Ce genre d'intervention ne s'oppose pas à une réintervention qui peut être effectuée sans inconvénients mais dans certains cas par voie d'approche intracapsulaire. Le nombre des réinterventions est notablement plus faible qu'après les techniques opératoires classiques.

Les conclusions sont basées sur une expérience de 324 cas traités sans mortalité, sans complications et avec d'excellents résultats.

Nuevas directrices en la cirugía del riñón litiásico

Resúmen. Después de un estudio crítico de los métodos que vienen siendo utilizados en la cirugía del riñón litiásico y en el que se destaca la inseguridad de los mismos, su ineficacia o su peligrosidad, particularmente frente a los coraliformes, los caliciales o los contenidos en pelvis de tipo intrarrenal se analizan las causas de la enfermedad litógena operatoria origen de bastantes recidivas.

La nueva cirugía en litiasis renal que proponemos, se basa en la utilización conjunta y simultánea de diversos principios, unos conocidos, como la cirugía del riñón «in situ», otros modificados, como la lumbotomía vertical posterior en sustitución a la clásica y lesiva lumbotomía oblicua y por último, otros nuevos, como son: la vía extracapsular de acceso al sinus renal, la incisión de pielotomía transversa intrasinusal y la incisión de calicotomía selectiva.

La exposición del sinus renal a través de esta nueva vía de acceso extracapsular es técnicamente fácil, permite la visualización de la totalidad de la porción intrarrenal de la pelvis y de los grandes cálices. Es completamente exangue.

La incisión de pielotomía intrasinusal transversa, descrita por vez primera es, por muy diversas razones, muy superior a la clásica pielotomía vertical.

Tambien la calicolitotomía intrasinusal es, así mismo, un nuevo termino que debe entrar en la nomenclatura y en la práctica quirúrgica.

El postoperatorio transcurre sin extravasación de orina – por lo que puede omitirse el drenaje de la celda lumbar – y carente de complicaciones. Los pacientes abandonan la clínica al séptimo día de la intervención, muchos lo hicieron entre el tercer y el cuarto día de la operación.

Este tipo de cirugía ha cambiado el pronóstico de la litiasis coraliforme y de la calculosis múltiple al lograrse su completa extracción sin lesionar el parénquima renal y sin traumatismo de las vías excretoras. Representa así mismo una importantísima ventaja para el enfermo portador de un simple cálculo piélico.

Cuando el enfermo fué anteriormente intervenido siguiendo las anteriores directrices, la reintervención no ofrece mayores dificultades.

El número de recidivas ha sido notoriamente muy inferior en relación con las que se observan siguiendo las técnicas clásicas.

Nuestras conclusiones están respaldadas por una estadística de 324 casos sin mortalidad, sin complicaciones y con excelentes resultados.

We must admit that our techniques now in use for the surgical treatment of renal lithiasis do not always allow an easy and safe removal of the calculi. They are not always innocuous to the kidney and leave a considerable number of postoperative complications.

Operations for recurrence are always difficult to carry out and sometimes the final result is the excision of a still functioning kidney.

The reccurrence as an operative sequel is much more frequent than it is believed, and it is not necessary to employ the term of 'lithogenous focus'.

Urological surgery has progressed in many aspects, however in the field of renal lithiasis our techniques are practically the same as those of the last century, with all the inconveniences.

The surgical procedure of the urologist in a renal calculus can be briefly described as follows: an oblique lumbotomy is performed, the kidney is released from its anatomical connections and it is usually pulled through the lumbar incision; then the pelvis is incised vertically in its extrarenal portion and forceps are introduced to remove the calculus. If this has emigrated to a calyx, or if there are multiple stones, or perhaps, a staghorn calculus, the surgeon performs one or several 'small' nephrotomies and in combination with the introduction of the finger through the pyelotomy, tries to localize and remove them. After these manipulations, which are often very tedious, always traumatic, and not always successful, the surgeon replaces the kidney in its cell, introduces several drainages, and sutures the lumbotomy. The immediate postoperative period is characterized by leakage of urine for some days or weeks, and after a period of 15 to 20 uncomfortable days, the patient leaves the hospital.

We can criticize the present renal surgery as follows:

The incision of oblique lumbotomy is the most lacerating of all the incisions performed on the human body: it sections transversely important muscles and nerves leaving as sequels lumbar hernias, paralysis of the hemi-abdomen and neuralgia. The size of the incision is disproportionate to the size of the stone to be removed, making any re-operation difficult. It also has some inconveniences: The surgeon has a good approach to the external border of the kidney, but a bad one to the internal edge of the renal hilus, where the pelvis and the pedicle are situated, i.e. the most important part for the surgeon.

The exteriorization of the kidney from its cell ist not only useless in most of the cases, but it has sequels. At the end of the operation, the kidney without its anatomical relations falls into the lumbar space, takes up defective positions and often becomes a ptosed kidney. This ptosis is the cause of vascular ailments—shown on the renogram—and makes the passing of urine difficult, which in turn is the cause of recurrence. If at the end of the lithotomy the surgeon

performs a nephropexy, the patient must remain in bed for 15 to 18 days in complete immobilization with all its inconveniences. A re-exposure of these kidneys, surrounded by a sclerose perinephritis is extremely difficult and dangerous.

The vertical pyelotomy has an important number of inconveniences. By the prolongation to the ureter, the union is often damaged and a post-operative stricture may result. The suture of a vertical pyelotomy does not avoid the leakage of urine and it is possible that the same sutures near the union are the cause of this stricture. This type of incision is antiphysiological because it sections the spiral muscular fibers of the pelvis transversely, impeding the contraction of the muscular layer.

The leakage of urine through the pyelotomy incision is normal, practically constant in the first few days, and has its inconveniences. Apart from the subjective ones for the patient himself, who sees his urine escaping from the wound, there is usually the obligation for a longer period of hospitalization, as well as the irritating action of the urine producing cellulitis which involves the kidney, the pelvis and the ureter. This cellulitis causes a sclerose magma which fixes the kidney to the walls of its fossa, making any future operation very difficult.

Now, the complications and difficulties of surgery as previously described are manifold in the staghorn and calicial, or multiple pyelocalicial calculi, as well as in those contained in an intrarenal pelvis, and offer important technical difficulties. The decision for a surgical intervention is, in many cases, made in spite of the unsafe complete extraction and the risk of causing big damage to the kidney.

I believe that everyone will agree with me that if a staghorn could be removed in the same and easy manner as a single pelvic stone is extracted, i.e. with a safe extraction, no damage to the renal parenchyma and no trauma to the excretory tracts, nobody would be against surgical treatment, because all of us know that sooner or later, the staghorn will cause the destruction of the kidney.

We must admit that if we support surgical abstention in the staghorn, it is because we know the big risk the kidney runs—as well as the patient—i.e. the big valve nephrotomy.

And if sometimes we raise an objection about leaving a kidney with multiple stones, it is because we know that their extraction

will be incomplete or because the posterior pyelotomy in combination with a small nephrotomy will cause serious damage to the vessels and excretory tracts of the kidney.

We often insist on explaining to the bearer of caliceal lithiasis that his calculus is not the cause of his sufferings (for which he is consulting us, and by which we discover his disease), because by so doing, we avoid the big difficulties we should have to face at the operation, the localization and the extraction.

This is as far as the technical part is concerned. Concerning the prognosis the records show that the higher frequency of recurrences is produced by the pyelocalicial calculi and staghorns, being still higher after a nephrolithotomy than a pyelolithotomy. Its causes: free calculi, fragments overlooked or deliberately avoided stones; laceration and tearing of the mucosa of the neck of the calyx, or the renal papilae by the forceps; the tearing of the ureteropelvic junction by the vertical incision of the pelvis; or by the rough introduction of the surgeon's finger with the consequent cicatricial stricture, a real cause of a good number of recurrences. All this constitutes the post-operative lithogenous disease.

However the number of recurrences is, in some cases, inferior to what is expected, because of the formation of the postoperative renal insufficiency caused by the nephrotomy (infarct, arterio-

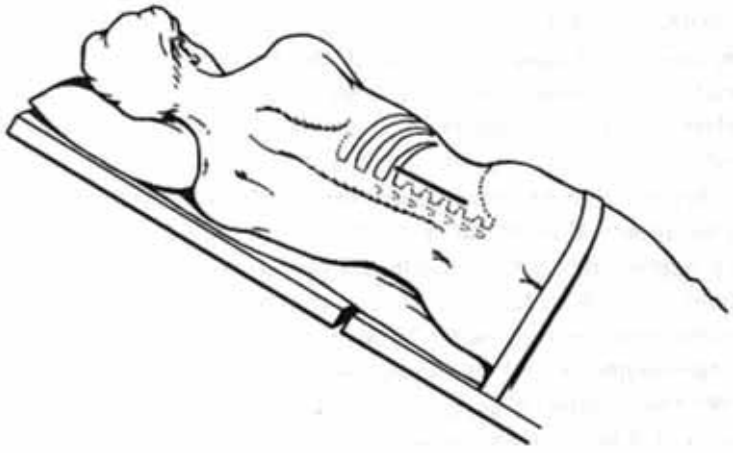

Fig. 1. Posterior vertical lumbotomy incision.
Abb. 1. Hintere vertikale Lumbotomieincision.
Fig. 1. Incision de la lombotomie verticale postérieure.

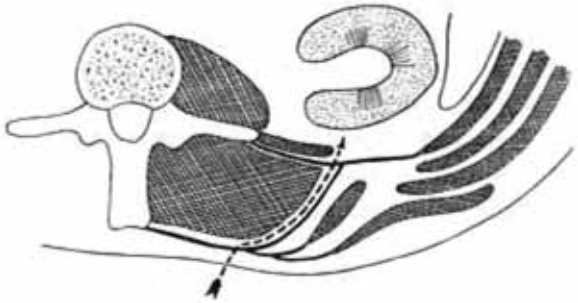

Fig. 2. Schematic section. The arrow points to the approach to be followed in the posterior vertical lumbotomy.

Abb. 2. Schematischer Querschnitt. Der Pfeil zeigt den Zugang, dem man bei der hinteren vertikalen Lumbotomie folgt.

Fig. 2. Coupe schématique. La flèche pointillée montre la voie d'accès suivant la lombotomie verticale postérieure.

Fig. 3. Once the sacrolumbar group of muscles and the quadratus lumborum muscle have been retracted, the kidney is tilted, its hilus appearing perpendicular to the surgeon.

Abb. 3. Wenn erst die sacro-lumbale Muskelgruppe und der Quadratus lumborum auseinandergedrängt sind, dreht sich die Niere, so daß der Nierenstiel zum Chirurgen zeigt.

Fig. 3. Après écartement du groupe des muscles sacro-lombaires d'une part et du carré des lombes d'autre part, le rein est récliné de façon à présenter le hile.

venous fistulae, aneurysm by excision, etc. when the concentration power of the kidney is reduced.

Our surgical attitude in this matter is at present based on the simultaneous utilization of the following surgical procedures: *posterior vertical lumbotomy, surgery of the kidney 'in situ', intrasinusal approach to the pelvis and renal calices, transverse pyelotomy and longitudinal calicotomy.*

The posterior vertical lumbotomy (fig. 1, 2, 3) has the following advantages over the oblique lumbotomy:

- muscular fibres are not sectioned, only the aponeurosis; so that there is no risk of hernias.
- Generally, no nerve is sectioned, neither the twelfth intercostal, nor the abdomino-genital; there are no sensitive, nor motor complications of the wall, no parathesia, nor paralysis of the oblique muscles.
- The pelvis and the upper ureter are reached posteriorly, i.e. directly and perpendicularly over the pelvis, thus not being necessary to free the kidney. This way leads directly to the renal pelvis.
- The operative area is quite sufficient, even for very stout patients and eventually the incision of the superior border may be increased laterally, ventrally and forwardly.

All these manipulations are carried out instrumentally; the surgeon's hand, having no contact with the kidney and the exteriorization is avoided.

- The fact that by this approach, many cases can easily be reoperated is of great importance, i.e. having been previously operated by the same approach.
- This lumbotomy is not painful and the patient can leave the bed and walk within 24 h after the operation.

The surgical technique of this lumbotomy has been previously published. In brief, it is a personal modification of the primitive operation performed by SIMON. The vertical incision is made parallel to the spinal processes, 2 inches distant, and over the sacrolumbar mass, and starts from the border of the twelfth rib to one or two inches from the iliac bone going through the following layers: skin and aponeurosis of the latissimus dorsi, leaving the sacrolumbar mass exposed whose external edge is retracted towards the vertebral column. Then the posterior aponeurotic leaf of the transversalis muscle appears which is extremely thin and covers the posterior aspect of the quadratus lumborum muscle which must be longitudinally incised very close to the insertion in the transverse processes; the aponeurosis is then freed from the quadratus, appearing totally exposed, and when its external edge is retracted towards the vertebral column one enters into the lumbar fossa.

The suture is made in two layers: the transversalis aponeurosis and the latissimus dorsi. The approach we describe differs from

Simon's in that the quadratus is not sectioned and the operation is therefore simplified, the muscular fibres are not injured and the operative exposure is larger.

The surgery of the kidney 'in situ' has well known advantages for removing stones from the pelvis or calices; there is no need to free the kidney from ist natural means of support nor to exteriorize it. This renal manipulation carries along a certain morbidity rate. If we want to remove a pelvic stone, we must free exclusively the posterior wall of the pelvis avoiding the manipulation of the ureteropelvic junction, and of course the lumbar ureter. This surgery, highly atraumatic and selective, will make any possible reoperation easier and will not upset the secretory and exretory function of the kidney.

The approach to the renal sinus. L. SURRACO in 1939 described—for the first time—the approach to the renal sinus. BABICS, HELLSTRÖM and ABOULKER presented afterwards modifications in this approach. All of them agree by calling attention to the difficulties in removing the big stones and the ones situated in the intrarenal pelvis, emphasizing the fact that the classic techniques forcing sectioning of the renal parenchyma (PAPIN, MARION, PRATHER) involve a serious danger causing damages to the vessels of the kidney, and decreasing its functional capacity. They described an approach to the sinus to avoid these complications.

Surraco's technique consists in the decapsulation of the posterior aspect of the kidney reflecting this part of the capsule towards the hilus, he places a blunt retractor retracting the posterior edge of the kidney giving the surgeon a view of the intrarenal part of the pelvis and of the initial part of the major calices. The way of access to the sinus is intracapsular, i.e. between the fibrous capsule and the parenchyma.

Doubtless this procedure makes the access to the renal pelvis possible, and constitutes an important improvement, but anyway the area is very limited and, only in the most fortunate cases, permits visibility of the initial part of the major calices, because the retractor cannot forcibly retract the renal border without the risk of tearing the parenchyma, which, when decapsulated, loses consistency and its injury causes profuse hemorrhage interfering with surgical manipulations. It is nearly impossible to remove a staghorn by this approach, so that it must be associated with nephrotomy with all its inconveniences, or the retropyelic artery must be

sectioned, thus causing a large infarct of the posterior half and the inferior pole of the kidney.

The approach to the renal sinus, later on described, is very different from the former ones, it is extracapsular, i.e. inside the retropyelic artery with no risk of damaging this artery or its branches; among other advantages it offers greater visibility to the renal sinus permitting the access to all the calices up to the fornix; it is bloodless and harmless to the parenchyma and excretory tracts. It often permits the extraction of an inarticulated staghorn in one piece, the localization of calicial stones by sight, not by touch, instrumental contact or by chance.

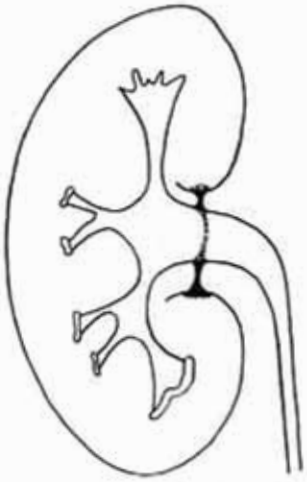

Fig. 4. The fibrous capsule sends a bunch of fibres which surround and adhere to the pelvis, forming the capsular diaphragm that closes the entrance to the renal sinus. (From NARATH: Renal pelvis and ureter. Grune & Stratton, ed. New York 1951.)

Abb. 4. Die fibröse Kapsel sendet ein Netzwerk von Fasern aus, welches das Nierenbecken umgibt und an ihm haftet und so ein kapsuläres Diaphragma bildet, das den Eingang zum Nierenhilus verschließt. (Aus NARATH: *Renal pelvis and ureter. Grune & Stratton, ed. New York 1951.)*

Fig. 4. La capsule fibreuse présente des faisceaux de fibres disposées tout autour du bassinet et qui forment un diaphragme s'opposant à l'entrée dans le sinus rénal.

Nowadays, whatever the type of calculi may be, even the easiest ones, those in extrarenal pelvis—we approach them this way —which in combination with the transversal pyelotomy offers a very short postoperative period, without complications and no leakage of urine through the wound from the start.

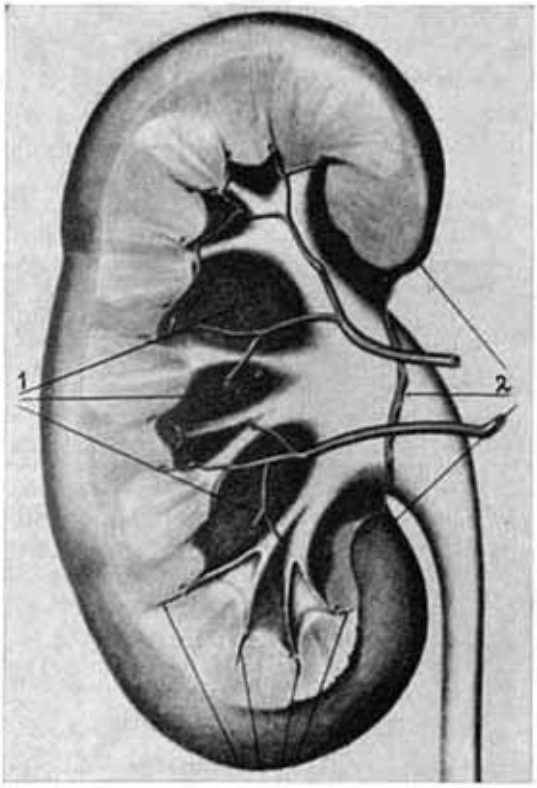

Fig. 5. (1) Renal sinus and its relation with the vessels, calices and pelvis. (2) The entrance to the hilus is closed by an area of capsular adhesions. (From NARATH: Renal pelvis and ureter. Grune & Stratton, ed. New York 1951.)

Abb. 5. (1) Nierensinus und die Beziehungen zu den Gefäßen, Kelchen und Nierenbecken. (2) Der Eingang zum Hilus ist durch ein Gebiet von kapsulären Adhäsionen verschlossen. (Aus NARATH: *Nierenbecken und Harnleiter, Grune & Stratton, ed. New York 1951.)*

Fig. 5. (1) Sinus rénal et ses rapports avec les vaisseaux, les calices et le bassinet. (2) L'entrée du hile est fermée par des adhérences fibreuses.

This way of access is based on the topographic situation of the elements of the pedicle at the entrance to the renal hilus and their relation with the parenchyma inside the sinus. The hilus is formed by vessels, nerves, lymphatics, renal pelvis and fat, the latter being a part of the perirenal fat which accompanies all the elements of the pedicle way up inside the intraparenchymal space called the renal sinus, and it is occupied by the intrarenal portion of the pelvis, the calices, vessels, lymphatics and nerves, surrounded by

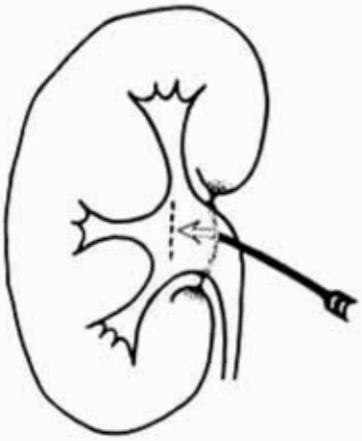

Fig. 6. Drawing shows our extracapsular approach to the sinus renalis. The arrow points to the approach to be followed when performing an intrasinusal pyelotomy: you have to get in between the capsular diaphragm and the renal pelvis.

Abb. 6. Die Zeichnung zeigt unseren extrakapsulären Zugang zum Sinus renalis. Der Pfeil zeigt den Zugang, dem man bei einer Pyeolotomie im Sinus folgt: Man muß zwischen kapsulärem Diaphragma und Nierenbecken eingehen.

Fig. 6. Schéma montrant notre approche extracapsulaire jusqu'au niveau du hile. La flèche montre la voie d'approche qui est suivie par une pyélotomie intrasinusale. Il convient de passer entre le diaphragme capsulaire et le bassinet.

adipose tissue with connective fibre, which provides the easiness for the free movements of the calices in the sinus. An important deduction: *there is no firm adherence between the renal parenquima on one side, the pelvis and major calices on the other, because the internal leaf of the fibrous capsule gets in between them.*

This fibrous capsule of the hilus sends a thick bunch of fibres (fig. 4, 5) that surrounds and adheres to the pelvis, forming the capsular diaphragm which closes the entrance to the sinus, and isolates it from the retroperitoneal space (DISE).

According to the classical anatomical and surgical texts, the renal hilus is too narrow to enable a complete exploration, both anatomical and surgical of the kidney, and its study could only be done by the use of nephrectomy or histological sections.

The sinus, therefore, offers itself as an unexplorable space for simple inspection.

'The agglomeration of the vessels opposes any intent for intrasinusal pyelotomy and limits the external debridement of the extrahilar posterior pyelotomy' (F. PAITRE).

However, our findings show that this concept is not exact and that a perfectly definite individualized space exists between the adventitia of the posterior aspect of the renal pelvis and the capsular diaphragm surrounding it. Crossing this diaphragm the surgeon (fig. 8) enters the intrasinusal space which can be totally explored without injuring any vessel.

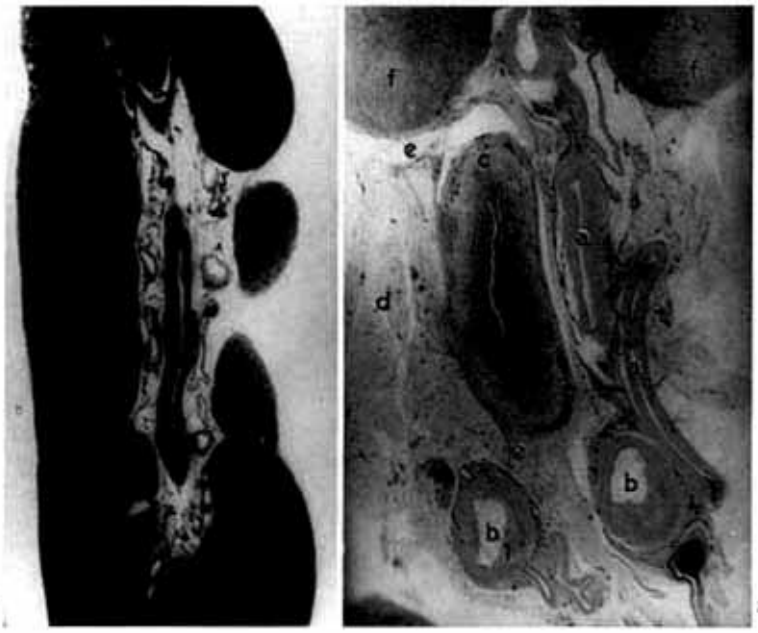

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Fig. 7. Microphotography of a sagittal section of the kidney at the sinus level. Note how the pelvis and the vessels are surrounded by a lax cellular tissue which isolates them from the fibrous capsule (Internal leaf).

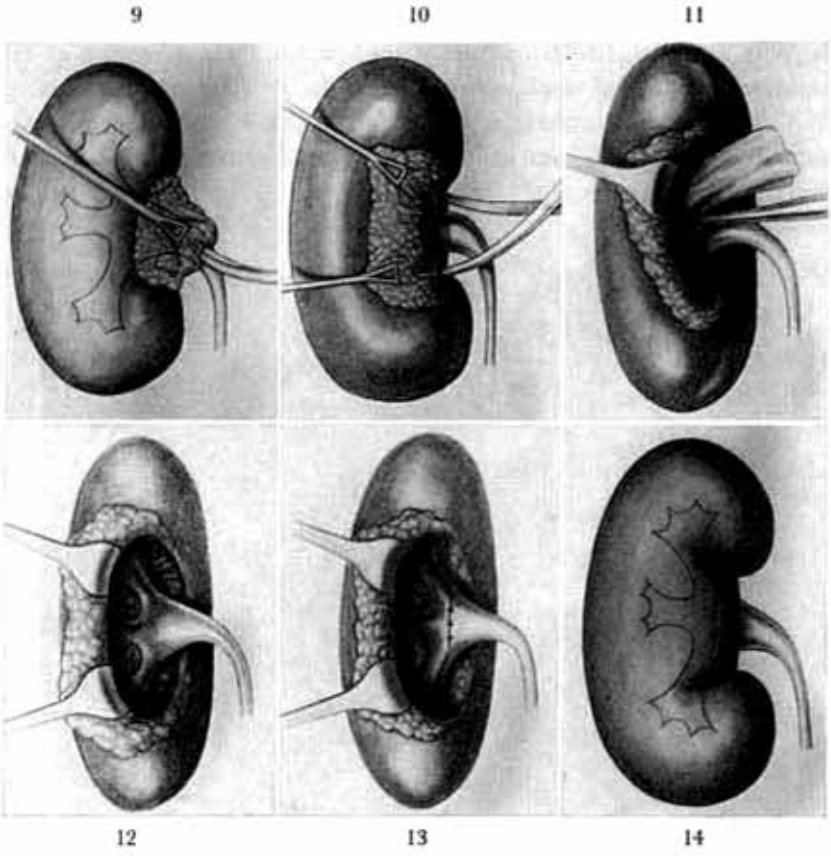
Fig. 8. Sagittal section of the kidney very close to its internal edge. a, vein; b, arteries; c, renal pelvis; d, adipose tissue entangled by connective fibres; e, capsular diaphragm surrounding the pelvis; f, renal parenchyma.

Abb. 7. Mikrophotographie eines Sagittalschnittes der Niere am Sinusniveau. Man beachte, wie das Becken und die Gefäße von einem lockeren zellulären Gewebe umgeben sind, das sie von der fibrösen Kapsel isoliert (inneres Blatt).

Abb. 8. Sagittalschnitt der Niere, sehr dicht zum Medialrand. a) Vene, b) Arterien, c) Nierenbecken, d) Fettgewebe eingeschlossen von Bindegewebsfasern, e) kapsuläres Diaphragma, das das Becken umgibt, f) Nierenparenchym.

Fig. 7. Microphotographie d'une section sagittale du rein au niveau du sinus. A noter que le bassinet et les vaisseaux sont entourés d'un tissu cellulaire lâche les isolant de la capsule fibreuse (feuillet interne).

Fig. 8. Section sagitalle d'un rein montrant la fermeture étanche au niveau de son bord interne: a) veine, b) artères, c) bassinet, d) tissus adipeux bordés par les éléments fibreux, e) diaphragme capsulaire entourant le bassinet, f) parenchyme rénal.



The technique which we present is extremely simple. It consists in identifying the ureteropelvic junction reflecting towards the kidney the peripelvic cellular tissue with the aid of very curved blunt-pointed scissors. By a blunt dissection the adventitia of the pelvis is freed from the peripelvic adipose cellular tissue (fig. 7).

The scissors must enter in direct contact with the adventitia. When entering underneath the capsular diaphragm the scissors will be opened energetically thus tearing the diaphragmatic circle which offers a slight resistance. We are at present at the entrance of the sinus placing immediately an adequate retractor which pulls the mass of the peripelvic adipose tissue, the internal lip of the posterior edge of the kidney and the retropelvic vessel: the whole of this is reflected upwards without danger of tearing the parenchyma, which, being protected by the capsule and the peripelvic

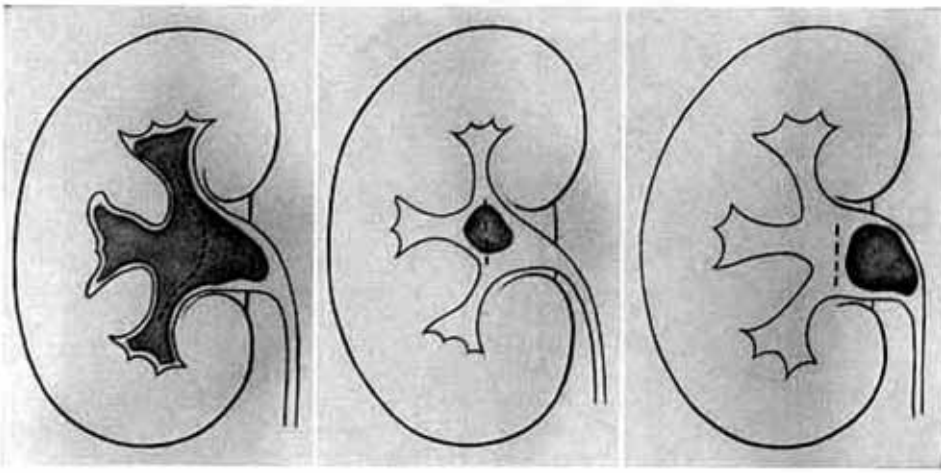


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Fig. 15. Pyelocalicial incision for the removal of staghorn calculi.
Fig. 16. Transverse intrasinusal incision of intrarenal pelvis.
Fig. 17. Transverse intrasinusal incision for the removal of a calculus from an extrarenal pelvis.

Abb. 15. Nierenbeckenkelchschnittführung zur Entfernung von Ausgußsteinen.
Abb. 16. Intrasinusaler Querschnitt eines intrarenalen Nierenbeckens.
Abb. 17. Quere intrasinusale Incision zur Entfernung eines Steines aus einem extrarenalen Nierenbecken.

Fig. 15. Incision pyélo-calicielle pour extraction d'un calcul coralliforme.
Fig. 16. Incision transversale intrasinusale du bassinet.
Fig. 17. Incision transversale intrasinusale pour extraction d'un calcul dans un bassinet extrarénal.

fat has a great resistance and elasticity. At this moment a wet and unfolded gauze is progressively introduced into the sinus until filling it, the gauze is withdrawn and another retractor of the same or smaller size is introduced. With both retractors the posterior half of the kidney is firmly lifted up making the organ turn, so that the sinusal space offers itself perpendicularly to the surgeon with a complete view of the pelvis and the posterior aspect of the major calices (fig. 10). When this manipulation is correctly carried out, it is completely bloodless.

At the same time that the retractors open the sinusal space, they simultaneously pull the kidney towards the surgeon.

In the case of a pyelitis, which is rather frequent, this approach is somewhat more difficult. In this case the lancet must section the thick sclero-lipomatose stratum in the posterior aspect of the pelvis

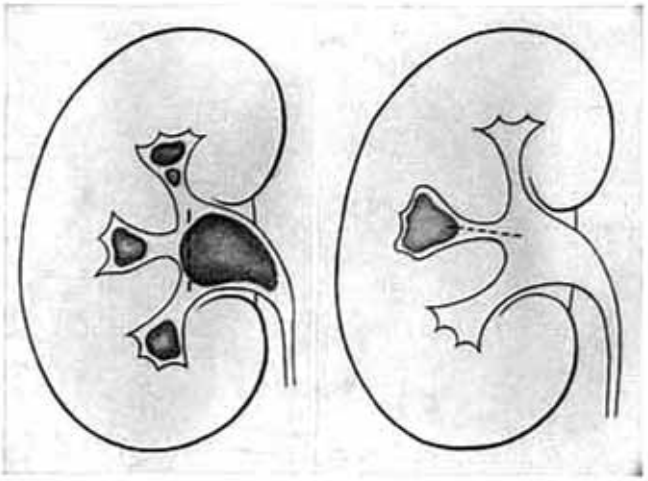

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Fig. 18. Transverse incision of the upper edge of the pelvis for multiple pyelocalicial calculi.

Fig. 19. Calicotomy intrasinusal incision.

Abb. 18. Querschnitt am oberen Beckenrand wegen mehrerer Becken- und Kelchsteine.

Abb. 19. Intrasinusale Calicotomieschnittführung.

Fig. 18. Incision transversale à la limite supérieure du bassinet dans un cas de calculs pyélocaliciels multiples.

Fig. 19. Tracé d'incision d'une calicotomie intrasinusale.

until the pelvic adventitia is reached, where the blunt dissection already described is started, or the highest portion of the ureter is identified and from there by blunt retrograde dissection we can follow on until the sclerolipomatose magma, going across the detachable space between the pelvic adventitia and the peripyelitis. It is advisable to free the pelvis from the sclerose stratum which covers it to simplify the peristaltic movements in the future.

Not all the calculi can and must be extracted by this approach, per expl. the big ones, ball-shaped, situated in the caliceal space covered by a thin stratum of parenchyma which can easily be felt on the outer surface of the kidney and logically will be extracted by nephrotomy because the sections being bloodless, owing to the atrophy of the parenchyma, practically prevent an infarct. Or, in the case of a solitary stone, with big dilatation of the inferior calyx and atrophy of the corresponding parenchyma, a polectomy and uretero caliceal anastomosis may be advisable (Reggeti).

Not even in surgical interventions for recurrences, because, after the first operation, the walls of the renal pelvis and calyces

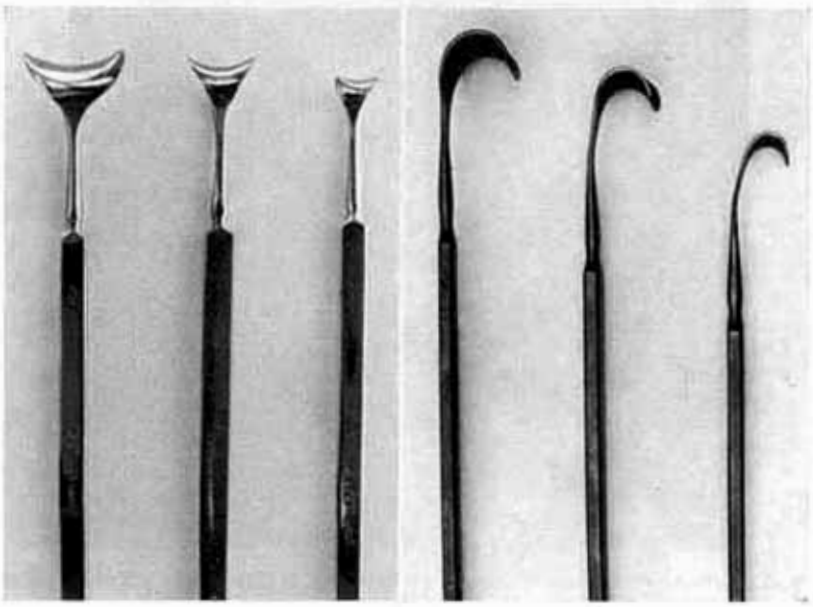

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Fig. 20. Renal sinus retractors, front view.
Fig. 21. Renal sinus retractors, side view.

Abb. 20. Nierensinushaken, Vorderansicht.
Abb. 21. Nierensinushaken, seitliche Ansicht.

Fig. 20. Ecarteurs du sinus rénal. Vue antérieure.
Fig. 21. Ecarteurs du sinus rénal. Vue latérale.

adhere firmly to the inner sheet of the fibrous capsule and then it is not possible to reenter the 'sinus renalis' through the extracapsular approach.

In the cases of a very closed and small hilus this approach offers a good enough view and manipulation area to ensure an easy extraction of a large calculus, but this is less frequent.

In the exploratory lumbotomies for unknown hematurias, the exploration of the sinus space by this approach has given us the chance to find, in one case, two serum cysts, and in another, an angioma, both intrasinusal, and in which the kidney, even decapsulated, was of normal appearance. No exploratory lumbotomy should be ended without the inspection of the renal sinus.

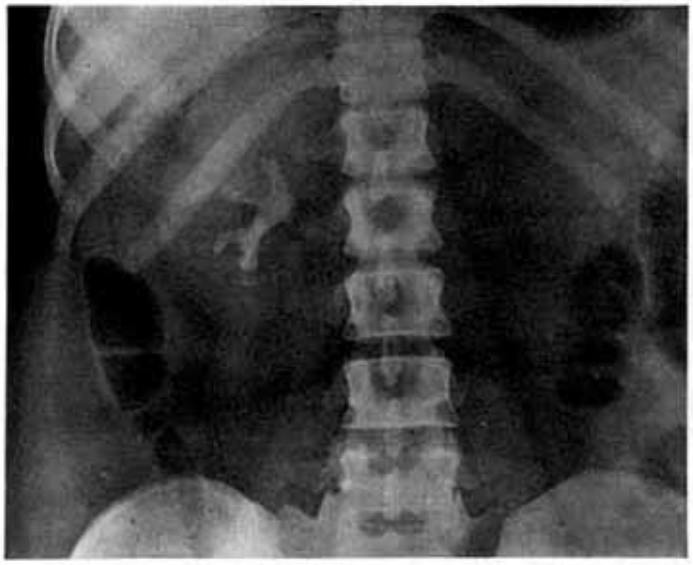

Fig. 22. (Obs. 1). Flat plate of the abdomen (K. U. B.). Staghorn calculus in right kidney.

Abb. 22. (Obs. 1) Abdomenleeraufnahme (K. U. B.) Ausgußstein in der rechten Niere.

Fig. 22. (Obs. 1). Cliché à vide A. P. Calcul coralliforme du rein droit.

The Incision of the Intrasinusal Transverse Pyelotomy

The vertical pyelotomy is the type of incision usually used. We have already mentioned the complications and the sequels of this type of incision. It is not anatomical. The interesting works of GOMEZ BOSQUE on the functional anatomy of the ureteric musculature show that the muscular stratum is constituted by a spiral system that runs along the organ, in different directions, crossing each other.

This system of spiral muscular bundles assumes a different inclination according to the level of the excretory tract, so that in the superior end there are only 'circular fibres', i.e. muscular spirals of little inclination. Therefore the transverse pyelotomy is the most logical and anatomic incision if performed in the same direction as the pyelic musculature, (fig. 11) with no risk of tearing towards the ureter. On the other hand, this incision which must be carried out in the upper part of the pelvis, i.e. intrasinusally, permits a view to the entrance of the calices, easing the exploration of all of them. The ureteropelvic junction is so easily explored and

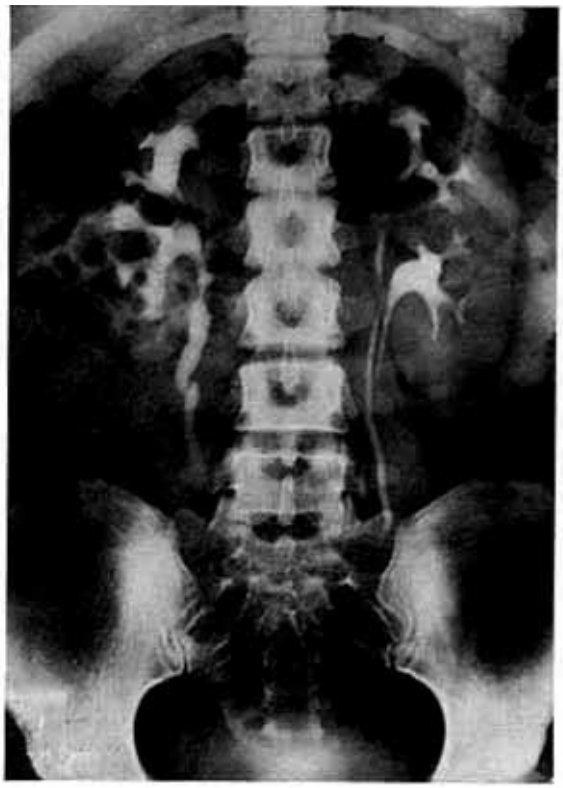

Fig. 23. (Obs. I). Preoperative I. V. P.
Abb. 23. (Obs. I) Präoperatives intravenöses Pyelogramm.
Fig. 23. (Obs. I). U.I.V. préopératoire.

in the case of a stricture a pyeloplasty may be performed independently of the transverse pyelotomy incision.

After the extraction of the calculi, some sutures can be placed using a very fine atraumatic needle with catgut 00000 to confront the edges of the pelvis.

This transverse intrasinusal incision is more physiological, has no complications, there is no leakage of urine and the hospitalization days are shortened. *Some patients leave the hospital on foot, the third day after the operation.*

In large caliceal stones we perform a longitudinal incision along the caliceal infundibulum (fig. 17). *This calicotomy incision* does not affect the mobility of the calyx because its muscular stratum is longitudinal. The incision which affects the 'musculus sphincter

Fig. 24. (Obs. I). Surgical specimen.
Abb. 24. Operationspräparat (Obs. I).
Fig. 24. (Obs. I). Pièce opératoire.

calycis' also brings solution to the dysfunction in the caliceal dis-ectasis when performing the sphincterotomy of the pyelocaliceal union.

The longitudinal incision of the calyx must not reach the fornix because the pericaliceal veins would bleed and, although the lesion is not important, this would reduce visibility.

Abb. 25. (Obs. I) Postoperative Nieren- und Blasenübersichtsaufnahme, vollständige Entfernung des Steines.
Abb. 26. (Obs. I) Postoperatives intravenöses Pyelogramm.
Abb. 27. (Obs. II) Leeraufnahme. Zahlreiche Becken- und Kelchsteine in der rechten Niere.
Abb. 28. (Obs. II) Praeoperatives intravenöses Pyelogramm. Deutliche Funktionseinschränkung der rechten Niere.
Fig. 25. (Obs. I). Cliché à vide postopératoire. Le calcul a été extrait en totalité.
Fig. 26. (Obs. I). U.I.V. postopératoire.
Fig. 27. (Obs. II). Cliché à vide. Multiples calculs pyélocaliciels à droite.
Fig. 28. (Obs. II). U.I.V. préopératoire. Important déficience fonctionellen du rein droit.

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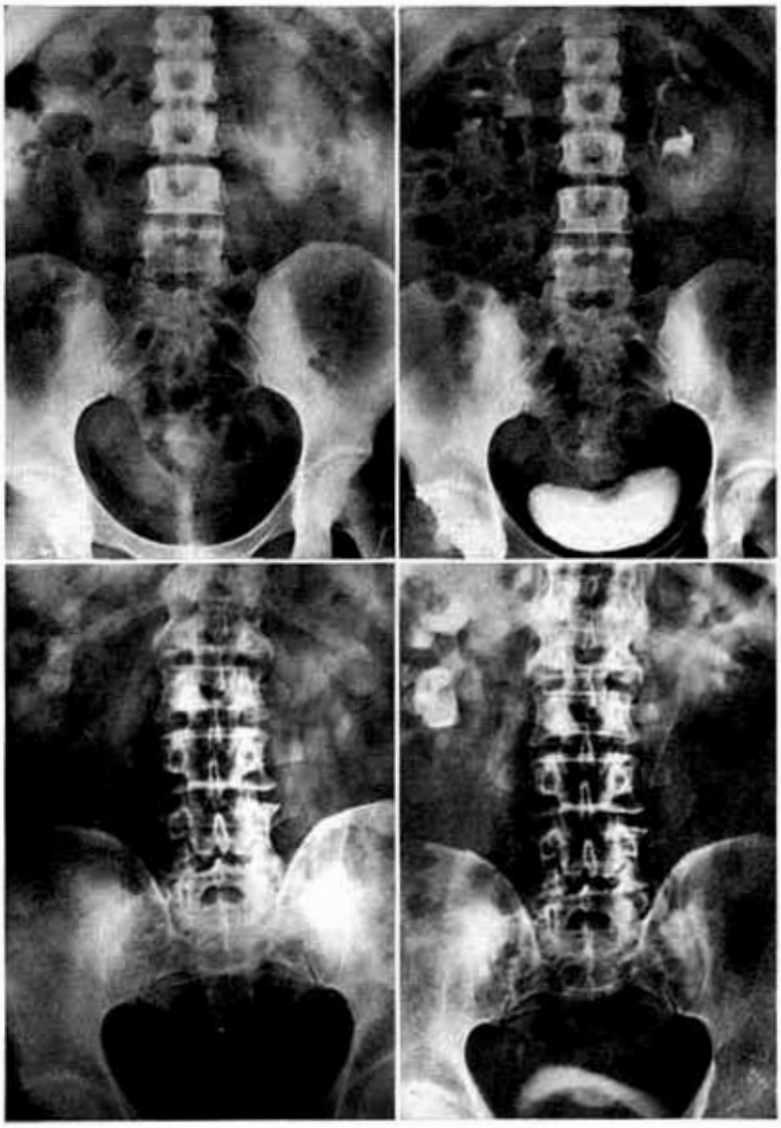

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Fig. 25. (Obs. I). Postoperative K. U. B. Complete removal of the calculus.
Fig. 26. (Obs. I.) Postoperative I. V. P.
Fig. 27. (Obs. II). K. U. B. Multiple pyelocalicial calculi in the right kidney.
Fig. 28. (Obs. II). Preoperative I. V. P. Important functional deficit from the right kidney.

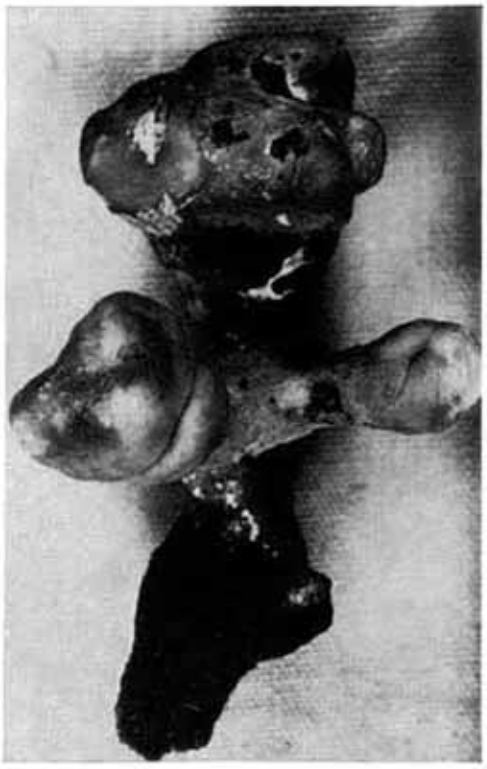

Fig. 29. (Obs. II). Calculi specimen.
Abb. 29. (Obs. II) Steinpräparat.
Fig. 29. (Obs. II). Pièce opératoire.

When the renal hilus is closed or too small, the calicotomy is difficult, and sometimes impossible to perform. These cases are very odd, and the calculus is then removed by transverse intrasinusal pyelotomy which permits the identification of the caliceal opening and the introduction of the forceps therein.

We usually make one or two sutures of the calyx but if it happens to be ectasic it must not be sutured and the posterior epithelialization starting from the borders will provide a larger calibre to the caliceal neck which will permit emptying with no difficulty.

On principle, the incision of the pelvis or the calices will depend on the type of the calculus and its situation.

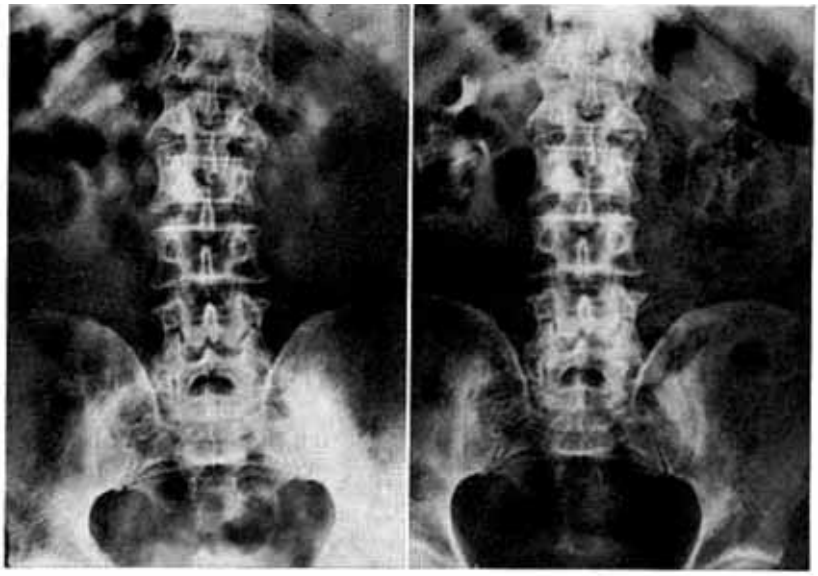

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Fig. 30. (Obs. II). Postoperative K. U. B. Total and complete removal of calculi.
Fig. 31. (Obs. II). Postoperative I. V. P. Functional recovery of the right kidney.

Abb. 30. (Obs. II) Postoperative Leeraufnahme. Vollständige Steinentfernung.
Abb. 31. (Obs. II) Postoperatives intravenöses Pyelogramm. Funktionale Erholung der rechten Niere.

Fig. 30. (Obs. II). Cliché à vide postopératoire. Les calculs ont été extraits en totalité.
Fig. 31. (Obs. II). U. I. V. postopératoire. Récupération fonctionnelle du rein droit.

When a staghorn is extracted a curved incision is performed (fig. 13), that will go from the upper calyx to the lower one, across the pelvis. This large incision which is longitudinal on all the upper calyx is continued transversally in the pelvis, being longitudinal again in the lower calyx. The pelvic part of the stone is freed by the aid of a stylet used as a lever from the pelvis and it is extracted with its ramifications by gentle manipulation. Usually the hour-glass-shaped calculi are easily extracted by sectioning the whole caliceal infundibulum as well as its union with the pelvis, nephrectomy not being necessary. If it is articulated or there are multiple stones, when the pyelicea calculus is removed the orifices of the medium calices are visible and the extraction of the calculi is made under eye control. If the stone is larger than the infundibulum, this must be dilated by the aid of forceps, and if it still refuses to pass through, a longitudinal incision of its neck must be carried out.

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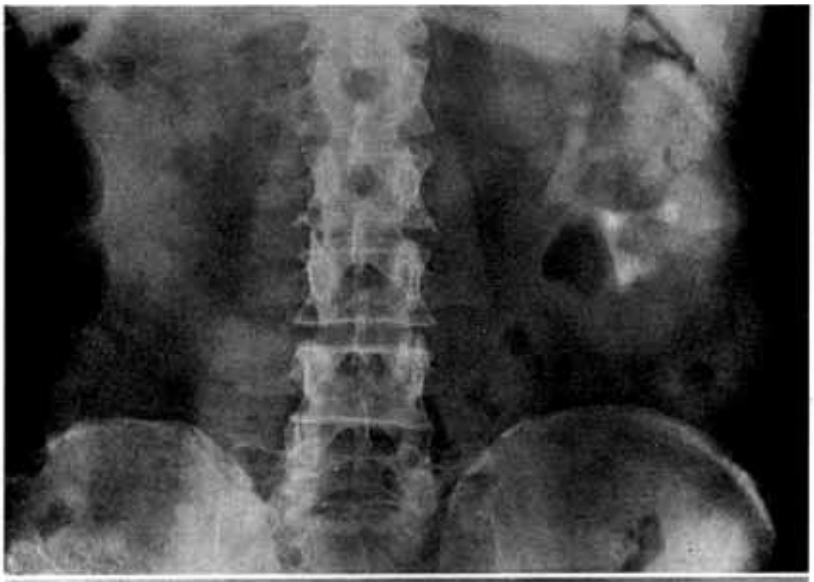

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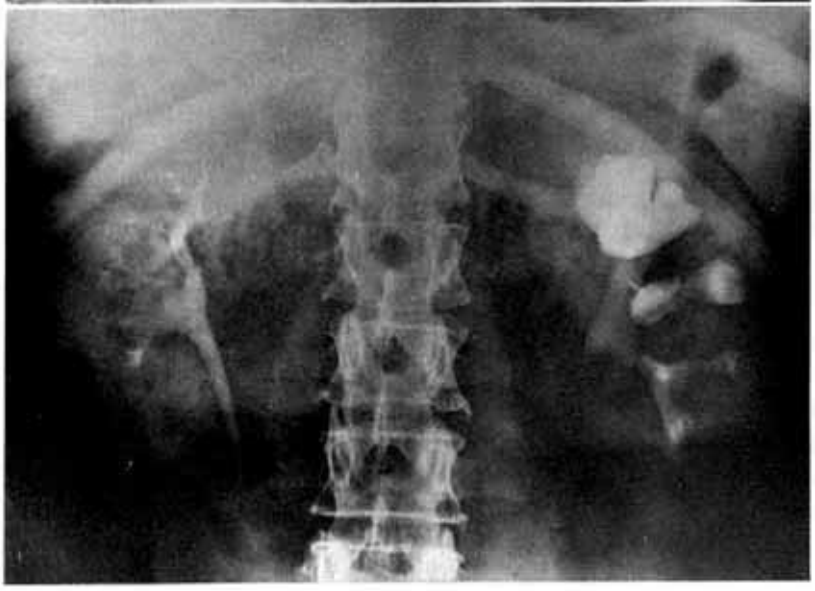

Fig. 32. (Obs. III). K. U. B. Staghorn calculi in left kidney.
Fig. 33. (Obs. III). Preoperative I. V. P.

Abb. 32. Obs. (III) Nierenleeraufnahme. Ausgußstein in der linken Niere.
Abb. 33. (Obs. III) Praeoperatives intravenöses Pyelogramm.

Fig. 32. (Obs. III). Cliché à vide. Calcul coralliforme du rein gauche.
Fig. 33. (Obs. III). I.U.V. préopératoire.

Fig. 34. (Obs. III). Surgical specimen. The staghorn calculus removed in toto plus several calicial calculi.

Abb. 34. (Obs. III) Chirurgisches Präparat. Der in toto entfernte Ausgußstein und einige Kelchsteine.

Fig. 34. (Obs. III). Pièce opératoire. Le calcul coralliforme a été extrait en totalité, de même que les calculs caliciels.

The operation must not be ended without taking a peroperative X-ray film. The X-ray intensifier of images can be very useful in certain cases. The caliceal and pelvic cavities and the ureter must be irrigated with normal saline solution at rather high pressure. ABOULKER uses a metal syringe—usually used for biliary tracts—with a long flexible and atraumatic point which is introduced in each one of the calices and ureter. It is very useful and serviceable.

After closure, and when the retractors of the renal sinus are withdrawn, the posterior edge of the kidney covers the incisions completely, both the pyelotomy and the calicotomy. *As the renal*

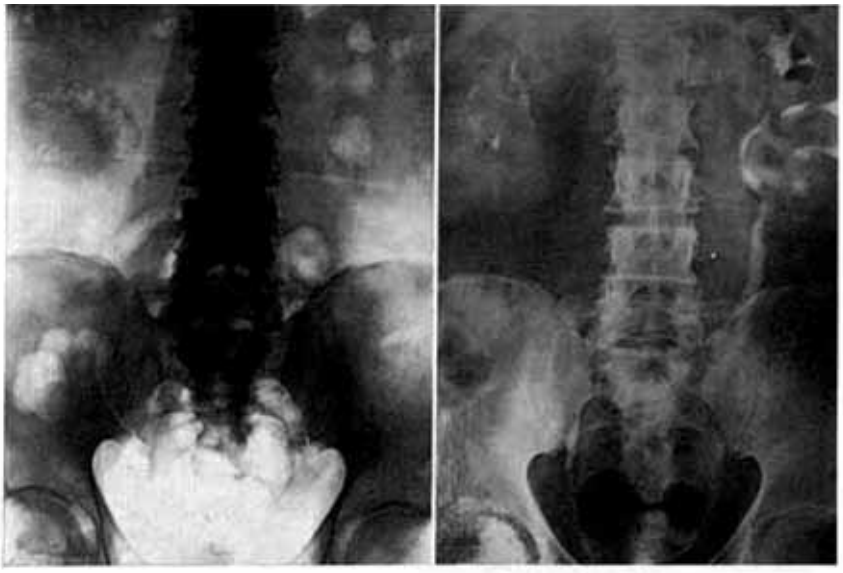

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Fig. 35. (Obs. III). Postoperative K. U. B. Complete removal of calculus.
Fig. 36. (Obs. III). Postoperative I. V. P. Complete recovery of left kidney.

Abb. 35. (Obs. III) Postoperative Leeraufnahme. Komplette Steinentfernung.
Abb. 36. (Obs. III) Postoperatives intravenöses Pyelogramm. Vollständige Erholung der linken Niere.

Fig. 35. (Obs. III). Cliché à vide postopératoire. Les calculs sont extraits en totalité.
Fig. 36. (Obs. III). U. I. V. Récupération fonctionnelle totale du rein gauche.

parenchyma acts as a cover and the peristalsis being immediately reestablished, there is no leakage of urine not even in the first few hours after the operation, which is of great importance, not only subjectively, for the patient himself, but because it considerably shortens the postoperative period for the patient who can leave the hospital between the third and seventh day after operation. Moreover it avoids the constrictive perinephritis (Hypertensive factor), the extrinsic strictures of the union (stasis factor) especially because it simplifies any future re-operation.

As there is no leakage of urine, it is not necessary to drain the lumbar space; in 14 cases we left no drainage at all, and the post-operative period was completely normal. Usually we leave a permanent suction tube (Redon type) which is removed after two or three days. We are against urine diversion, both ureteral and by a nephrostomy tube.

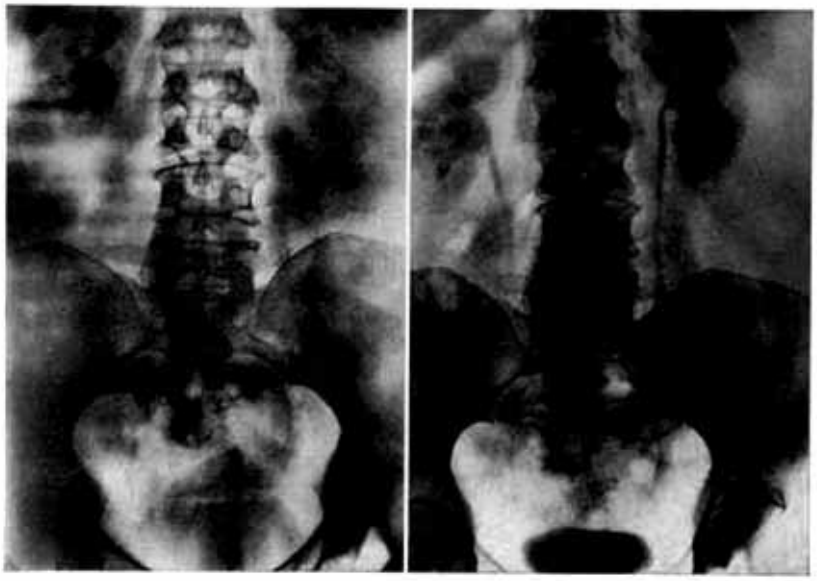

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Fig. 37. (Obs. IV). Preoperative K. U. B. Staghorn calculus in right kidney.
Fig. 38. (Obs. IV). Preoperative I. V. P.
Abb. 37. (Obs. IV) Präoperative Leeraufnahme. Ausgußstein in der rechten Niere.
Abb. 38. (Obs. IV) Präoperatives intravenöses Pyelogramm.
Fig. 37. (Obs. IV). Cliché à vide. Calcul coralliforme du rein droit.
Fig. 38. (Obs. IV). U.I.V. préopératoire.

The Problem of Recurrence from a Surgical Point of View

The tactics as well as the operative technique to be used in the removal of recurrent renal calculi, constitute one of the big problems which the urologist has to face.

The first problem is the type of kidney approach.

The second, how the pyelolithotomy is to be performed.

The surgical approach depends on whether the patient was previously operated on, following the above mentioned indications, i.e. posterior vertical lumbotomy, kidney 'in situ', extracapsular pyelotomy through the 'sinus renalis', or if following the classical operative techniques.

In the first case, the surgical approach to the lumbar fossa will be the same, i.e. the posterior vertical kidney incision, because the surgeon will not find distortion as far as the anatomical layers are concerned.

Fig. 39. (Obs. IV). Staghorn calculus removed in one piece.
Abb. 39. (Obs. IV) Der in einem Stück entfernte Ausgußstein.
Fig. 39. (Obs. IV). Le calcul coralliforme a été extrait en une seule pièce.

Once the lumbar fossa has been reached, only a small portion adherent to the deep layers will be found, which corresponds in fact to the place where the renal pelvis is located, the only structure which was freed during the first surgical intervention. As previously pointed out, following the first extracapsular pyelolithotomy

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Abb. 40. (Obs. IV) Postoperative Leeraufnahme. Keine Steine zurückgeblieben.
Abb. 41. (Obs. IV) Postoperatives intravenöses Pyelogramm. Funktionelle und morphologische Erholung der Niere.
Abb. 42. (Obs. V) Nierenleeraufnahme, Zahlreiche Becken- und Kelchsteine der linken Niere.
Abb. 43. (Obs. V) Präoperatives intravenöses Pyelogramm. Deutliche Funktionsstörung der linken Niere.
Fig. 40. (Obs. IV). Cliché à vide postopératoire. Pas de calculs à droite.
Fig. 41. (Obs. IV). U.I.V. postopératoire. Récupération fonctionnelle et morphologique du rein droit.
Fig. 42. (Obs. V). Cliché à vide. Calculs pyélocaliciels multiples du rein gauche.
Fig. 43. (Obs. V). U.I.V. postopératoire. Déficit fonctionnel important du rein gauche.

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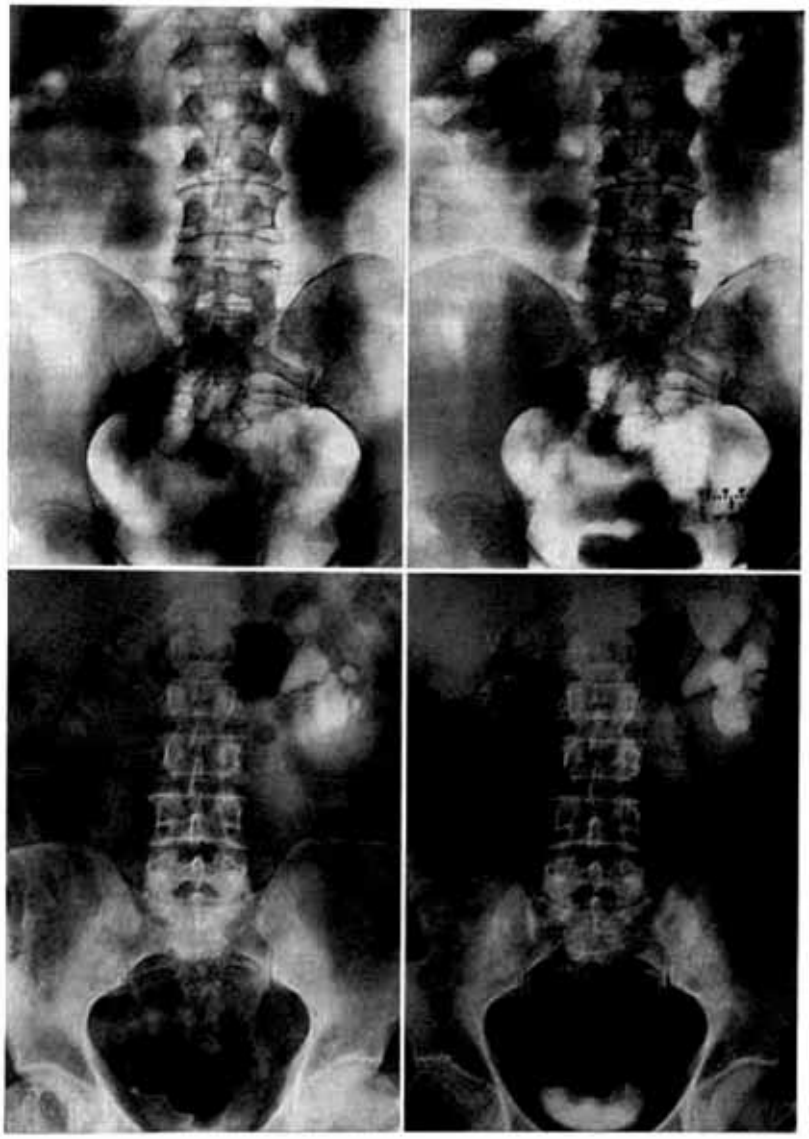

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Fig. 40. (Obs. IV). Postoperative K. U. B. No stone fragments left.
Fig. 41. (Obs. IV). Postoperative I. V. P. Functional and morphological recovery of the kidney.
Fig. 42. (Obs. V). K. U. B. Multiple pyelocalicial calculi in left kidney.
Fig. 43. (Obs. V). Preoperative I. V. P. Important functional deficit from the left kidney.

Fig. 44. (Obs. V). Calculi specimen.
Abb. 44. (Obs. V) Steinpräparat.
Fig. 44. (Obs. V). Pièce opératoire.

through the 'sinus renalis' strong quick developing adhesions take place between the posterior wall of the renal pelvis and the inner sheet of the fibrous capsule of the kidney so as to make a second access to the 'sinus renalis' impossible following the same approach. Two possibilities may be taken into consideration: if the kidney pelvis is of an extrarenal type, a classical pyelotomy must be performed, although in a transverse manner. If pyelitis makes it difficult to identify the renal pelvis, or if the kidney pelvis is of an intrarenal type, then a complete decapsulation of the kidney must be performed, entering the 'sinus renalis' by way of Surraco's intracapsular approach, but with an important difference, which is, that once the inner edge of the kidney has been carefully retracted with the aid of retractors 'ad hoc', a transverse incision must be used

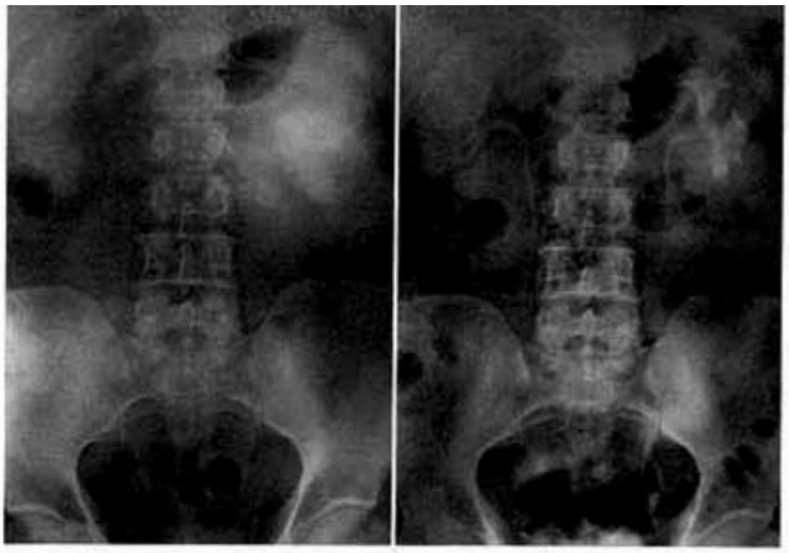

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Fig. 45. (Obs.V). Postoperative K.U.B. Total and complete removal of calculi.
Fig. 46. (Obs.V). Postoperative I.V.P. Functional recovery of the left kidney.

Abb. 45. (Obs. V) Postoperative Leeraufnahme. Vollständige Entfernung der Steine.
Abb. 46. (Obs. V) Postoperatives intravenöses Pyelogramm. Funktionelle Erholung der linken Niere.

Fig. 45. (Obs. V). Cliché à vide postopératoire. Les calculs sont extraits en totalité.
Fig. 46. (Obs. V). Récupération fonctionnelle du rein gauche.

instead of a longitudinal one, which will incise the inner sheet of the fibrous capsule and the kidney pelvis firmly adhered to it. This type of incision must be made parallel to the retropelvic artery with no danger of injuring it, which does not occur with the vertical incision.

In the second case, i.e. when the patient has been operated on in accordance with the classical techniques, then, usually, the entrance to the lumbar fossa using the oblique lumbotomy is preferred, and there is no waste of time trying to free the kidney, when one performs right from the start a complete decapsulation and procedes with the above-mentioned technique of intracapsular pyelotomy. This shortens remarkably the surgical intervention and makes it safer.

Briefly, the surgeon who is familar with the use of these two approaches to the 'sinus renalis' has important resources at hand enabling him to deal with the most difficult cases.

Instruments

For this type of surgery, besides a fine aspirator, the use of *retractors for renal sinus* is indispensable. Their type is usually used in ophthalmology and vascular surgery, their depth, amplitude and curvature being modified so that they may adapt themselves to the lip of the parenchyma, their edges being very blunt.

We have manufactured three different sizes (fig. 20 and 21), which are used according to the type of the hilus; large or small, opened or pointed. *These retractors keep the kidney very firm and when drawn apart, present the renal hilus perpendicularly to the surgeon.* If the operation is prolonged, it is advisable to loosen the retractors, as they may compress the retropyelic vessels, this is noticeable by a change of colour of the posterior aspect of the kidney.

Results

Staghorn calculi. 19 staghorn calculi have been extracted. Three of them in one piece with no fragmentation. No nephrotomy. Only in one case a caliceal calculus was left, owing to a sudden failure of the X-ray apparatus. Now, after three years, the stone is well tolerated, its size has not increased; in all of the other cases, no fragment or calculus was left, at least visible in the X-ray picture.

No recurrence in 18 cases up to date. The other case presented a real recurrence after a few months; it was in a very dilated and infected kidney.

The extraction of a bilateral staghorn was performed in one operative stage; it was on a young patient and it was well tolerated.

Multiple pyelocaliceal calculi. 27 cases were operated on. In 25 the extraction was complete, in 2 cases an X-ray transparent calculus was left; one was spontaneously expulsed, the other operated on. Two cases of multiple bilateral calculi were operated on in one operative stage with good tolerance.

Caliceal calculi. A total of 41 cases, 36 of which were easily extracted, and the incisions, in most of them, were made on the infundibulum of the lithiasic calyx (calicolithotomy). The other

5 cases were of large calculi occupying the whole caliceal system, with multiple ramifications, easily felt through the thin cortical parenchyma and were extracted by nephrotomy.

Pyelic calculi. These were the most in number, a total of 237 cases. In 12 the pyelic lithiasis coexisted with a stricture of the ureteropelvic junction, a pyelolithotomy and a plastic operation, Foley type, were simultaneously performed. One case of recurrence after one year of the operation, and two between the second and the third year after the operation.

From experience of a total of 324 cases, only two staghorn cases reported leakage of urine through the wound, caused by the ureteral obstruction with sand or by a small clot forcing the catheterization of the ureter. The remaining 322 cases did not report any leakage of urine, not even during the first few hours after the operation, so that *in some of the cases we do not drain the lumbar fossa.*

When the posterior vertical lumbotomy is combined with the intrasinusal approach, the patient walks in the first 24 h and can be discharged between the 5th and the 7th day after the operation. *We insist on the fact that a good number of patients should leave the hospital between the third and fourth day after the operation.*

In the staghorn cases where the oblique lumbotomy as well as freeing of the kidney and its pexy are usually forced to, the average time of hospitalization was 15 days, with no leakage of urine or complications. The postoperative care consisted in assuring abundant diuresis from the start, acidification of the urine, antibiotics of wide-spectrum, associated with chemotherapy, repeated cultures of urine and antibiograms, massive dosis of vitamins A and C and early mobilization. The administration of antibiotics must be followed until a complete sterilization of the urine, especially when Proteus B exists, is reached.

We do not pretend to assert that by following this technique recurrences are avoided, but it is a fact, that if we succeed in obtaining the total extraction of the calculi and if we avoid the traumatism of the intrarenal excretory tracts—all this without damaging the kidney—there is no doubt that we shall reduce the number of lithiasis recurrences resulting from surgical manipulations which occur in a much higher percentage of cases than is generally believed.

We use the posterior vertical lumbotomy with the exception of the big staghorn cases in which the renal fossa must be reached by the classic lumbotomy and the kidney must sometimes be freed and even exteriorized; and of the multiple pelvic or calicial stones. This incision, associated with the intrasinusal approach, the transverse pyelotomy or longitudinal calicotomy and the principle of surgery of the kidney 'in situ' constitutes the firm basis upon which *this new surgery is secured for renal lithiasis: undoubtedly less traumatic, more selective, more efficacious and safer than that which is performed nowadays.*

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TECHNIQUE FOR CONSTRUCTION OF A FUNCTIONING ARTIFICIAL BLADDER

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Having been very much impressed by the results obtained with use of a segment of the sigmoid colon as a partial substitute for the urinary bladder (colo-cystoplasty), we decided to perform total substitution of the cancerous bladder with an isolated segment of the sigmoid colon anastomosed to the urethra, thus obtaining a functional artificial bladder from which the patient would void through this natural outlet and also be spared the severe complications frequently observed after ureterosigmoidostomy without diversion of the fecal stream.

We are aware of experimental and clinical data from several authors who have been using ileum in such cases, although the results are not too encouraging. These results are explained when we consider the inadequacy of the small bowel not only as a total substitute for the urinary bladder but also for enlargement, as in ileo-cystoplasty.

Our experience now covers 15 cases of malignant tumors of the bladder and recurring diffuse papillomatosis; we are also presenting the techniques used in these cases.

This surgical technique planned by us is entirely different, as far as we know, from those described up to the present time. It was conceived after seeing the results obtained from other types of colo-cystoplasty for the enlargement of the so-called contracted bladder with a segment of the sigmoid colon. We have performed our type of colo-cystoplasty in a series of 30 cases without mortality and with perfect restoration of normal micturition. These results made us realize the tremendous possibilities of an isolated segment of the sigmoid colon, so much so that in the near future we think we might have a proper substitute for the cancerous bladder from both the anatomical and functional standpoints.

We consider of capital importance the selection of the intestinal segment, because from its activity depends the postoperative functional results. We positively disregard the use of ileal segments for construction of an artificial bladder because of their poor contractile power to expel urine, small volumetric capacity, excessive mucus formation, possibilities of invagination and of obstruction, the electrolyte disturbances due to reabsorption (typical physiological property of the ileum) and shortness of the mesentery. A comparative study of the use of the sigmoid in the execution of enterocystoplasty has already been published in collaboration with R. Gosalbez.[1]

Technically speaking, the ideal intestinal segment for the construction of an artificial bladder is the sigmoid colon.

The first case of total cysto-prostato-vesiculectomy, followed by construction of a functioning artificial bladder with an isolated sigmoid segment anastomosed to the membranous urethra, was done in a one stage operation, on June 4, 1957 in the Department of Urology, School of Medicine, University of Barcelona, on a 64-year-old patient with bladder carcinoma. The postoperative results were very satisfactory, as we now see this patient at the present time with a completely normal urinary tract and perfect voiding control (figs. 1 and 2).

We have already performed this operation on 15 patients. The late results obtained will be the subject of another paper. Here, we shall limit discussion to a description of the different techniques that have been followed.

Preoperative preparation of the intestine is the same as for a segmental colectomy or a colocystoplasty.

It is of great importance to study and have a perfect control of the pre- and postoperative status of the patient, from which will depend the good postoperative results. A perfect water and electrolyte balance should be obtained with a determination of plasma protein as well. On the other hand, we have to avoid the imbalance that

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* Translated into English by Dr. J. P. Bourque, Montreal, Canada.

[1] Gil Vernet, J. M. and Gosalbez, R.: Ileo-cystoplasties ou colo-cysto-plastie. J. d'urol., **63**: 466, 1957.

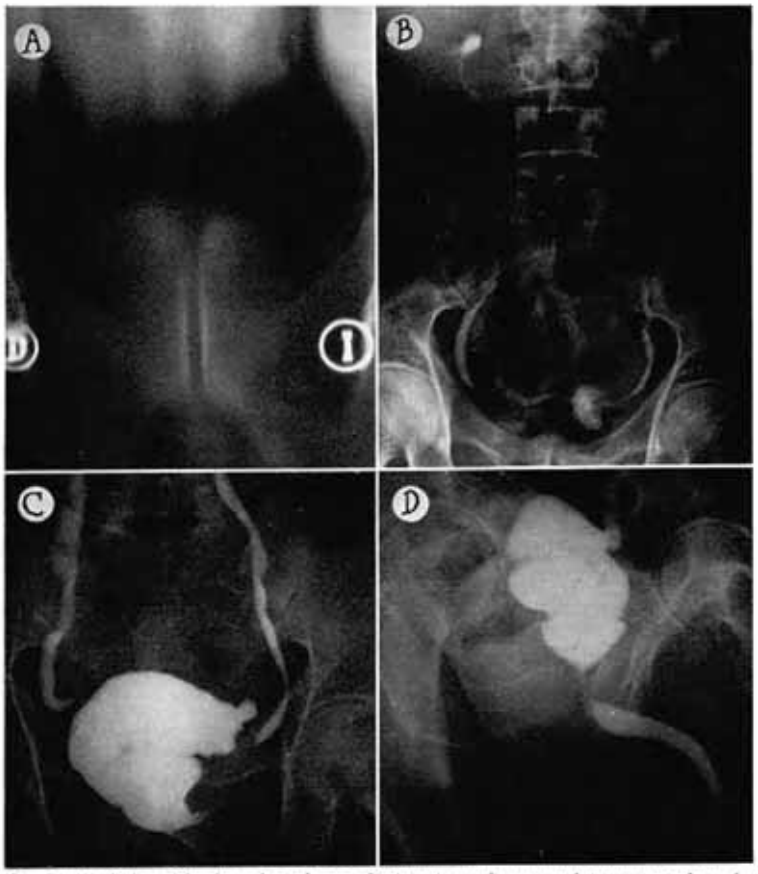

FIG. 1. Case 1. Technique No. I used (end-to-end). Anastomosis at membranous urethra. *A*, preoperative planigraphy. Infiltrating tumor of dome, neck and vesical trigone. *B*, excretory urogram 1 year after operation. *C*, retrograde cystourethrogram to maximum capacity (1 year following operation). Slight ureteral reflux. *D*, oblique cystourethrogram.

goes along with the postoperative illness, the renal involvement (with an already diseased kidney in many cases) and the continuous suction; parenteral nourishment and hydration have to be kept under control for they can promote by themselves failure of the operation performed with perfect technique. These precautions which are per se very important in any given abdominal operation are far more important in this instance when the large bowel is being used as a bladder substitute.

Finally, the patient's nutritional status, usually an elderly person in the majority of cases, in poor general condition due to tumor, is a fact that has to be considered in order to choose the proper time for the operation; also, we have to be very careful in the preparation and more so in the immediate postoperative period of 10 to 15 days.

The intestinal segment can be anastomosed to different urethral levels according to the type of vesical lesions and their localization; therefore, in performing the block excision of the bladder and of the prostate gland, the division of the urethra can be performed as follows (fig. 3):

A) Immediately above the verumontanum, thus not disturbing the seminal vesicles.

B) Below the verumontanum, anastomosing the bowel segment to the apex of the prostate.

C) Immediately below the apex of the prostate with the anastomosis made to the membranous urethra.

Anastomosis between the urethra and the intestinal segment can be either end-to-end or end-to-side.

TECHNIQUE NO. 1 (EXTRAPERITONEAL END-TO-END ANASTOMOSIS)

A) *Excision.* A midline abdominal incision from the umbilicus to the symphisis pubis is made and held by a large autostatic retractor, lymphadenectomy of the internal iliac chains is carried out, and total cysto-prostato-vesiculectomy[2] is performed by our extraperitoneal technique taking care to do an elliptical division of the ureters as they enter into the bladder (fig. 4). When the prostatic urethra appears to be intact, because the tumor is far away from the vesical neck, we prefer to salvage the apical portion of the prostate in order to preserve the integrity of the external sphincter. In some cases the excision is complete and the intestinal segment is being anastomosed to the membranous urethra, thus preserving also a good urinary control.

In cases where there is a large prostatic adenoma, it is better to do the enucleation so as to avoid any obstruction to the new bladder. If the seminal vesicles are being excised it is better to avoid the prostato-peritoneal aponeurosis and also the serosa which protects the rectum because they can be used for the first posterior layer thus suturing the serosa of the intestinal segment to the aforementioned aponeurosis. When the excision has been finished, both ureters are catheterized and ureteral catheters are placed outside the operating field. The pelvic cavity is then packed with a warm moist laparotomy pad dipped in normal saline solution.

B) *Construction of the intestinal segment into a neo-bladder.* The same intestinal technique that we follow in performing simple colo-cystoplasty is used with the exception that for construction of the artificial bladder, the length of the intestinal segment must be longer, that is, 28 or 30 cm.

Although in simple colo-cystoplasty, the sigmoidal segment can be anastomosed to the trigone without tension due to the fact that the remaining portion of the bladder has some mobility which permits it "to go" to the intestinal segment, in this operative technique it is the intestinal segment which has to move forward more deeply until it reaches the membranous urethra or the apex of the prostate; in this

[2] Gil Vernet, J. M.: Cystectomie totale et implantation urétéro-rectale par voie extra-péritonéale. J. d'urol., **58**: 359, 1952.

FIG. 2. Case 1. Patient during act of micturition (1 year following operation). Micturition every 2 to 3 hours. Perfect urinary control at daytime and slight noeturnal incontinence.

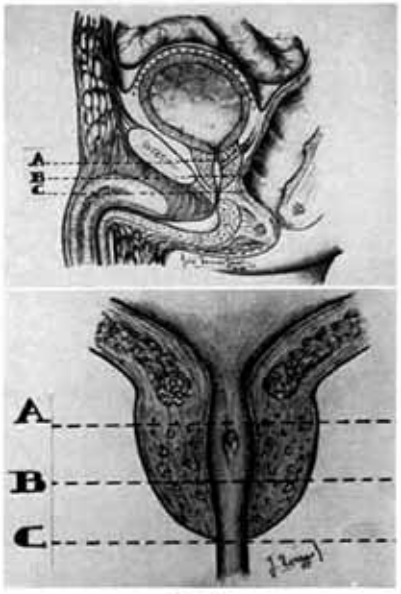

FIG. 3

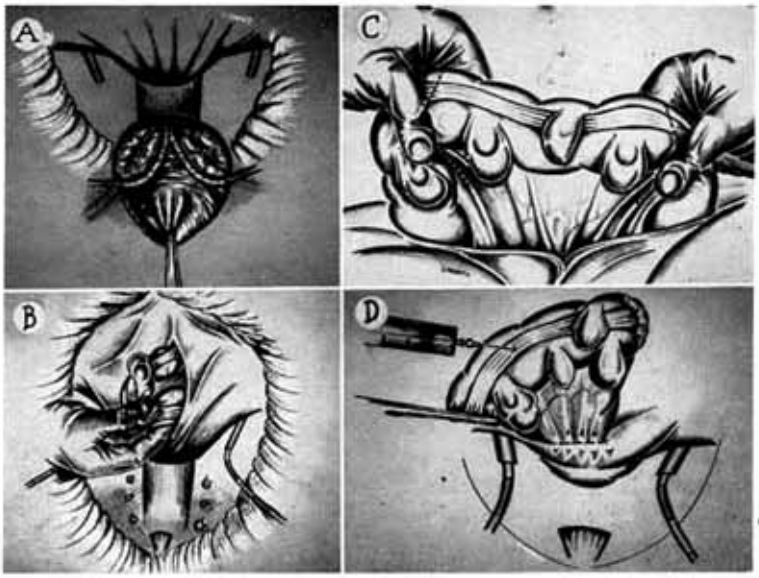

FIG. 4. *A*, total cysto-prostato-vesiculectomy. *B*, pulling out ileopelvic colon. *C*, dotted line shows section of intestinal segment. *D*, extraperitonealization of intestinal segment by suturing two serosal leafs of its meso to anterior parietal peritoneum.

eventuality it is essential, in the majority of cases, to lengthen the mesosigmoid, executed by transverse section of the serosa on both sides of the mesosigmoid (fig. 5). This technique has been described in a preceding publication[3] and with its possibilities, we have always been able to anastomose the intestinal segment to the urethra without undue tension. We know that the ileopelvic colon sometimes, due to some fecal stagnation, can enlarge up to a point where it can get to measure 25 to 30 cm. or even more; it is this fact that explains, according to Testut, the frequency of volvulus formation in this segment of the bowel both in the adult and in the aged.

Once the intestinal segment is isolated, the ends are closed, and a highly concentrated non-irritating bacteriostatic solution (chloramphenicol) is injected into it to avoid excessive mucus formation, which will render difficult the drainage of the intestinal segment in the immediate post-operative period, with possible formation of urinary fistulas; by this process we obtain bacteriologic cleansing of the intestinal segment (fig. 4, *D*).

[3] Gil Vernet, J. M.: Colo-cysto-plasties. J. d'urol., **64**: 301, 1958.

This stage is followed by closure of the peritoneal cavity with extraperitonealization of the intestinal segment; this is one of the most important operative stages, for intraperitoneal rough surfaces or areas should never be left, if we want to avoid postoperative intestinal obstruction. All the mesosigmoid of the intestinal segment without its serosa must be completely extraperitonealized. For this reason we have to be careful to close the anterior parietal peritoneum around it (fig. 4, *D*), that is, from the cul-de-sac of Douglas to the descending colon. In this way, the meso of the intestinal segment will be free, mobile and completely extraperitoneal; on the other hand, the abdominal cavity is completely closed and isolated from the pelvic excavation.

C) *Ureterosigmoid anastomosis.* The technique to be followed depends of any one's personal choice. If the ureter has been cut near the bladder, the Nesbit technique can be used, although we personally prefer the anastomosis in "trunk of an elephant" (fig. 11, *F*). If we are dealing with diffuse papillomatosis and the outlet of one or of the two ureters is far away from the tumor, we prefer to free the ureter with all its intramural

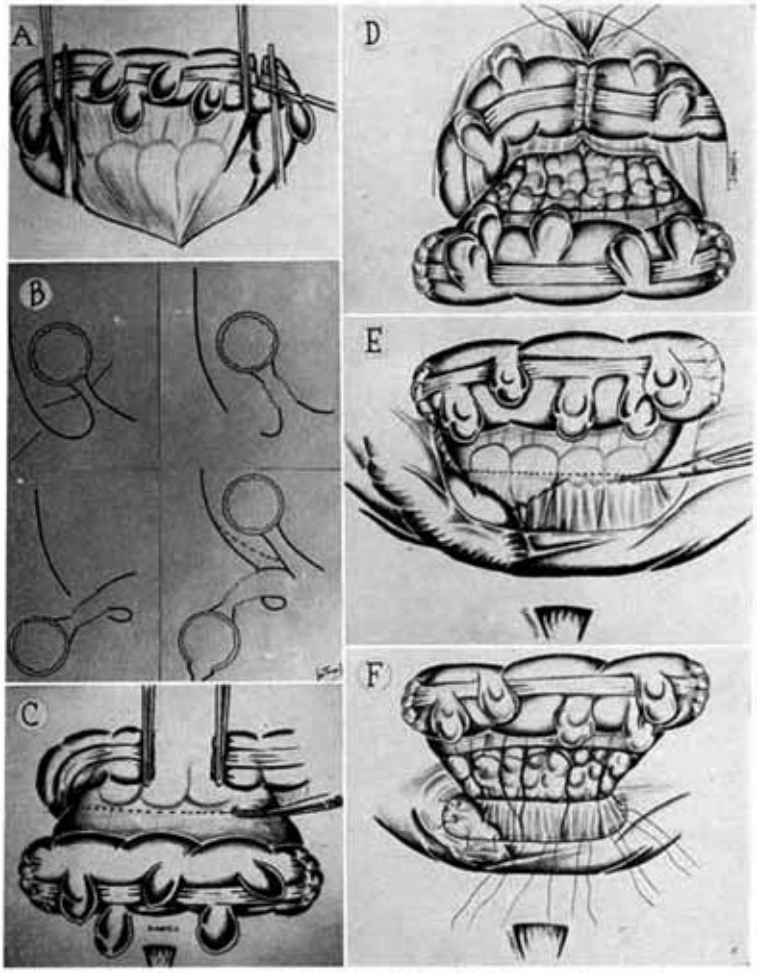

FIG. 5. *A*, obtaining intestinal segment. *B*, schematic drawing shows enlargement maneuver of mesosigmoid and extraperitonealization of intestinal segment. *C*, end-to-end anastomosis of colon from above meso of intestinal segment. Sectioning superior serosal leaf (enlargement maneuver). *D*, end-to-end anastomosis of colon completed and superior leaf incised. *E*, sectioning of inferior serosal leaf of intestinal segment at level of coalescence process and intersigmoid fossa. *F*, enlargement maneuver of mesosigmoid completed. Extraperitonealization of inferior surface.

portion, thus preserving the ureteral orifice together with a small portion of the mucosa around the ureteral meatus. This modifiation of the Bergenhem's technique[4] prevents stenosis of the ureterosigmoid anastomosis, and also preserves normal ureteral peristalsis in its total integrity.

In order to perform ureterosigmoid anastomosis, a curved clamp is passed through the intestinal lumen, thus protruding on the cintilla longitudinalis in the selected place for the implant, in which case the left ureter will be very close to the proximal end of the intestinal segment; all the intestinal layers are incised, the

[4] Gil Vernet, J. M.: Anatimofisiologia de los diferentes metodos de implantation ureterointestinal Nuevas tecnicas. Cir. Ginec. y urol., vol. 3, 1952, page number not available.

forceps pulling the catheter until it appears in the lumen of the intestinal segment. The right ureter is anastomosed approximately in the mid-portion of the intestinal segment. After the anastomosis, the intestinal segment shows some degree of kinking (fig. 8, *D*) which can render difficult complete emptying of the newly formed bladder with this end-to-end technique.

D) *Urethrosigmoid anastomosis.* The end-to-end anastomosis between the urethra and the distal end of the intestinal segment can present some difficulties because of the different calibers between them. In some cases it will be necessary to make a cuneiform resection of the intestinal end (fig. 6) which will diminish its caliber. The anastomosis will be performed in two layers with interrupted sutures of 0000 chromic catgut.

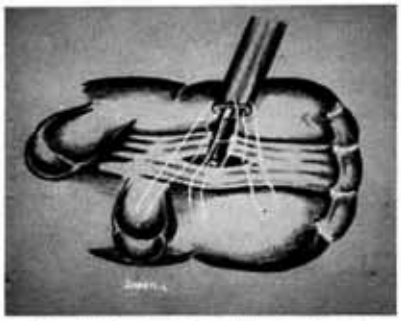

FIG. 6. Urethrosigmoidal anastomosis

First, posterior layer: It will join the serosa of the intestinal segment with the prostatic or periurethral structures (fig. 7, *C*). The stitches will not be tied until all of them are placed.

Second, posterior layer: This one will join the urethral and intestinal ends by means of total and interrupted sutures (fig. 7, *C*).

After this last layer is completed, a urethral catheter is passed and the tips of the ureteral catheters are introduced into its lumen and pulled outside through the urethra; the urethral catheter is again reintroduced and left indwelling (fig. 8, *A*) in order to drain some of the mucus or urine from the intestinal segment if one or both of the ureteral catheters become obstructed. Following this stage the first anterior layer (fig. 8, *A*

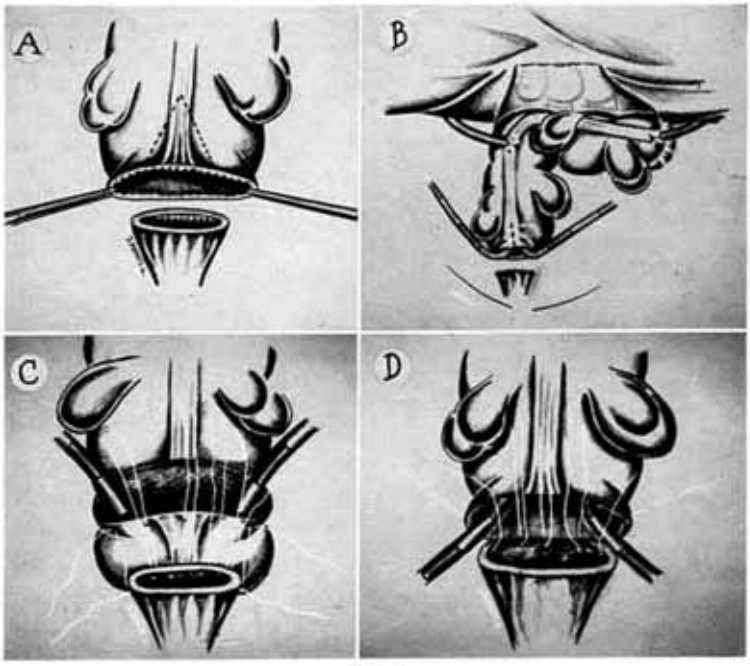

FIG. 7

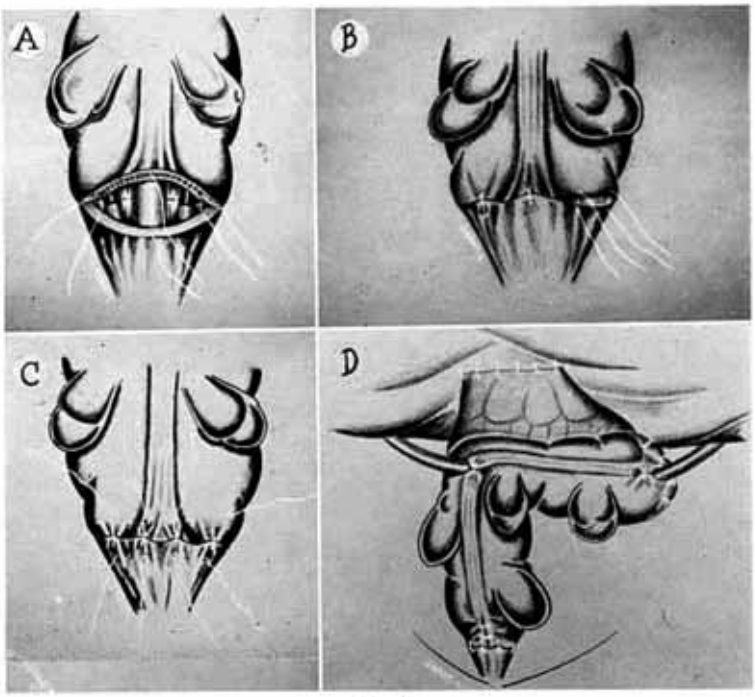

FIG. 8

FIG. 9

and *B*), will be followed by the closure of the second anterior layer.

After all the anastomoses are finished, we proceed with irrigation and distention of the new bladder with a normal saline solution added of penicillin in order to verify the water-tight line of sutures.

Only one drainage tube is left in the retropubic space and exteriorized outside of the laparotomy wound (fig. 9).

The abdominal wall is closed in layers and with continuous wire sutures, because catgut sutures are not well tolerated by the traumatized edges of the laparotomy wound held for several hours under forceful tension by an autostatic retractor. The skin is closed with interrupted sutures of mane. All the catheters are secured and a forceful dilatation of the anus is done.

We do not consider it necessary to keep the colon at rest by a diversion colostomy.

TECHNIQUE NO. II (EXTRAPERITONEAL END-TO-SIDE ANASTOMOSIS)

The excision stage (isolation of the intestinal segment, extraperitonealization and transplantation of the ureters) is the same as for technique No. I, the only difference being in the end-to-side anastomosis between the urethra and the intestinal segment.

Once the intestinal segment is obtained, both ends are closed, and the bacteriostatic solution is injected into it (fig. 10, *C*).

In the midportion of the intestinal segment and

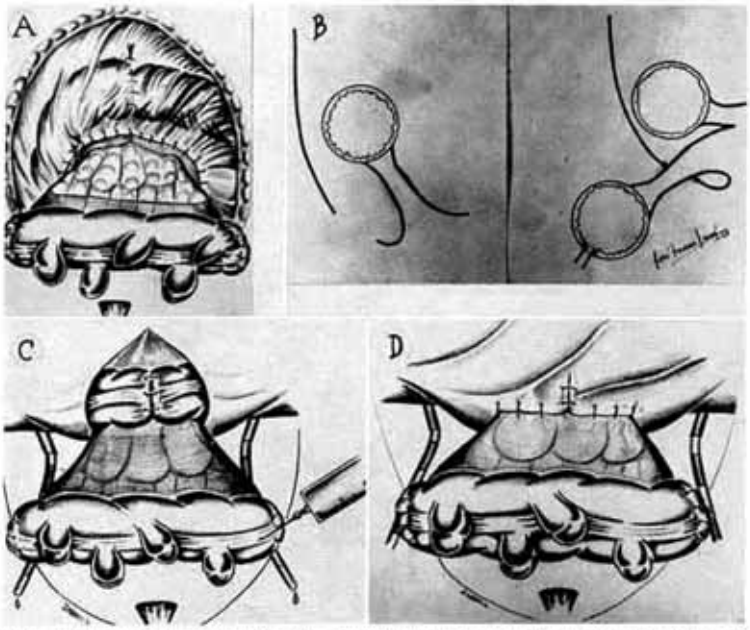

FIG. 10. *A*, suturing of anterior parietal peritoneum around meso of end-to-end anastomosis of colon. *B*, schematic drawing shows extraperitonealization of intestinal segment in cases of large meso where enlargement is not needed. *C*, large mesosigmoid which has no need for enlargement. *D*, suturing of anterior parietal peritoneum to serosal leaf of mesosigmoid which had no need for enlargement.

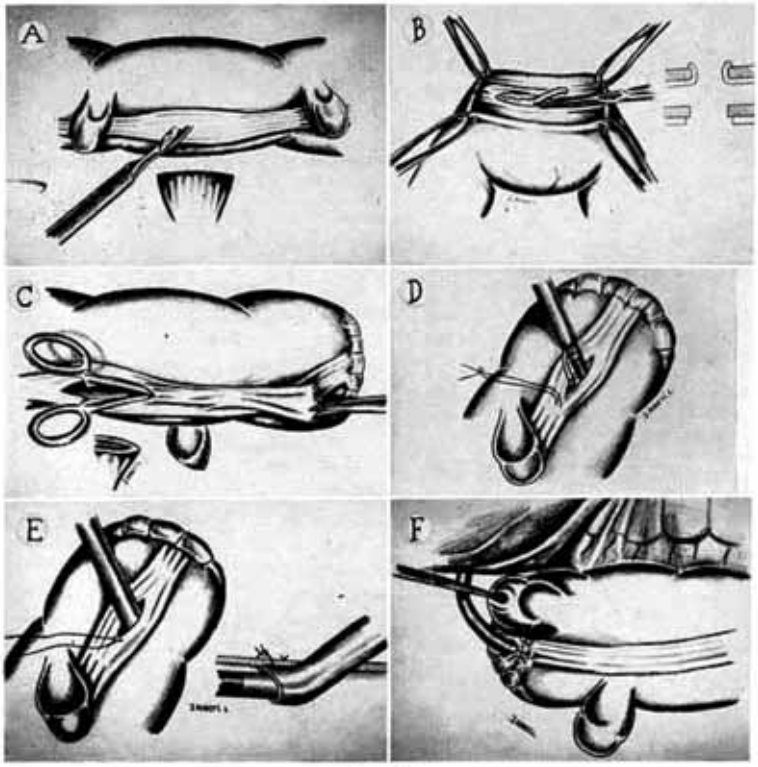

FIG. 11

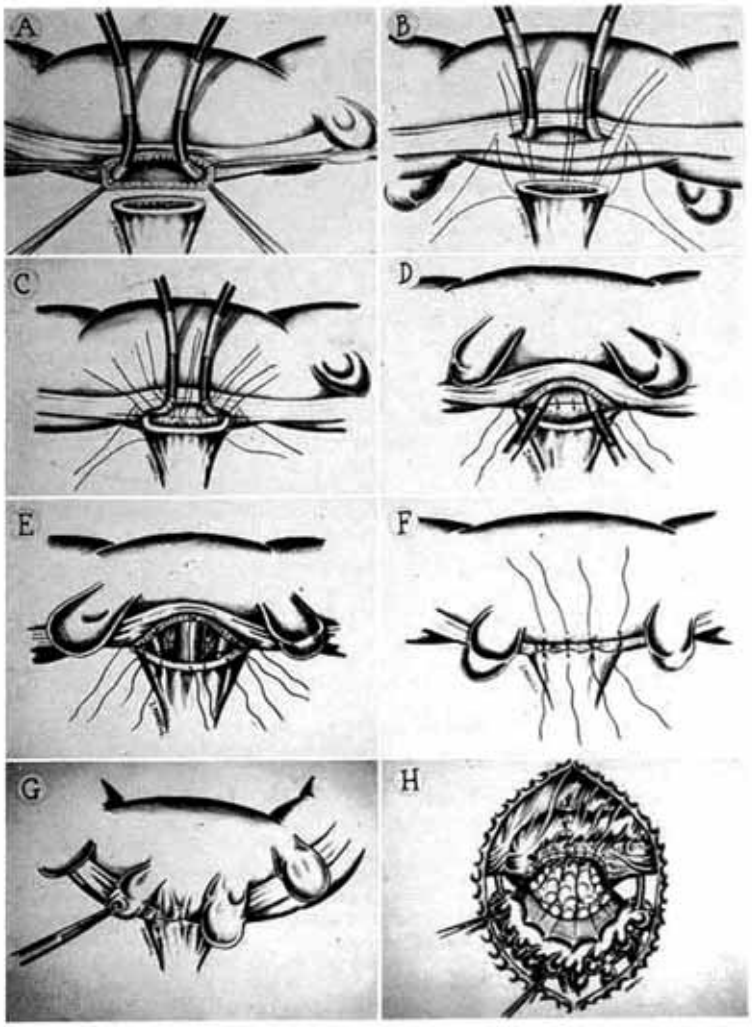

FIG. 12

on its antemesenteric edge, an incision of 1½ to 2 cm. in length is made; this will be the new intestinal ostium. To avoid extrusion of the intestinal mucosa (fig. 11, *B*) which could produce urinary fistulas, we proceed with resection of 2 cm. of the mucosa (fig. 11, *B*) that surrounds the intestinal ostium.

The curved clamp is introduced through the incision (fig. 11, *C*) and we proceed to transplantation of the ureters. Each one of the ureters will be transplanted in the end zones of the intestinal segment.

The side-to-end anastomosis of the urethra and of the intestinal segment will be done in two layers (fig. 12).

Once the operation is completed, the new bladder will show a U shaped curve without any kinking. Technique No. II is more laborious and more complex than No. I but the postoperative results are likely to simulate a normal bladder and

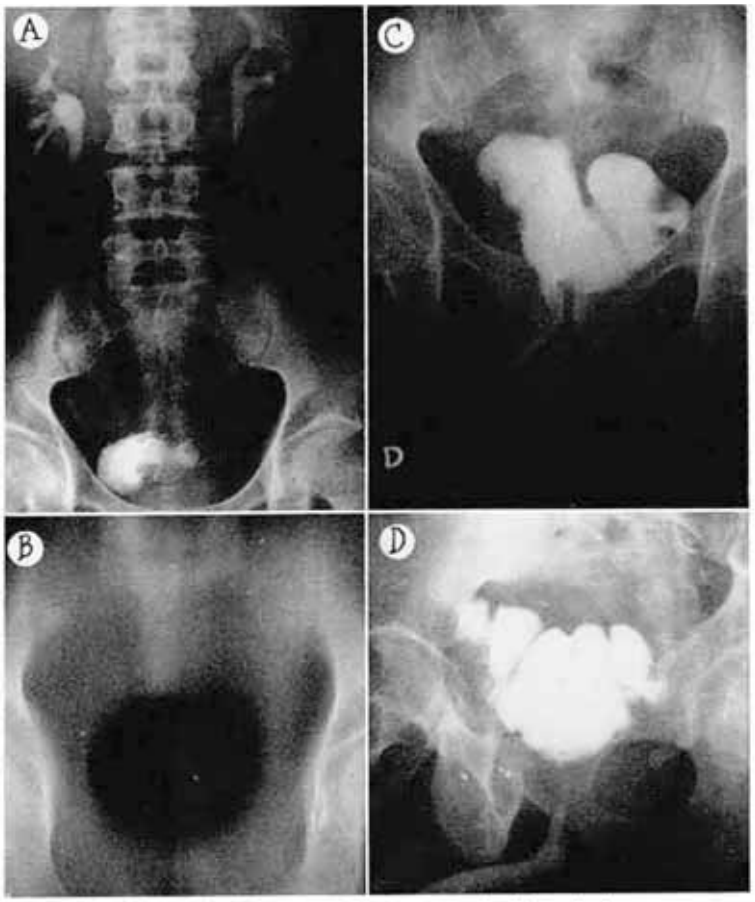

FIG. 13. Case 11. Technique No. II (end-to-side). Anastomosis of intestinal segment to membranous urethra. *A*, preoperative excretory urogram. Note vesical filling defect. *B*, vesical planigraphy. Infiltrating tumor of left lateral wall. *C*, oblique urethrocystogram (3 months after operation). *D*, retrograde cystogram 30 days after operation, in full stage of accommodation.

the course of the ureters is maintained in a normal position. Functionally, the results also appear superior to those obtained by technique No. I. (Figs. 13-15 are from a case that was operated upon by this last technique.)

A Wangensteen drainage tube is maintained during the first two or three days. The newly formed bladder is irrigated three times daily with a normal saline solution and so are the ureteral catheters as well; these will be removed on the sixth or seventh postoperative day, thus enabling the patient to get out of bed. Forty eight hours after operation, a laxative is prescribed to liquify the feces and to permit their passage through the colonic anastomosis. The urethral catheter will be removed on the tenth or twelfth postoperative day, and the patient discharged. Antibiotics and chemotherapeutic drugs are given periodically for the first few months depending on the bacterial flora of the newly formed bladder which is generally made up of B. proteus, which does not seem to have any pathological influence even though it is present for a long time.

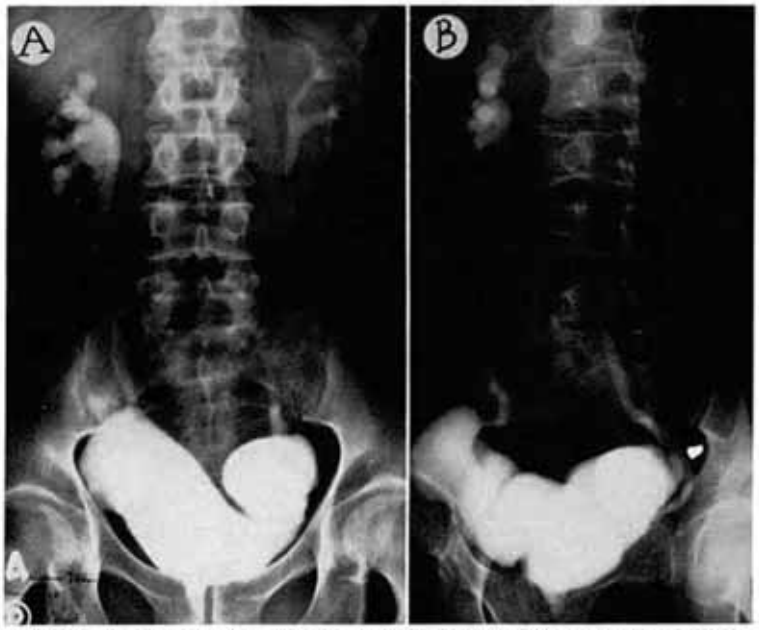

FIG. 14. Case 11. *A*, excretory urogram combined with retrograde cystogram (1 year after operation). Slight ureteral reflux on right side after maximum distention of "new bladder." *B*, excretory urogram with patient in oblique position (1 year after operation).

FIG. 15. Case 11. Patient during micturition (1 year following operation). Perfect diurnal and nocturnal continence; voiding every 4 to 5 hours.

The mucus formation from the intestinal segment is practically not seen while the bladder is "dry" but once the new bladder gets to function, there is an increased amount of mucus due to the irritative action of the urine; however the mucus formation is at a much lesser degree than when the ileum is used and as the time goes by, there is a remarkable decrease of it.

TOLERANCE AND OPERATIVE TIME

In spite of the length of time that it takes for the operation, which is performed upon patients with malignant tumors in poor general condition, some of them being reoperated on in ages varying between 60 to 75 years of age, all have tolerated the procedure well and none have shown symptoms of shock.

The average operative time was 3½ hours.

COMPLICATIONS

Having in mind that we are working on a cancerous patient, the possibility of complications is logically greater than with colo-cystoplasty for a small tuberculous bladder.

The complications encountered in a total of 15 operative cases were:

Urinary fistulas: 4 cases, located at site of the urethrosigmoid anastomosis. They were reoperated upon and recovery was uneventful. The

other cases healed just by leaving an indwelling urethral catheter for as long as 20–25 days.

Intestinal complications: 1 case of intestinal obstruction because of a peritonealization defect.

MORTALITY

Among the total number of 15 cases there have been 3 deaths: 2 patients died on the fourth and on the eighth postoperative day respectively, because of myocardial damage after good tolerance of the surgical intervention; the third died from intestinal obstruction due to a technical defect in the extraperitonealization process; in fact in this case, surgical intervention was extremely laborious because of scar tissue and the patient's obesity (110 kg.). The patient was reoperated upon but he died.

The results obtained in all other cases are really satisfactory and hopeful and will be the subject of a detailed revision in a forthcoming publication.

SUMMARY

An entirely new operative technique for construction of a functioning artificial bladder has been used in a small series of 15 patients. Indications for and techniques of the operation have been discussed.

□ □ □

EXCMO. SR. DR. BJÖRN O. NILSSON

Discurso de presentación

Dra. M. dels Àngels Calvo Torras
Académica de Número de la Real Academia Europea de Doctores

Excelentísimo Profesor Alfredo Rocafort, Presidente de la Real Academia Europea de Doctores,
Dignísimas Autoridades,
Sras. y Sres. Académicos,
Señoras y Señores,

La Real Academia Europea de Doctores se complace hoy, en dar la bienvenida como Académico de Honor al Prof. Björn Olof Nilsson, y me honra al solicitar que les dirija un breve discurso con motivo de su solemne ingreso en nuestra querida y docta corporación.

Permítanme que mis primeras palabras sean para expresar mi gratitud a la Junta directiva, y especialmente al Sr. Presidente de la Real Academia Europea de Doctores, Prof. Alfredo Rocafort por haberme encargado la presentación del nuevo académico. Por primera vez desde mi ingreso a esta corporación tomo la palabra para dar cumplimiento a la solicitud recibida y aunque puedo decir que para mí representa un gran honor, una satisfacción e incluso un legítimo

orgullo, estos sentimientos están en equilibrio con la responsabilidad que representa llevar a cabo con acierto tan distinguido y estatutario encargo.

El profesor Bjórn Olof Nilsson nació el día 11 de enero de 1956 in Sollentuna, Stockholm, Suecia y en su persona convergen una excelente formación como bioquímico y su notable liderazgo a nivel empresarial. Desde agosto de 2008 ostenta el cargo de Profesor Presidente de la *Royal Swedish Academy of Engineering Sciences* (IVA) de Stockholm, Suecia.

El Profesor Nilsson desarrolló y defendió su doctorado en Bioquímica en el Royal Institute of Technology (KTH) en Estocolmo, Institución en la que hoy ocupa un cargoo de profesor adjunto asociado.

La importancia de su Tesis titulada "*Fusions to the gene encoding staphylococcal protein A*" se demuestra claramente por el hecho de que a partir de sus estudios en esta área ha profundizado en el conocimiento de la Proteína A, capaz de unirse a la región constante de los anticuerpos humanos, siendo la responsable de que las cepas de *Staphylococcus aureus* puedan protegerse y escapar a la respuesta del sistema inmune. El mecanismo y la estructura de la superficie de interacción fueron estudiados por su equipo de investigación y basándose en este conocimiento diseñaron un dominio sintético de esta proteína A.

Asimismo fueron capaces de construir una biblioteca combinatoria que expresaron en bacterias y que ha sido denominada "Affibody". El concepto de Affibody fue objeto de una de las ocho patentes propuestas y descritas por el profesor Nilsson y su equipo, y como él mismo nos comentará en su discurso ha dado origen a una importante empresa, líder en el desarrollo de nuevas generaciones de bi y multi específicos anticuerpos miméticos y más de 10 programas, útiles en múltiples indicaciones terapéuticas.

El profesor Nilsson ha desempeñado una amplia variedad de funciones de gestión y ejecutivas en la industria biotecnológica y farmacéutica, principalmente dentro del grupo de empresas Pharmacia, entre las que destacan sus responsabilidades como Presidente y Vicepresidente de compañías públicas. Asimismo y durante un período de cuatro años fue Presidente de la Organización de la Industria Biotecnológica sueca (SwedenBIO) y hoy en día, junto a la presidencia de la *Royal Swedish Academy of Engineering Sciences*, se desempeña como miembro de diversas Juntas de Gobierno, incluyendo la presidencia

de la Junta directiva de la compañía biotecnológica BioInvent Internacional AB (OMX Small Cap) y es miembro del equipo de dirección de la empresa de ingeniería AF (OMX Mid Cap).

El profesor Nilsson en la actualidad forma parte de las Juntas y del Comité de dirección y es miembro de un amplio y selecto número de organizaciones internacionales, nacionales, gubernamentales y no gubernamentales en áreas tales como: Ciencia, Educación e Innovación.

En el año 2016, el profesor Nilsson fue elegido miembro del Consejo de Administración del Instituto Europeo de Innovación y Tecnología (EIT) del Gobierno sueco y colabora activamente en el grupo de referencia para la preparación del proyecto de ley de investigación del gobierno sueco.

En la relación con sus publicaciones, en su *curriculum vitae*, se citan con detalle más de 60 artículos de investigación, en revistas de alto índice de impacto, como ejemplo seleccionado de su labor investigadora, podremos leer tres de sus trabajos de investigación en la revista que se entregará al final de este Solemne Acto de recepción

Su trayectoria científica y su labor gestora han sido reconocidas con Honores y Premios entre los que destacan:

- *Post- Doc. Fellowship award from the Swedish Research Council Engineering Sciences (TFR)* (1986-1988).
- *Genentech Recognition Award* (1988).
- *Pharmacia & Upjohn Science and Medicine Award* (1996).
- *Recipient of a Bulgarian presidential award* (2016).
- *Recipient of H.M. The King of Sweden's Medal of the 8th size with the ribbon of the Order of the Seraphim (*2016).

Junto a los méritos científicos del Prof. Nilsson, queremos significar una faceta de su vida que sin duda ha marcado su trayectoria científica muy positivamente. El profesor Nilsson es un atleta destacado, digno del más alto reconocimiento nacional e internacional. Un aplaudido corredor de media distancia entre finales de los 70 y principios de los 80, su deportividad no sólo le permitió obtener premios meritorios sino que ha cristalizado en su nombramiento como

Presidente de la *Swedish Athletic Association,* desde Marzo de 2016. Su ejemplo nos permite insistir en la famosa frase: "*Mens sana in corpore sano"* del poeta romano Juvenal, que expresa la aspiración de una mente equilibrada en un cuerpo equilibrado.

El Prof Nielsson leerá a continuación su discurso titulado: "*Promoting science, technology and innovation*", fruto de su experiencia y profunda reflexión a lo largo de los años dedicados a fomentar la relación entre estos tres conceptos en los diversos cargos desempeñados por el recipiendario en diversos fórums de discusión tanto a nivel nacional como internacional.

La promoción en ciencia, tecnología e innovación es esencial para el desarrollo económico y el progreso social. La investigación y el desarrollo fomentan el desarrollo sostenible, sin embargo, para ser eficaces, el desarrollo de la infraestructura, la transferencia de tecnología y la Investigación pública y privada necesitan ser fomentados y regulados a través de políticas eficaces. A fin de asegurar que el progreso científico se tenga en consideración en todos los niveles de la toma de decisiones gubernamentales, la UNESCO promueve el desarrollo de estructuras y mecanismos que incluyen asesores científicos, oficinas de política científica y tecnológica y comités científicos parlamentarios. Cada dos años, la UNESCO y sus asociados organizan el Foro Mundial de la Ciencia, una cita bienal que aspira a convertirse para la Ciencia en lo que el Foro Económico Mundial de Davos representa para la Economía. Dado que la innovación es fundamental para traducir los conocimientos científicos y tecnológicos en productos, servicios y empleo útiles, la UNESCO fomenta los vínculos más estrechos entre las universidades y la industria dentro de su programa de asociaciones entre la universidad y la industria.

Además de su papel en la provisión de bienes públicos mundiales, La ciencia, la tecnología y la innovación, son factores cruciales para incrementar la prosperidad y mejorar la competitividad nacional. La conexión entre las necesidades tecnológicas locales con las oportunidades tecnológicas internacionales es un reto para muchos países en desarrollo.

La ciencia, la tecnología y la innovación elevan el bienestar de los ciudadanos mediante el desarrollo social, económico y empresarial de un país.

"La ciencia es el alma de la prosperidad de las naciones y la fuente de vida de todo progreso". Las palabras de Louis Pasteur resumen cuál es y debe ser el principal motor del conocimiento científico y tecnológico: servir a la sociedad

En nombre de todos los presentes reciba, Profesor Nilsson, nuestra más cordial bienvenida, deseamos que su incorporación que representa para nosotros un honor, fructifique en una amplia colaboración entre las dos Academias a las cuales desde este momento pertenece la *Royal Swedish Academy of Engineering Sciences* y la Real Academia Europea de Doctores y que tienen previsto celebrar su primer acontecimiento científico en común en el mes de julio del próximo año.

Enhorabuena.

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Prof. Alfredo Rocafort, Chairman of the Royal European Academy of Doctors.
Distinguished Academics
Ladies and Gentlemen

The Royal European Academy of Doctors is pleased today to welcome Prof. Nilsson as Honorary academician and I am honored to be asked to give the words on the occasion of your solemn admission to our beloved and learned corporation.

Let me begin by expressing my gratitude to the Chairman of the RAED, Prof. Alfredo Rocafort for having entrusted me with the task of introducing this new academic. For the first time since I joined this corporation I am addressing you to comply with the request received and although I can say that for me represents a great honor, satisfaction and even a legitimate pride, these feelings are in balance with the responsibility that represents successfully carrying out such a distinguished and statutory commission.

Professor Bjórn Olof Nilsson was born on January 11, 1956 in Sollentuna, Stockholm, Sweden and in his person converge an excellent training as a biochemist and his remarkable leadership at the corporate level. Since August 2008 he has held the position of Professor President of the Royal Swedish Academy of Engineering Sciences (IVA) of Stockholm, Swede

Professor Nilsson developed and defended a PhD in Biochemistry from the Royal Institute of Technology in Stockholm, where he today holds an Adjunct position.

The importance of his thesis entitled "Fusions to the gene encoding staphylococcal protein A" is clearly demonstrated by the fact that from his studies in this area he has deepened the knowledge of Protein A, able to bind to the constant region of human antibodies, being responsible that the strains of *Staphylococcus aureus* to protect themselves and escape the response of the immune system. The mechanism and structure of the interaction surface were studied by their research team and based on this knowledge a synthetic domain of this protein A was designed.

They were also able to construct a combinatorial library that they expressed in bacteria and which has been called "Affibody." The concept of "Affibody" was the subject of one of the eight patents proposed and described by Prof. Nilsson and his team, and as he will comment in his lecture has given rise to an important company, leader in the development of new generations of bi and multi specific mimetic antibodies and more than 10 programs, useful in multiple therapeutic indications.

Professor Nilsson has held a wide range of management and executive roles in the biotechnology and pharmaceutical industry, mainly within the Pharmacia group of companies, including his responsibilities as President and Vice President of public companies. He also served as President of the Swedish Biotechnology Industry Organization (SwedenBIO) for four years and today, in addition to the IVA presidency, he serves as corporate Board member, including Chairman of the Board of the public biotechnology company BioInvent International AB (OMX Small Cap) and a board director of the engineering company AF (OMX Mid Cap).

Professor Nilsson is currently a member of the Boards and Committee of a large and select number of international, national, governmental and non-governmental organizations in areas such as Science, Education and Innovation.

In 2016, Professor Nilsson was elected as a member of the Governing Board of the European Institute of Innovation and Technology (EIT) and he is actively involved in the Reference group for the preparation of the Research Bill of the Swedish government.

More than 60 research articles in high impact factor journals are cited in detail in his *curriculum vitae*, as a selected example of his research work, and we can read three of his research papers in the book which will be delivered at the end of this Solemn Act of Reception.

He has received a lot of Honors and Awards in the course of her career:

- Post- Doc. Fellowship award from the Swedish Research Council Engineering Sciences (TFR) (1986-1988)
- Genentech Recognition Award (1988)
- Pharmacia & Upjohn Science and Medicine Award (1996)
- Recipient of a Bulgarian presidential award (2016)
- Recipient of H.M. The King of Sweden's Medal of the 8th size with the ribbon of the Order of the Seraphim (2016).

In addition to the scientific merits of Prof. Nilsson, we would like to highlight a facet of his life that has undoubtedly marked his scientific trajectory very positively. Professor Nilsson is an outstanding athlete, worthy of the highest national and international recognition. A medium distance runner between the late 70's and early 80's, his sportsmanship not only allowed him to achieve such meritorious awards but also crystallized in his appointment as President of the Swedish Athletic Association, since March2016. His example allows us to insist on the famous phrase *Mens sana in corpore sano* from the Roman poet Juvenal, which expresses the aspiration of a balanced mind in a balanced body.

Prof. Nilsson will then proceed to read his lecture entitled: "Promoting science, technology and innovation", result of his experience and deep reflection over the years dedicated to promoting the relationship between these three concepts in the various positions held by the new Academic in various forums for discussion at both national and international levels.

Promoting science, technology and innovation is essential for economic development and social progress. Research and development promote sustainable development, but to be effective, infrastructure development, technology transfer and public and private research need to be regulated through effective policies. In order to ensure that scientific progress is taken into account at all levels of government decision-making, UNESCO promotes the development of structures and mechanisms including scientific advisors, science and technology policy offices and parliamentary scientific committees. Every two years, UNESCO and its partners organize the World Science Forum, a biennial event that aims to become science what the World Economic Forum in Davos represents in economics. As innovation is fundamental for translating scientific and technological knowledge into useful products, services and employment,

UNESCO promotes closer links between universities and industry within its program of university-industry partnerships.

In addition to its role in providing global public goods, science, technology and innovation are crucial factors in increasing prosperity and improving national competitiveness. The connection between local technological needs and international technological opportunities is a challenge for many developing countries.

Science, technology and innovation raise the well-being of citizens through the social, economic and business development of a country

"Science is the soul of the prosperity of nations and the source of life for all progress." These words of Louis Pasteur summarize what is and should be the main driver of scientific and technological knowledge: serving society.

On behalf of all those present, receive, Prof. Nilsson, our warmest welcome, we hope that your incorporation, which represents an honor for us, will be fruitful in a wide collaboration between the two Academies to which from now you belong, Royal Swedish Academy of Engineering Sciences and Royal European Academy of Doctors which are planning to hold their first joint scientific event in July next year.

Congratulations.

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Discurso de ingreso

Dr. Björn O. Nilsson
Presidente de la Real Academia Sueca de Ciéncias de la Ingeniería

Dear academy fellows, ladies and gentlemen,

I am delighted to deliver this acceptance speech on behalf of becoming an Academician of the Royal European Academy of Doctors.

My speech will have as a main theme to connect knowledge derived from basic scientific research with technical applications for society, including business. It will be based on my own personal professional experience of research and promoting science, technology and innovation nationally as well as internationally.

More than 50 years ago, the US aerodynamics scientist of Hungarian origin, Theodore von Karman, elegantly canned the expression: “Science studies what is. Engineering creates what never was”. Thus, science will, out of curiosity, generate new data and knowledge through research. The engineers will use the scientific knowledge to create something brand new. And the process when knowledge come to use, it’s today referred to as *innovation*.

Our society is dependent on that the process of innovation is working. The importance of innovation in the formation our modern society cannot be exaggerated. The fuel of innovation is science. Technology, or the process of engineering, is necessary to create and invent new solutions to our grand challenges and to create competitive enterprises that generate jobs and prosperity. In fact, these two separate needs are highly connected since the new solutions to resolve societal challenges will also provide new business opportunities.

So, who am I? My own professional framework is mainly based on being the president of the Royal Swedish Academy of Engineering Sciences, IVA. IVA was the first engineering academy in the world and it's also one of the largest of its kind in terms of activities, projects, staffing and turnover. My professional framework in the academy includes advising society on scientific and technical issues. The academy IVA conducts large projects in areas such as energy, life science, health, innovation, research policy, young peoples interest for science & technology, etceteras. Indeed, any applied science area of relevance to society. As an academy, we are always evidence based and we are independent. In addition, I'm serving on a number of governmental and non-governmental academic committees and board. To mention a few, I am the chairman of the large Swedish Foundation for Strategic Research, chairman of the Stockholm Science City, member of the government's reference group for the national research bill. Since July 2016, I also serve as a director of the governing board of the European Institute of Innovation and Technology (EIT), which is the main dedicated innovation granting body of Horizon 2020 of the European Commission. Grants are provided to sizeable so called Knowledge Innovation Communities connecting universities and institutes with existing and new companies. Finally, my professional framework includes involvement in the business sector. For instance, I serve on two Swedish public company boards of which one is as chairman of BioInvent International, an antibody therapeutics biotechnology company headquartered in southern Sweden. In addition, I serve as a board director of AF, a major engineering consultancy company with 10.000 employees and activities in more than 100 countries.

Taken together, this landscape of activities and responsibilities have knowledge as the common denominator and they connect into the national and European systems to promote 3 a knowledge society in terms of innovation. I spend most of my days to bridge the university sector, the business sector and the political sphere. It's often quite a challenge but also very rewarding.

In this brief acceptance speech, I will mainly cover an example of innovation from my own research, that lead to many applications in business, in particular the formation of the company Affibody in Stockholm. Thereafter, I will end my speech by looking into the role of science to promote innovation in a society.

Early in my career, I studied the basic molecular biology of a pathogenicity protein named staphylococcal protein A, derived from the pathogenic bacterium *Staphylococcus aureus*. Protein A binds to the constant region of human antibodies, and by doing that the pathogenic bacterium can facilitate its defense to escape the immune system. Protein A is displayed on the surface of the pathogenic bacterium as a kind of protruding antennas binding the back side of antibodies.

In brief, each Protein A molecule consists of five individual domains of 58 amino acid residues, each one of them capable of binding to human antibodies. The mechanism and structure of the interaction surface were studied for many years by my team and others, also trying to address how this interaction contributes to bacterial pathogenicity.

We also used protein A fusions to manufacture challenging protein products of interests, also at large scale (see references 1-35).

Based on this detailed knowledge about structure and binding mechanism, a synthetic consensus domain of staphylococcal protein A was designed (36, 37). A combinatorial library was constructed where the 13 amino acid residues, known to be involved in antibody binding were randomized *in vitro* at the genetic level. The library was expressed in bacteria and the library of so called ”affibody” molecules were displayed on bacteriophages forming a combinatorial library from where binders to essentially any target molecule could be fished out.

The affibody concept was patented and the company Affibody was formed. It has for many years worked on applying the patented affibody technology into several biotechnology application areas such as diagnostics, medical imaging and more recently also into therapeutics, which by far is the commercially most interesting area. Affibodies have many unique advantages over for instance antibodies. Affibody application areas have continued to expand eg. forming bispecific divalent affibodies capable of simultaneously binding two separate antigens and combining the technology with other fusions to expand half-life in man.

Today, Affibody is a successful and profitable company with a recognized proprietary platforms for protein therapeutics (~30 patent families). The company has a newly developed next-generation bi- and-multi-specific antibody mimetics and more than 10 programs (proprietary & partnered) in multiple therapeutic indications. Affibody has started two clinic trials in 2016 and six additional products are predicted to be in clinical testing by 2018. The company has succeeded in bringing a number of international partners onboard such as Abclon, Biotest, Daewoong, Daiichi-Sankyo, Medimmune, Nordic Nanovector and Sobi.

So, what can we learn from the Affibody success story?

1. Basic knowledge generated through curiously driven research can be highly applicable. The application landscape can often not be understood when the scientific questions are answered.

2. When he affibody concept had been technically developed, and the Affibody company had been formed, there were still more than 10 years before the first commercial applications were ready for business partners.

So, for the future, we must prioritize to form a knowledge culture where education, basic research and innovation promoting activities are equally recognized. We'll need them all for the future, and perhaps not linearly applied, but more together.

Knowledge has never been more important in society.

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Trabajos aportados por el nuevo Académico de Honor

Folding of Insulin-like Growth Factor I Is Thermodynamically Controlled by Insulin-like Growth Factor Binding Protein†

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ABSTRACT: Insulin-like growth factor I (IGF-I) is thermodynamically unable to quantitatively form its native disulfides under reversible redox conditions *in vitro* [Hober et al. (1992) *Biochemistry 31*, 1749–1756]. These results prompted the question of how IGF-I may overcome this energetic problem in its folding *in vivo*. Here, we report that an IGF-I precursor, IGF-I-E_a, shows disulfide-exchange folding properties similar to those of mature IGF-I and, thus, is concluded not to overcome the identified folding problem of mature IGF-I. However, correct disulfide bonds are formed very efficiently when insulin-like growth factor binding protein 1 is added in equimolar amounts to IGF-I to the refolding mixture. On the basis of these results, we propose that one important function of at least one of the six homologous insulin-like growth factor binding proteins is to assist in the formation and maintenance of the native disulfides of IGF-I. To our knowledge, this is the first example where the folding of a mammalian protein or peptide in circulation has been demonstrated to be thermodynamically controlled by its binding protein. Speculatively, this could provide a mechanism to regulate the half-life of IGF-I *in vivo* by altering the interaction with insulin-like growth factor binding proteins.

Human insulin-like growth factor I (IGF-I)[1] is a three-disulfide 70 amino acid residue peptide growth factor (Humbel, 1990) with a defined three-dimensional structure in solution (Cooke et al., 1991). Recently, we (Hober et al., 1992) and others (Owers Narhi et al., 1993) reported that IGF-I is unable, as a result of its disulfide-exchange thermodynamic folding properties, to quantitatively form the native disulfides *in vitro*. The detailed analysis of the folding of IGF-I demonstrates the presence of two distinct three-disulfide-bonded forms (Hober et al., 1992): the native form (disulfides 6–48,[2] 18–61, and 47–52) and a non-native form designated "mismatched" (disulfides 6–47, 18–61, and 48–52). These two forms are equally represented in a disulfide-exchange equilibrium mixture (Hober et al., 1992), and they possess similar free energies of conformational stability (Owers Narhi et al., 1993). However, these two folding variants of IGF-I show significant differences in secondary structure contents (Hober et al., 1992), and only the native form is biologically active on the type 1 receptor (Raschdorf et al., 1988). This presence of two different, but thermodynamically equivalent, folding forms from one polypeptide chain is in conflict with the "thermodynamic hypothesis" of protein folding, as first proposed by Anfinsen (1973), and states that protein folding is expected to be energetically driven to a single native state. We have recently found that the thermodynamic folding problem of IGF-I is present not only at a redox potential comparable to that found in secretory vesicles (≈−200 mV) (Hober et al., 1992; Hwang et al., 1992) but also in more oxidizing environments (data not shown), similar to those found in plasma (≈0 mV) (Jellinek et al., 1992). Thus, the disulfides of IGF-I are thermodynamically unstable even in the oxidizing serum conditions. Since glutathione is abundant in serum (Shaheen & Hassan, 1991), the result would suggest that the folding behavior of IGF-I must be considered not only in the process of forming the disulfides but also in maintaining the native structure in the circulation.

It is unlikely that the formation of correct disulfides in IGF-I could be guided by classical protein folding chaperones, e.g., Hsp70, or folding enzymes, e.g., protein disulfide isomerase, since these have been shown only to affect the kinetic process of folding but not the folding thermodynamics (Nilsson & Anderson, 1991; Gething & Sambrook, 1992; Weissman & Kim, 1993). Instead, at least two different mechanisms can be proposed to act *in vivo* to quantitatively form and maintain correct disulfide bonds in IGF-I (Hober et al., 1992). First, the precursors of IGF-I, IGF-I-E_a and IGF-I-E_b (Rotwein, 1986), may show thermodynamic folding properties favoring the native disulfide bonds. Since these IGF-I precursors are not present in the circulation, the pro form of IGF-I could not function to maintain native disulfide configuration in the circulation, but it is still possible that folding would be promoted in this pro form in the secretory vesicles. This mechanism would be analogous to the enhanced folding properties of precursor forms of microbial proteases, e.g., subtilisin (Zhu et al., 1989), α-lytic protease (Baker et al., 1992), and carboxypeptidase Y (Winther & Sørensen, 1991). However, in all these cases, the propeptides seem to act at the kinetic level by accelerating the rate of folding, as was first demonstrated for α-lytic protease (Baker et al., 1992). A second hypothesis is that insulin-like growth factor binding proteins (IGFBPs) [for recent reviews, see Hintz (1990) and Shimasaki and Ling (1992)] assist in the formation of correct

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[1] Abbreviations: DTT, dithiothreitol; GSH, reduced glutathione; GSSG, oxidized glutathione; IGFBP, insulin-like growth factor binding protein; IGF-I, insulin-like growth factor I; IGF-I-E_a, insulin-like growth factor I precursor containing the C-terminal E_a peptide extension of 35 amino acid residues; PDMS, plasma desorption mass spectrometry; PFPA, pentafluoropropionic acid; RP-HPLC, reverse-phase high-performance liquid chromatography.

[2] In this paper, amino acid residues are numbered according to their position in the corresponding protein from its N-terminus, and disulfide bonds are designated as connected residue numbers, e.g., 18–61 as the disulfide connecting cysteine residues 18 and 61.

disulfide bonds by their binding to IGF-I. Both of these hypotheses have now been tested and are reported in this paper.

MATERIALS AND METHODS

Preparation of Native, Mismatched, and Reduced Forms of IGF-I. Native and mismatched forms of IGF-I were produced as a fusion protein in *Escherichia coli* and purified essentially as described [see Moks et al. (1987) and Forsberg et al. (1990)]. Reduced IGF-I was prepared by incubating the native protein in a buffer containing 10 mM reduced DTT and 8 M urea. After reduction, the buffer was changed to 10 mM HCl using gel filtration chromatography (Hober et al., 1992).

Production and Preparation of IGF-I-E_a. IGF-I-E_a was produced intracellularly in *E. coli* and purified as a fusion protein with an analogue (*Z*) of an IgG-binding domain of staphylococcal protein A based on the efficient expression system described by Altman et al. (1991). After production, cells were disrupted by incubation for 2 h at 20 °C in 6 M GuHCl, 50 mM phosphate buffer, pH 6.5, 150 mM NaCl, and 0.5 mM EDTA. The protein solution was diluted six times in 10 mM Tris, pH 8, 0.05% Tween 20, 200 mM NaCl, and 1.25 mM EDTA and subsequently purified by IgG affinity chromatography. The eluted fusion protein was immediately subjected to Mono S cation-exchange chromatography (Pharmacia Biotech, Uppsala, Sweden). After the protein was washed with 40 mM ammonium acetate, pH 5.5, containing 15% acetonitrile, Z-IGF-I-E_a was eluted with 2 M ammonium acetate, pH 5.2, containing 15% acetonitrile. The fusion protein was chemically cleaved with hydroxylamine as described in Moks et al. (1987). Released IGF-I-E_a was separated from uncleaved fusion protein (Z-IGF-I-E_a) as well as the fusion partner (Z) by a second passage through the IgG affinity column. The final purification of IGF-I-E_a was performed using Mono S cation-exchange chromatography. The column was preequilibrated in starting buffer (40 mM ammonium acetate, pH 7.0, 6 M urea, and 10 mM reduced DTT). Before application onto the column, the protein was reduced by incubation (1 h) in starting buffer at 37 °C. The reduced IGF-I-E_a was eluted with 1 M ammonium acetate, pH 7.0, 6 M urea, and 10 mM reduced DTT. The buffer was subsequently changed to 10 mM HCl by gel filtration chromatography using a Sephadex G25 column (Pharmacia Biotech, Uppsala, Sweden).

Production and Preparation of IGFBP-1. Recombinant IGFBP-1 was produced in DON cells transfected with a bovine papilloma viral vector containing an expression cassette with the cloned human IGFBP-1 gene (Luthman et al., 1989). IGFBP-1 was purified by IGF-I affinity purification followed by cation-exchange chromatography (details will be published elsewhere).

Protein Analysis. Purified proteins were analyzed by SDS–PAGE (Laemmli, 1970) using the Phast system (Pharmacia Biotech, Uppsala, Sweden). The concentrations of IGF-I, IGF-I-E_a, and IGFBP-1, respectively, were determined by quantitative amino acid analysis or by measuring the absorbance at 280 nm using the specific absorption constant A_{280} (1%, 1 cm) = 2.1 for IGF-I (Hober et al., 1992) and 2.4 for IGF-I-E_a.

Molecular masses of the different forms of IGF-I were determined using ^{252}Cf plasma desorption mass spectrometry (Bio-Ion 20, Applied Biosystems, Foster City, CA) (Hober et al., 1992). Molecular masses of IGF-I-E_a, and derivatives thereof, were determined using a JEOL SX102 (Tokyo, Japan) mass spectrometer equipped with an electrospray unit.

Disulfide-Exchange Reactions of IGF-I and IGF-I-E_a. The disulfide-exchange reactions were performed as described

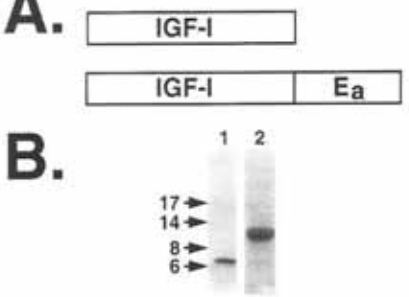

FIGURE 1: (A) Schematic representation of IGF-I and IGF-I-E_a. IGF-I is a protein of 70 amino acid residues, and IGF-I-E_a represents a pro form of IGF-I, having a C-terminal extension of 35 amino acid residues (Rotwein, 1986). (B) SDS–polyacrylamide gel electrophoresis analysis of purified recombinant IGF-I and IGF-I-E_a: lane 1, IGF-I; lane 2, IGF-I-E_a. Molecular mass, as measured by mass spectrometry, of purified recombinant IGF-I was 7650 ± 5 Da (calculated 7650 Da) and of purified recombinant IGF-I-E_a was 11682 ± 3 Da (calculated 11 684 Da).

(Hober et al., 1992) at an IGF-I concentration of 30 μM and at an IGF-I-E_a concentration of 5.5 μM at 37 °C for 1 h, which is at least 30 times that needed to reach equilibrium of reduced mature IGF-I under the conditions used (data not shown). The concentrations of oxidized (GSSG) and reduced (GSH) glutathione were 1 and 10 mM, respectively. The reactions were terminated by alkylating free thiols using 160 mM vinylpyridine as described (Hober et al., 1992). The method will efficiently pyridylethylate free thiols but not yield any detectable alkylation of non-thiol groups in IGF-I under the conditions used (Hober et al., 1992). After alkylation, the different forms of IGF-I and IGF-I-E_a, respectively, were separated on reverse-phase HPLC (Hober et al., 1992). IGFBP-1 is apparently unaffected in the alkylation reactions, and it elutes as a single peak at a retention time of ≈30 min in the HPLC system used (data not shown).

RESULTS AND DISCUSSION

Refolding of IGF-I-E_a. A recombinant IGF-I precursor, IGF-I-E_a (Figure 1A), having a C-terminal extension of 35 non-cysteine amino acid residues, was produced in *E. coli* and purified (Figure 1B). The identity of the purified IGF-I-E_a polypeptide chain was confirmed by mass spectroscopy. The thermodynamic properties of its disulfide-exchange folding was studied in a redox buffer containing reduced and oxidized glutathione. The redox potential in the experiment is comparable to that described for secretory vesicles of mammalian cells (Hwang et al., 1992), and these conditions were previously used to study the disulfides of IGF-I (Hober et al., 1992). In addition, these conditions are expected to favor the formation of protein disulfides and are typically used *in vitro* to generate reversible thiol-exchange conditions to study kinetics and thermodynamics of protein disulfide bond formation (Saxena & Wetlaufer, 1970; Creighton, 1984). The components in the refolding mixture were trapped by thiol alkylation using vinylpyridine. Separation of the different IGF-I-E_a forms was performed by RP-HPLC (Figure 2). The materials in the different peaks were collected and analyzed by mass spectrometry. These determined masses, and the absorbances at the pyridylethyl chromophore at 254 nm, were utilized to calculate the number of covalently attached pyridylethyl or glutathione groups. The relative amounts of

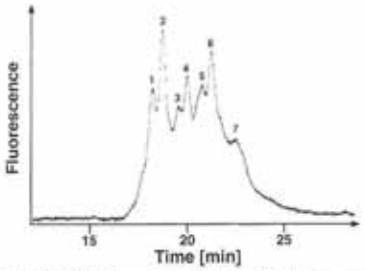

FIGURE 2: RP-HPLC separation of different IGF-I-E_a components after incubation in a redox buffer followed by pyridyl ethylation of free thiolate groups (Hober et al., 1992). The elution was monitored by a fluorescence detector and a diode array detector (data not shown) in series.

IGF-I-E_a polypeptide in the different peaks were determined by integrating their respective fluorescence (data not shown). The analysis of fractionated materials from the different peaks indicated that the major peak (peak 2 in Figure 2) is a two-disulfide-bonded form with two attached pyridylethyl groups. In the two peaks eluting before (peak 1) and after (peak 4) the major peak are three disulfide-bonded forms (data not shown). If the order in which the different forms of IGF-I and IGF-I-E_a elute from the reverse-phase HPLC system is maintained between IGF-I and IGF-I-E_a, the two-disulfide major peak (peak 2) of the chromatogram would correspond to IGF-I-E_a lacking the disulfide bond corresponding to 47–52 in native IGF-I, preceded by the peak containing mismatched IGF-I-E_a (peak 1) and followed by IGF-I-E_a with native disulfides (peak 4). A one-disulfide IGF-I-E_a species elutes late in the chromatogram (peak 6), which is also consistent with the elution profile of vinylpyridine-trapped forms of the mature IGF-I under these conditions (Hober et al., 1992). Even though the detailed analysis of the different IGF-I-E_a forms in the mixture remains to be performed by peptide mapping, the experiment implies that, under these redox conditions, IGF-I-E_a possesses a similar thermodynamic folding problem as previously described for IGF-I (Hober et al., 1992). Thus, we conclude that the analyzed precursor of IGF-I, IGF-I-E_a, fails to guide quantitative formation of native disulfides under the conditions used. However, it should be mentioned that only a single refolding time point (1 h) was used. Even though this refolding time exceeds the time needed for IGF-I to reach equilibrium by at least 30 times under the conditions used (data not shown), it is still possible that the IGF-I precursor indeed favors native disulfides but that the disulfide exchange rates become extremely slow by the precursor peptide extension. However, we find this possibility less likely in light of the results below and in light of the disulfide-exchange rates found *in vitro* for other disulfide-containing proteins (Creighton, 1984).

Refolding of IGF-I in the Presence and Absence of IGFBP-1. The majority of IGF-I and IGFBP-1 in circulation is coexpressed in the liver (Humbel, 1990), while in the plasma IGF-I is carried mainly by a high molecular weight form of IGFBP-3 (Hintz, 1990). Thus, one function of IGFBP-1 is thought to be the transport of newly synthesized IGF-I from the liver out into the circulation. We decided to study if IGFBP-1 additionally assists in folding by directing the formation of correct disulfides in IGF-I. Native, mismatched, and reduced IGF-I, respectively, were incubated in a glutathione redox buffer in the presence or absence of IGFBP-1. Separation of the different IGF-I components was performed

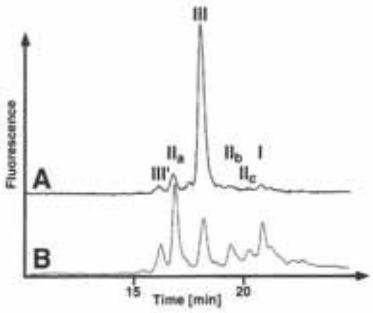

FIGURE 3: RP-HPLC separation of different IGF-I forms after incubation in the presence (A) or in the absence (B) of IGFBP-1 in a glutathione redox buffer. Here, the refolding was performed by starting from reduced IGF-I, but the results were indistinguishable by starting from either mismatched or native IGF-I (data not shown). IGFBP-1 elutes at ≈30 min (data not shown).

Table 1: Relative Amounts of the Different Forms of IGF-I in the Presence and Absence of Variable Amounts of IGFBP-1[a]

reaction conditions			forms of IGF-I					
IGF-I	IGFBP-1 (μM)	BSA (μM)	III (%)	III' (%)	IIa (%)	IIb (%)	I (%)	O (%)
O	30	0	89	<4	11	<4	<4	<4
O	0	0	22	11	30	12	25	<4
O	0	30	23	10	30	11	26	<4
III'	30	0	84	5	11	<4	<4	<4
III'	0	0	22	10	31	12	25	<4
III	30	0	86	4	10	<4	<4	<4
III	3	0	29	9	30	8	24	<4
III	0.3	0	26	11	32	8	23	<4
III	0	0	22	10	32	9	27	<4

[a] The IGF-I concentration was 30 μM in all experiments. The amount of IGF-I in each peak was determined by integrating the fluorescence signal from the HPLC chromatogram, which has been shown to be consistent (within 5%) with quantitative amino acid analysis of isolated materials for all these peaks of IGF-I (Hober et al., 1992). Peak designations for **III**, **III'**, $\mathbf{II_a}$, $\mathbf{II_b}$, and **I** are as described in the text. **O** is the form with six pyridylethyl groups. As a control experiment, disulfide-exchange reactions were performed in equimolar amounts of bovine serum albumin. The IGF-I column shows the form of IGF-I (**O**, **III**, or **III'**) that was used as starting material in the different redox reactions.

on RP-HPLC (Figure 3). The identity of each peak was confirmed by its retention time in the HPLC system as well as by mass spectrometry analysis of isolated peak materials. Most of these peaks have previously been analyzed in detail by peptide mapping techniques (Hober et al., 1992). Roman numbers in the chromatogram represent the different trapped forms of IGF-I (Hober et al., 1992). **I** is a form with only the native disulfide between amino acid residue 18 and 61, $\mathbf{II_a}$ is a form with two of the three native disulfides present between amino acid residues 18–61 and 6–48, $\mathbf{II_b}$ corresponds to a variant of IGF-I with two disulfide bonds of which one is native (18–61) and the other (6–52) is not present in native IGF-I, and peak $\mathbf{II_c}$ contains an IGF-I variant with two disulfide bonds that remain to be analyzed. **III'** is the mismatched IGF-I which is a form with three disulfides of which one is native (18–61) and the other two (6–47 and 48–52) are not present in the native and biologically active molecule, and **III** is the native molecule with disulfides 6–48, 18–61, and 47–52. It was found that only in the presence of IGFBP-1 does IGF-I quantitatively attain its native three-disulfide conformation (Figure 3, Table 1). Refolding experiments with an excess of IGF-I over IGFBP-1 (Table 1) suggest that equimolar amounts of IGFBP-1 and IGF-I are

necessary to quantitatively form and maintain the native disulfides of IGF-I. This would indicate that the molecular mechanism by which IGFBP-1 is assisting in the folding of IGF-I is a mass-action effect by recognition of only the native IGF-I molecule in forming the heterodimeric complex. Thus, complex formation with IGFBP-1 can overcome the previously described thermodynamic problem in quantitatively forming correct disulfides in IGF-I.

In serum and in other extracellular fluids, IGF-I is carried by at least six distinct IGFBPs, and there are a large number of reported different activities of these homologous molecules (Hintz, 1990; Shimasaki & Ling, 1992), and the most important of these are (i) to protect IGF-I from clearance and proteolytic degradation, (ii) to transport IGF-I to specific tissues, (iii) to play a role in hormone regulation, (iv) to prevent hypoglycemia by inhibiting the binding of IGF-I to the insulin receptor, (v) to increase the potency of IGF-I by interacting with the cell surface, (vi) to remove IGFs from tissue and circulation, and (vii) to inhibit the biological activity of IGFs. On the basis of our results, we propose that an additional and important function of the IGFBPs is to form and maintain native disulfides in IGF-I. Since the majority of IGF-I in circulation is carried by IGFBP-3, the question of whether this, and other IGFBPs, could also act in forming and maintaining the native IGF-I disulfides arises. Because of the significant homologies between the different IGFBPs and the identified problem to maintain IGF-I disulfides in the circulation, we find this likely.

IGFBP-1 contains 18 cysteine residues furnishing 9 intramolecular disulfide bonds (Hintz, 1990). Do these disulfides participate in the refolding of IGF-I? In fact, we have recently addressed this issue and found that IGFBP-1 significantly accelerates the refolding rate of reduced IGF-I (S. Hober and B. Nilsson, data not shown). However, the mechanism of this acceleration could be found in other explanations than IGFBP-1 acting as a disulfide isomerase. Even though the folding problem of IGF-I that has been addressed in this paper is of thermodynamic and not of kinetic nature, we find this effect on the IGF-I refolding rate interesting.

Can IGFBP-1 act also at the precursor level? When the pro form IGF-I-E_3 (Figure 1) was allowed to fold *in vitro* in the presence of IGFBP-1, IGF-I-E_3 with disulfide bonds corresponding to those in native IGF-I was quantitatively formed (data not shown). This result demonstrates that IGFBP could act to promote correct folding of IGF-I not only at the level of the mature polypeptide chain but also at the precursor level.

The results presented in this paper suggest that IGFBP assists in the folding of IGF-I *in vivo*, which would solve both the forming and the maintenance problem of the energetically unfavorable native disulfides present in the noncomplexed IGF-I molecule. Thus, thermodynamically, IGF-I could be considered as the receptor binding subunit in a heterodimeric complex with an insulin-like growth factor binding protein. Speculatively, the binding of IGF-I to IGFBP provides a mechanism to regulate the half-life of IGF-I in the circulation, since the stability of noncomplexed IGF-I molecules can be predicted to be low due to the instability of its disulfide bonds under serum redox conditions. A truncated form of IGF-I, lacking the three N-terminal amino acid residues, is present in brain (Sara et al., 1986) and in other tissue (Ogasawara et al., 1989). This form of IGF-I, des(1–3)-IGF-I, binds poorly to IGFBP-1 but is apparently more potent in receptor binding than IGF-I (Ballard et al., 1987). From our results we suggest that the significance of this posttranslationally modified form of IGF-I is to exclusively act locally and to become rapidly inactivated in the circulation.

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IMMOBILIZATION AND PURIFICATION OF ENZYMES WITH STAPHYLOCOCCAL PROTEIN A GENE FUSION VECTORS

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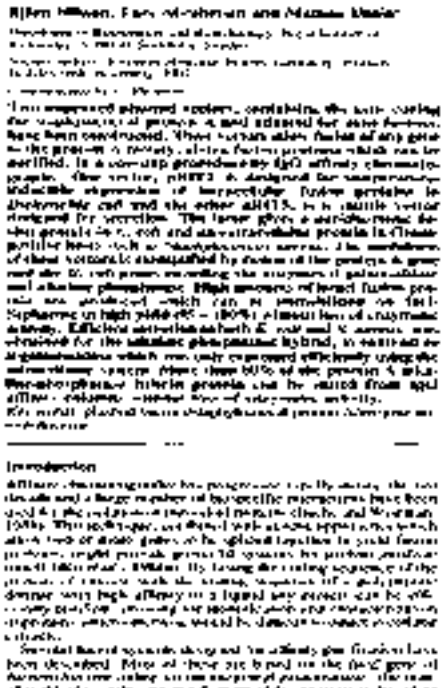
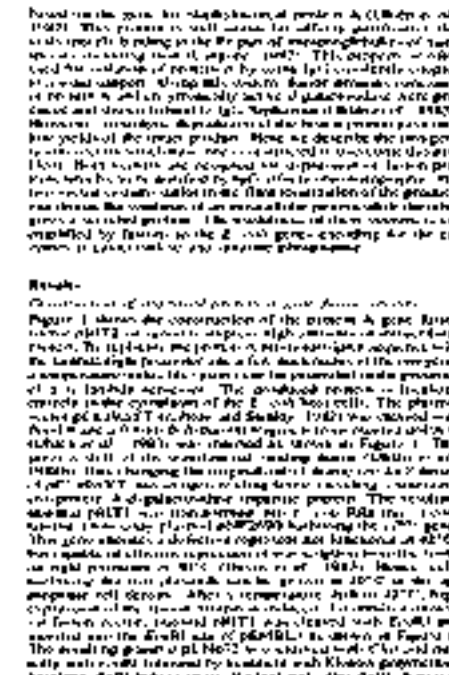
Presidente de la Real Academia Sueca de Ciencias de la Ingeniería





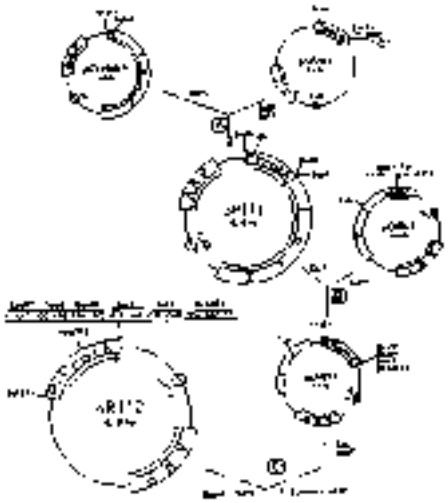


Fig. 1. Construction of the vector pRIT2. A few restriction sites are indicated. Boxes show the relative position of genes coding for protein A (Prot A), β-lactamase (Amp), chloramphenicol acetyltransferase (cat), phospholipase inhibitor, Staphylococcus aureus DNA fragment coding for the N-terminal part of β-galactosidase (lacZ') and the N-terminal part of Bacillus subtilis neutral protease (nprE). Hatched areas indicate signal sequences and direction of replication (ori) is also indicated. The hydrophobic sequence of the cloning vector is shown.

fusion point provides a guide for aligning reading frames to obtain the correct hybrid protein.

Figure 2 shows the construction of a gene fusion vector allowing replication in *E. coli* and a number of Gram-positive bacteria, such as *S. aureus*, *S. xylosus* and *S. epidermidis* (Uhlén et al., 1984c). The resulting vector pRIT5 contains the promoter/signal sequence of the protein A gene, which is functional in all three species, giving a periplasmatic protein in *E. coli* and an extracellular protein in *S. aureus*. Plasmid pSPA16 (Uhlén et al., 1984c) was cleaved with *Taq*I and *Eco*RI and the 1.1 kb fragment, containing the protein A promoter and the coding region of the signal sequence and the IgG-binding region, was purified. This fragment was inserted between the *Cla*I and the *Eco*RI sites of pEMBL9 (Dente et al., 1983). The resulting plasmid pRIT2 (Figure 2), was linearized with *Pst*I and ligated with pC194 (Horinouchi, 1979) cleaved with *Hin*dIII and made blunt-ended with Klenow polymerase. The resulting plasmid pRIT4 (Figure 2) is a shuttle vector allowing transfer between Gram-positive and negative bacteria from Penky, one of the *Hin*dIII sites was deleted by partial cleavage with *Hin*dIII, making blunt-ends with Klenow polymerase and ligating the ends together. The resulting gene fusion vector pRIT5 is shown schematically in Figure 2. Note that the periplasmic region of vector pRIT5, as well as vector pRIT2 (Figure 1), contains unique restriction sites, thus facilitating insertions of foreign genes.

The alkaline phosphatase gene from *E. coli* without its signal sequence was inserted into the unique *Pst*I site of pRIT5 (Figure 2) and the plasmid was transformed into *E. coli*. The resulting

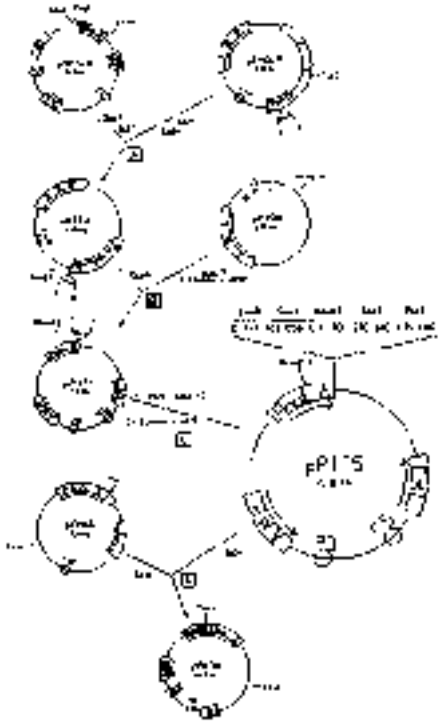
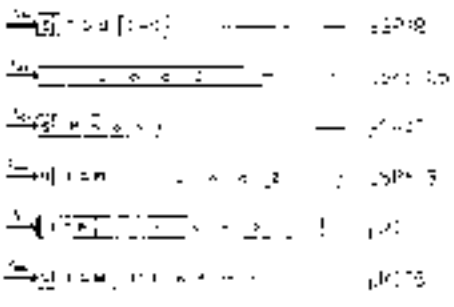


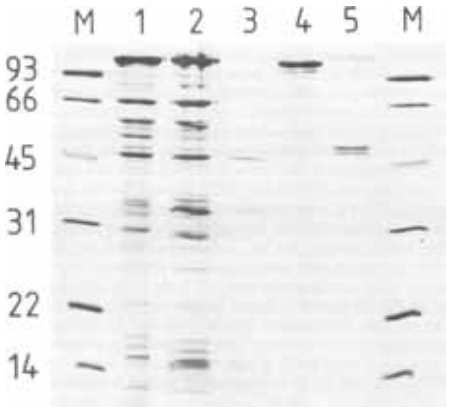
Fig. 2. Construction of the vector pRIT5. The abbreviations are as in Figure 1. The gene coding for alkaline phosphatase (phoA) and the origin of replication functional in *E. coli* (ori-E) and *S. aureus* (ori-S) are indicated.

plasmid pRIT6 encodes a fusion protein consisting of the N-terminal 271 amino acid residues of the mature protein A, a linker region of 11 residues and the C-terminal portion of the alkaline phosphatase, starting at residue 13. Clones were selected by plating the transformed cells on ampicillin plates containing a chromogenic substrate giving blue colonies when a functional alkaline phosphatase is expressed. In order to repress the endogenous alkaline phosphatase gene, 0.4% phosphate buffer was included in the plate media, to repress then the chromosomal phoA gene.

Expression and localization of β-galactosidase fusion proteins in E. coli

Table 1 shows the β-galactosidase activity in *E. coli* K-12 strains containing four different plasmids. The activity is determined both for whole cells after permeabilization of the cell membrane with toluene and for cell-free extracts prepared by sonication. The plasmids were pSPA5, encoding an intact protein A gene, pRIT5/106 expressing the β-galactosidase, pSPA12 with a protein A–β-galactosidase gene fusion containing the protein A signal sequence (Uhlén et al., 1983) and pRIT1 with a signalless gene





fusion-encoding protein A-β-galactosidase lacking the protein A signal sequence (Figure 1). The latter plasmid containing the efficient right promoter (P_R) of phage lambda, must be repressed during bacterial growth (Zabeau and Stanley, 1982). A temperature-sensitive cI^{ts} repressor was therefore provided in trans by a separate compatible plasmid, phi-2080. Cells harboring both plasmids were grown at OD_{550} = 0.5 at 30°C and then shifted to 42°C for 90 min, to derepress the promoter thus ensuring efficient transcription from the cro-protein A-β-galactosidase fusion gene. The induction caused a dramatic change in the morphology of the E. coli cells, as determined by phase contrast microscopy (not shown). Cell motility was reduced and cells with abnormal size and shape became predominant as previously observed with strains overproducing proteins under the control of the P_R promoter of phage lambda (Shirley, 1983, Remaut et al., 1981). Supernatants from sonicated cell extracts of cells harboring pRIT1 contained less than 1 unit (440 K) of beta-galactosidase activity, whereas cells containing the original pSPA13 gene fusion (Figure 4). The non-permeable fusion protein remains in the cell pellet, suggesting that a fraction of the fusion protein precipitate inside cells, as previously reported for other β-galactosidase fusion proteins (Goeddel et al., 1978, Zabeau and Stanley, 1982). The SDS-gel (Figure 4) even with less than 10 K protein at 30°C (lane 3) compared to 42°C in which the band becomes a major component (lane 2). Measurements of the β-galactosidase activity showed at least a 100-fold increase in activity upon induction (data not shown). The data in Table I also suggest that only just a small fraction of the precipitated fusion protein in pRIT1 cells. In contrast, the activity of sonicated

lysates from pSPA13 cells is less than the toluene-released activity, suggesting that the hybrid protein encoded by pSPA13 is membrane bound (Ullitz et al., 1983). A 1000-fold enrichment of activity was obtained comparing cells containing pRIT1 and cells containing the original pSPA13 gene fusion.

IgG-Sepharose affinity chromatography was used to purify the pRIT1 protein A fusion protein from a cell extract. SDS-gel electrophoresis of the purified protein reveals a major band of ~140 K, which is the expected size (Figure 4). The deduced amino acid sequence predicts that, for the cro-protein A and β-galactosidase components of, 7, 100 and 110 000, respectively, yielding up to a total mol. wt. of 141 000 for the fusion protein. In contrast, purification on IgG columns of the pSPA13 gene product revealed no or little full-length product (140 K). This suggests that the membrane-bound β-galactosidase-protein A is highly susceptible to proteolysis.

Expression and localization of alkaline phosphatase hybrid proteins in *E. coli* and *S. aureus*

Table II shows the alkaline phosphatase activity in *E. coli* cells

[illegible]

Plasmid	Gene	Phosphatase activity (U/ml)		Alkaline phosphatase activity (U/ml)		Relative amount of alkaline phosphatase bound to human IgG	
		Culture	Periplasm	Culture	Periplasm	supernatant	sediment
pRIT5	Protein A	2	1	<0.1	<0.1	+/-	+/-
pCH10	Alkaline phosphatase	<0.1	4.0	1	8	<0.2	100
pRIT6	Fusion	21	15	5	4	60	<0.2

Values are calculated per ml cell culture and 1 unit of activity is defined as in Materials and methods. The periplasmic enzymes were prepared by an EDTA-lysozyme treatment and the relative amounts of phosphatase bound to IgG-Sepharose were determined as described in Table I.

containing three different plasmids. The cells were grown in liquid medium containing 0.9% physiological buffer to repress endogenous alkaline phosphatase. The total activity was determined by sonication of cells in culture and the periplasmic activity by osmotic shock preparation. The plasmids were pRIT5 containing the whole protein A gene, pCH10 containing the alkaline phosphatase gene under constitutive transcriptional control by the β-lactamase promoter-signal sequence (C. Hoffman and A. Wright, personal communication) and pRIT6 containing the protein A-alkaline phosphatase gene fusion (Figure 3).

Determination of the protein A content in the cell lysates containing plasmids pSPA3 and pRIT6 revealed that the fusion protein (pRIT6) has been cleaved 16-fold compared with the protein A gene product (pSPA3) although its regulatory signals both at the transcriptional and the translational level are identical. Although factors such as mRNA stability or plasmid copy number could influence the steady state yield, the large difference suggests that the protein A moiety is protected from proteolysis by the alkaline phosphatase. This is supported by SDS-gel electrophoretic analysis of the affinity purified fusion protein revealing a major band which appears to have a mol. wt. of ~70 000 (Figure 5, lane 2). In contrast, mature protein A produced in *E. coli* (Uhlen *et al.*, 1984c) contains only minute amounts of the full-length protein (Uhlen *et al.*, 1984c). The protein A-alkaline phosphatase fusion protein encoded by pRIT6 contains the protein A signal sequence and the hybrid protein should therefore be translocated through the cytoplasmic membrane of the host cells. Measurements both of the protein A content and the alkaline phosphatase activity confirm that the fusion protein is indeed found in the periplasm, suggesting that a staphylococcal signal sequence can direct export of an enzyme in *E. coli*.

To investigate the yield of fusion protein in a Gram positive host, the plasmid pRIT6 was transformed to *S. aureus* RN4220 with standard unique techniques (Uhlen *et al.*, 1984c). SDS-gel electrophoretic of periplasmic fusion protein from the culture of *S. aureus* cells containing pRIT6 reveals very little intact protein (Figure 5, lane 1), in contrast to the same protein produced in *E. coli*. The alkaline phosphatase activity of the medium of *S. aureus* is also slightly lower than the periplasmic activity in *E. coli* (data not shown). These results suggest extensive degradation of the fusion protein in the Gram positive host. However the activity is found intracellularly which indicates that the secretion is functional although a heterologous protein has been fused to the protein A moiety.

Binding to IgG-Sepharose

Lysates from 1 ml *E. coli* cell cultures (Figure 3) containing the different plasmids were mixed with 0.1 ml of IgG-Sepharose. The mixture was rocked at room temperature for 1 h and the supernatants collected. The solid matrix were washed three times and the amounts of immobilized enzyme were determined as described in Materials and methods. The binding efficiencies of

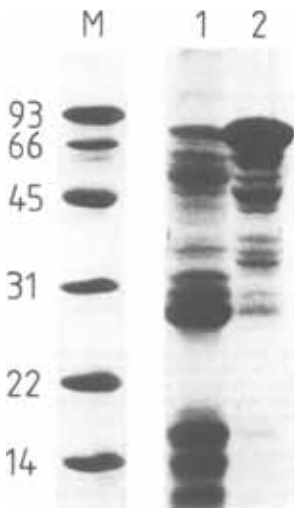


Fig. 5. SDS PAGE of affinity-purified alkaline phosphatase fusion protein from *E. coli* (1) and *S. aureus* containing plasmid pRIT6. Lane M = molecular weight standards with the size indicated. Lane 2 = fusion protein from the periplasm of *E. coli*. Lane 1 = fusion protein from the medium of *S. aureus* RN4220 containing pRIT6. The samples are incubated at 37°C in the presence of 1% SDS. The bands are visualized by Coomassie Brilliant Blue staining.

the β-galactosidase fusion proteins are shown in Table I. Since β-galactosidase alone is completely GKSRR does not bind to IgG-Sepharose. In contrast, >95% of the β-galactosidase from cells expressing fusion proteins bind to the gel. Measurements of the supernatants reveal no detectable protein from the pRIT1 gene product. Table II shows the immunogenicity of the alkaline phosphatase fusion proteins. All the activity encoded by pRIT6 binds to the gel as compared to the control (pCH10) which does not bind. Both enzymes can also easily bind to IgG-Sepharose when fused to protein A with little or no loss of enzymatic activity.

Affinity purification

Efficient elution of protein A molecules from IgG-Sepharose is performed with glycine buffer, pH 3.0. This was used for the analysis of the products on SDS-gels (Figures 4 and 5) and gave a pure product in high yield (> 95%). However, this procedure denatures the enzymes with loss of activity. The eluate must be

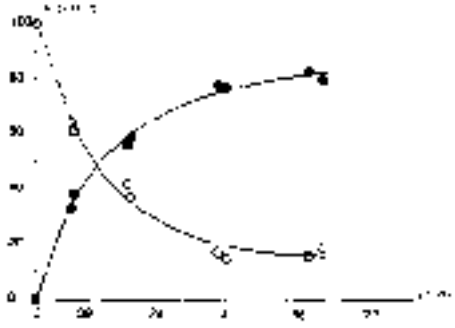


Fig. 6. Elution of alkaline phosphatase protein A fusion protein from IgG Sepharose with increasing concentrations of human immunoglobulin G. The relative activity of alkaline phosphatase bound to IgG at 1 mM shown (the other 3 were incubated with buffer alone).

a serious problem, for sequential analysis or immunization, but it hampers a functional study. It might, however, in simple cases be possible to construct the enzyme after elution.

The β-galactosidase activity is rapidly denaturated by the glycine buffer and ~60% of the alkaline phosphatase activity was inactivated after an elution from an IgG column followed by immediate change of pH. Alternative elution methods have therefore been investigated. We have earlier shown that >50% of the protein can be eluted by competing with pure protein A (Uhlén et al., 1983). Using IgG from sheep applied to Sepharose it was possible to bind and elute ~30% of the β-galactosidase activity with a glycine buffer at pH and 5 M NaSCN (Nilsson and M. Uhlén, unpublished) presumably because the affinity between protein A and IgG of this species is weaker (Langone, 1982). For the alkaline phosphatase fusion protein it was possible to use lithium diiodosalicylate (LiS) buffer, giving efficient elution without affecting the enzymatic activity. Figure 6 shows the activity bound and eluted after 5 min of incubation with increasing concentration of the LiS elution buffer. More than 80% of the fusion protein can be recovered with intact enzymatic activity.

Discussion

This paper has described the construction of two gene fusion vectors allowing expression of fusion proteins which can be affinity purified by a one-step procedure. The purification is based on protein A which under physiological conditions binds to the Fc region of human IgG with a dissociation constant of 2×10^{-8} (Sjöquist and Kronvall, 1980). Fusion proteins with protein A moiety can therefore rapidly be recovered from a crude lysate under conditions which do not denaturate the proteins. In this report we describe how this system can be used to purify enzymes with high efficiency. Obviously, other applications also exist and we have successfully been able to produce and affinity purify fusion proteins for direct amino acid sequencing (M. Hellman and M. Uhlén, unpublished) or to obtain specific antibodies (raised in the foreign gene product) (B. Lovgren-Bengtsson and B. Nilsson, unpublished).

The first vector, pRIT2 (Figure 1), allows for periplasmic translocation of heterologous fusion proteins in *E. coli* with the second, pRIT5 (Figure 2), directs the synthesis of fusion proteins which are transported into the periplasmic space of *E. coli* and are secreted into the medium of Gram-positive hosts, such as *S. aureus*. Protein synthesized by the latter can be collected by osmotic shock (E. Nilsson et al., manuscript in preparation) or directly from the culture medium (*S. aureus*) without the need to disrupt the cells. Secretion also allows the formation of disulfide bridges which cannot be formed in the reducing environment of most bacteria (Nilsson et al., 1985). An additional advantage with the shuttle vector pRIT5 is that the promoter/signal sequence of protein A is functional in several bacteria, allowing for transfer of the fusion construct into different hosts. The pRIT2 vector is based on the phage lambda P_R promoter and produces only monocistronic hybrid proteins (Oberkowski, 1983). As the disadvantage that cytosolic bridges cannot form, the cells must be disrupted before purification and only *E. coli* can be used as host. However, in many cases extracellular accumulation might be the only alternative since most intracellular proteins will not be transported across the membrane even when the protein contains a functional signal sequence. β-Galactosidase with such a sequence might be the most interesting protein to produce (Silhavy et al., 1977; Ullrich et al., 1981) and is therefore suitable to produce (Figure 4). This emphasizes that signal peptides cannot be used interchangeably for fusions. The two vectors must therefore be tested for each gene product separately.

Two *E. coli* genes were selected to test the vectors. The first, the *lacZ* gene, encodes β-galactosidase which is an intracellular enzyme consisting of four identical subunits each with a mol. wt of 130 000 (Kalnins et al., 1983). The second gene encodes alkaline phosphatase which is a periplasmic enzyme consisting of two identical subunits each with a mol. wt of 47 000 (Bradshaw et al., 1976). Using the intracellular expression system it was possible to overcome the problems encountered earlier (Uhlén et al., 1983), with the protein A-*lacZ* fusions. High production (pRIT2, Table I) and little degradation was observed (Figure 4). However, the alkaline phosphatase gene gave a functional hybrid protein which could be secreted to the periplasm of *E. coli* (Table II) and the medium of *S. aureus* (not shown). The fusion protein can thus be translocated through bacterial membranes with high efficiency. Affinity purification revealed that the gene fusion was susceptible to degradation in *S. aureus* (Figure 3), suggesting that *E. coli* is a better host for the construct. In contrast, *S. aureus* is probably better for expressing truncated proteins. A molecular (Uhlén et al., 1984a) or fusion program consisting of protein A and the mature part of hormones such as insulin-like growth factor I (Nilsson et al., 1985). This demonstrates the need for alternative expression vectors as well as host organisms for expressing recombinant proteins in bacteria.

During the last decade, the techniques for immobilizing enzymes have been greatly refined (Bucke and Wiseman, 1981). Involving methods such as covalent coupling, adsorption, entrapment and aggregation and has led to novel forms of biochemical catalysts which have been used for different applications. Thin cellulose enzyme electrodes (Guilbault et al., 1976), immobilized enzymes for therapeutic treatment (Chang, 1976) and large-scale enzyme technology where enzyme reactors can carry out industrial processes (Lilly and Dunnill, 1976). The use of the fusion gene has so far been limited. Nevertheless, the simplicity and efficiency of immobilizing enzymes on a solid support using the genetic approach might have an impact. A pure and immobilized enzyme can be obtained in large quantities simply by passing the cell lysate through an affinity column, giving efficient binding with little or no loss of enzymatic activity. In addition it is also possible to regenerate the column whenever the activity of the enzyme has decreased.

Materials and methods

Bacterial strains and plasmids

[illegible] pRIT5 (Pharmacia Biosensor, Uppsala, Sweden), pEZZ18 (Pharmacia LKB Biotechnology, Uppsala, Sweden), pUC18 (Stanley and Luzio, 1984), pUC19 (Yanisch-Perron et al., 1985), pUC118 and pUC119 (Vieira and Messing, 1987), pSK(SK+) (Stratagene, La Jolla, CA, USA), pBR322 (Bolivar et al., 1977), pBluescript II SK+ (Stratagene, La Jolla, CA, USA), pCK40 (Kolter and Helinski, 1978), pCK100900 (Lindberg et al., 1987), pEMBL18+ (Dente et al., 1983), pEMBL19+ (Dente et al., 1983), pRIT2T (Nilsson et al., 1985).

DNA manipulations

Restriction enzymes, T4 DNA ligase, Klenow DNA polymerase and other DNA modifying enzymes were used according to the suppliers' directions. Standard techniques were employed for transformation of *E. coli* and plasmid isolation by alkaline lysis (Maniatis et al., 1982). Small-scale preparations of DNA were performed as described by Holmes and Quigley (1981). Agarose gel electrophoresis was performed according to Sharp et al. (1973). DNA was isolated from agarose gels by electroelution (Dretzen et al., 1981). Ligations were performed overnight at 15°C using 400 U T4 DNA ligase (New England Biolabs, Beverly, MA, USA) per ml. Ligation mixtures were used directly for transformation of competent cells prepared by the calcium chloride method described by Cohen et al. (1972). Transformants were selected on LB-agar plates containing 100 μg ampicillin per ml.

Construction of plasmid pEZZ18

The plasmid pEZZ18 was constructed in a three-step procedure. In the first step, the 1.2 kb *Eco*RI–*Hin*dIII fragment from pRIT5 was ligated into pUC18 digested with the same enzymes. The resulting plasmid was digested with *Eco*RI and *Bam*HI, and the 1.2 kb fragment was ligated into pUC19 digested with the same enzymes. The resulting plasmid was digested with *Hin*dIII and *Bam*HI, and the 1.2 kb fragment was ligated into pUC118 digested with the same enzymes. The resulting plasmid was digested with *Eco*RI and *Hin*dIII, and the 1.2 kb fragment was ligated into pEMBL18+ digested with the same enzymes. The resulting plasmid was digested with *Eco*RI and *Bam*HI, and the 1.2 kb fragment was ligated into pEMBL19+ digested with the same enzymes. The resulting plasmid was digested with *Hin*dIII and *Bam*HI, and the 1.2 kb fragment was ligated into pUC119

A SYNTHETIC IGG-BINDING DOMAIN BASED ON STAPHYLOCOCCAL PROTEIN A

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A synthetic IgG-binding domain based on staphylococcal protein A was designed with the aid of sequence comparisons and computer graphic analysis. A strategy, utilizing non-palindromic restriction sites, was used to overcome the difficulties of introducing site-specific changes into the repetitive gene. A single mutagenized gene fragment was polymerized to different multiplicities, and the different gene products were expressed in *Escherichia coli*. Using this scheme, protein A-like proteins composed of different numbers of IgG-binding domains were produced. These domains were changed to lack asparagine–glycine dipeptide sequences as well as methionine residues and are thus, in contrast to native protein A, resistant to treatment with hydroxylamine and cyanogen bromide.

Introduction

Staphylococcal protein A (SPA) plays an important role in qualitative and quantitative immunology due to its specific binding to the Fc-portion of immunoglobulins from most mammalian species, including man (Langone, 1982). In addition, a large number of biological properties have been attributed to SPA, e.g. Fab binding, activation of the complement system, hypersensitivity reactions, cell-mediated cytotoxicity, interferon induction, activation of polyclonal antibody synthesis and mitogenic stimulation of lymphocytes (Sjöquist and Stahlenheim, 1969; Cowan *et al.*, 1979; Sjödahl and Möller, 1979; Romagnani *et al.*, 1980; Inganäs and Johansson, 1981; Catalona *et al.*, 1981). Because of its importance as an immunological tool and its diverse biological function, extensive structural and biochemical studies of SPA have been performed (Sjöquist *et al.*, 1972; Hjelm *et al.*, 1975; Sjödahl, 1977; Langone, 1982; Hanson and Schumaker, 1984). However, despite these extensive studies, several conflicting models to explain both the structure and function of the IgG–SPA complex exist (Inganäs and Johansson, 1981; Langone, 1982; Hanson and Schumaker, 1984).

Recently, the gene encoding SPA was cloned (Löfdahl *et al.*, 1983), sequenced (Uhlén *et al.*, 1984a) and expressed in heterologous hosts (Uhlén *et al.*, 1984b). The sequence revealed a remarkably repetitive protein with two structurally and functionally different parts (Uhlén *et al.*, 1984a). The N-terminal part of the mature protein, responsible for the binding to the Fc-portion of IgG, consists of five homologous domains each of about 58 amino acid residues (Moks *et al.*, 1986) which are individually IgG-binding (Sjödahl, 1977; Moks *et al.*, 1986). The C-terminal part is responsible for the association to the cell wall of *Staphylococcus aureus* (Guss *et al.*, 1984). Both ends of the primary sequence are hydrophobic corresponding to an N-terminal signal peptide (Abrahmsén *et al.*, 1984) and a C-terminal transmembrane region (Uhlén *et al.*, 1984c).

Using protein engineering it is now possible to try to resolve some of the unanswered questions concerning the SPA–IgG interaction. Such studies may be realized since the complex between region B of protein A and the Fc-fragment of IgG have been crystallized and the three-dimensional structure has been resolved to a resolution of 2.8 Å (Deisenhofer, 1981).

However, the repetitive structure of the native SPA gene makes site-specific mutagenesis technically difficult. After mutations of one of the repeats the mismatch primer will, in the next mutagenesis step, anneal more efficiently to a mutated than to a non-mutated repeat. In addition, multiple region mutants are hard to select from a single region mutant by hybridization. These problems severely limit the value of protein engineering in the study of protein A.

In this paper, we introduce an alternative and general approach to mutagenize repetitive genes. Using obligate head-to-tail polymerization of a synthetic DNA fragment, mutated gene products consisting of different multiplicities of a protein A-like fragment were obtained. The system was used to construct IgG-binding molecules which are resistant to specific chemical treatments, without changing the function of the molecule. The use of this mutagenesis method to study the structure–function relationship of the SPA molecule is discussed.

Materials and methods

Bacterial strains and plasmids

Escherichia coli HB101 (Boyer and Roulland-Dussiox, 1969) and *E. coli* JM103 (Messing *et al.*, 1981) were used as bacterial hosts. Plasmid and phage vectors were pRIT4 (Nilsson *et al.*, 1985a), pEMBL19, pEMBL8 (Dente *et al.*, 1983), M13mp8 (New England Biolabs) and phage f1 (Dente *et al.*, 1983).

DNA techniques and cell growth

Restriction enzymes (Pharmacia, New England Biolabs and Boehringer Mannheim) were used according to the recommendations of the suppliers. Transformation and growth of *E. coli* were performed as described by Abrahmsén *et al.* (1986). Single-stranded DNA from pEMBL-derived constructions for sequencing was prepared using f1 as helper phage as described by Dente *et al.* (1983).

DNA sequencing

The DNA sequencing was performed by the dideoxy method using reversed sequencing primer or the RIT-sequencing primer (Moks *et al.*, 1986) (KabiGen AB, Sweden). Deoxynucleotides, dideoxynucleotides (Pharmacia, Sweden), [α-^{35}S]dATP (Amersham International) and DNA polymerase large fragment (Boehringer Mannheim) were used. The labelled reaction mixtures were separated by electrophoresis on wedge shaped polyacrylamide gels (Olsson *et al.*, 1984) using the thin gel Macrophor system (LKB, Sweden).

Table I. A list of non-palindromic restriction sites yielding asymmetric sticky ends and the deduced amino acid sequences in the three possible reading frames

Restriction site	Recognition sequence 123456	Cleavage after no.	Deduced amino acid sequence							
			Frame 1		Frame 2			Frame 3		
			123	456	.12	345	6..	..1	234	56.
*Acc*I	GTAGAC	2	V	D	CGRS	R	HLPQR	I4	*	T
	GTCTAC	2	V	Y	CGRS	L	HLPQR	I4	S	T
*Aff*II	ACACGT	1	T	R	DHNY	T	CFLSWY*	I3	H	V
	ACGTGT	1	T	C	DHNY	V	CFLSWY*	I3	R	V
*Ava*I	CCCGAG	1	P	E	APST	R	ADEGW	I5	P	RS
	CTCGGG	1	L	G	APST	R	ADEGW	I5	S	G
*Ban*I	GGCACC	1	G	T	GRW	H	HLPQR	I4	A	P
	GGTGCC	1	G	A	GRW	C	HLPQR	I4	V	P
*Ban*II	GAGCCC	5	E	P	GR*	A	HLPQR	I4	S	P
	GGGCTC	5	G	L	GRW	A	HLPQR	I4	G	S
*Hgi*AI	GAGCAC	5	E	H	GR*	A	HLPQR	I4	S	T
	GTGCTC	5	V	L	CGRS	A	HLPQR	I4	C	S

The amino acids in the three possible reading frames are shown in a one letter code: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile, K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; *, Stop. The numbers in the deduced amino acids columns are: 13 = AEGIKLPQRSTV*, 14 = AEGKLMPQRSTVW* and 15 = ACDEFGHILNPRSTVY. There are also other restriction enzymes yielding asymmetric sticky ends, but recognizing 5 or 8 bp and therefore not included in the table, e.g. *Bgl*I, *Bst*EII, *Eco*RII, *Bst*NI, *Nci*I, *Sau*96I, *Mst*II and *Sfi*I.

DNA synthesis

Synthesis of oligodeoxynucleotides was performed using an automated machine (KabiGen AB, Sweden) as described previously (Chow *et al.*, 1981; Elmblad *et al.*, 1982). The deprotected oligomers were purified by PAGE.

DNA constructions

The derived DNA sequence for the synthetic gene fragment, designated Z, was divided into 10 oligodeoxynucleotides varying in length from 41 to 45 nucleotides and synthesized with overlaps of 6 bp. The oligonucleotides were phosphorylated separately using polynucleotide kinase (New England Biolabs). They were hybridized pair-wise, mixed and ligated to M13mp8 previously digested with *Eco*RI/*Hin*dIII, transfected to *E. coli* JM103, followed by DNA sequencing. The M13mp8 containing the Z fragment was designated M13Z.

The Z fragment was inserted in-frame after the promoter and signal sequence of the protein A gene by the use of the plasmid pHL3. The plasmid pHL3 was previously constructed by the insertion of the protein A gene contained on a *Taq*I/*Eco*RI fragment from pRIT4 (Nilsson *et al.*, 1985a) into *Cla*I/*Eco*RI of the plasmid pEMBL19 (Dente *et al.*, 1983) having the *Hin*dIII site filled in. The *Taq*I/*Cla*I site was filled in and a synthetic *Not*I linker 5′-AGCGGCCGCT-3′ (KabiGen AB, Sweden) was inserted. The plasmid pHL3 was cleaved with *Hin*dIII (in the protein A gene) and *Eco*RI. The Z fragment (*Hin*dIII/*Eco*RI) was isolated from M13Z and ligated to the cleaved pHL3. The resulting plasmid pHL3Z has the Z fragment out of frame but in the right orientation in the protein A gene. By cleaving pHL3Z with *Fsp*I followed by re-ligation and transformation, the plasmid pASZ could be isolated in which the Z fragment is in-frame after the signal sequence and promoter of the protein A gene. This construction was transformed into *E. coli* JM103 and sequenced over the Z fragment and the junction to the signal sequence.

To obtain high expression of the Z fragment, the orientation of the protein A derived fragment was reversed in the pEMBL vector (Abrahmsén *et al.*, 1986). A *Not*I/*Bam*HI fragment of pASZ was ligated to *Not*I/*Bam*HI cleaved pEMBL8, having the *Acc*I filled-in, and the *Eco*RI linked to the *Not*I site. After transformation the plasmid vector pEZ could be isolated (Figure 4). This vector encodes the signal sequence of staphylococcal protein A fused to the Z region but, in contrast to pASZ, the Z gene is transcribed in the same direction as the transcription of the *bla* gene and as the direction of replication of the plasmid.

Protein purification

The Z-derived proteins were purified by IgG affinity chromatography using IgG Fast Flow Sepharose (Affinity Chromatography Workshop, Pharmacia Biotechnology, Uppsala, Sweden). The column was equilibrated with TST (50 mM Tris pH 7.4, 150 mM NaCl, 0.05% Tween 20). After passage of the growth medium, the column was washed with 10 column volumes TST, 2 volumes TS (50 mM Tris pH 7.4, 150 mM NaCl) and 2 volumes 5 mM NH_4Ac pH 5.5. Elution was performed by 1 M acetic acid titrated to pH 2.8 using NH_4Ac. The eluted material was lyophilized prior to SDS–PAGE analysis.

Protein analysis

Protein A was quantitated by ELISA using Fab fragment of rabbit anti-protein A antibodies conjugated to β-galactosidase (a kind gift from Dr M.Inganäs, Pharmacia, Sweden) (Moks *et al.*, 1986). The analysis was performed by 8–25% gradient SDS–PAGE using the Phast system (Pharmacia, Sweden). The gel was stained with Coomassie Brilliant blue R-250 (LKB, Sweden).

Computer graphic analysis

The computer graphic analysis and simulations were performed using the program package FRODO (Jones, 1978, 1982, 1985, 1986) run on an Evans and Sutherland PS330.

Results

Design of a synthetic IgG-binding fragment

To facilitate mutagenesis of the IgG-binding part of SPA, we designed a synthetic monovalent IgG-binding domain, which could be polymerized at the DNA level in an obligate head-to-tail fashion using non-palindromic restriction sites. After site-directed mutagenesis of the single region, polymerization to different multiplicities can be performed and the mutation will be distributed to all regions.

A comprehensive list of convenient non-palindromic sites, which can be used for DNA fragment polymerization, is shown in Table I. The deduced amino acid sequences of the three alternative reading frames spanning each restriction site are also shown. The table can be used to find a non-palindromic site which

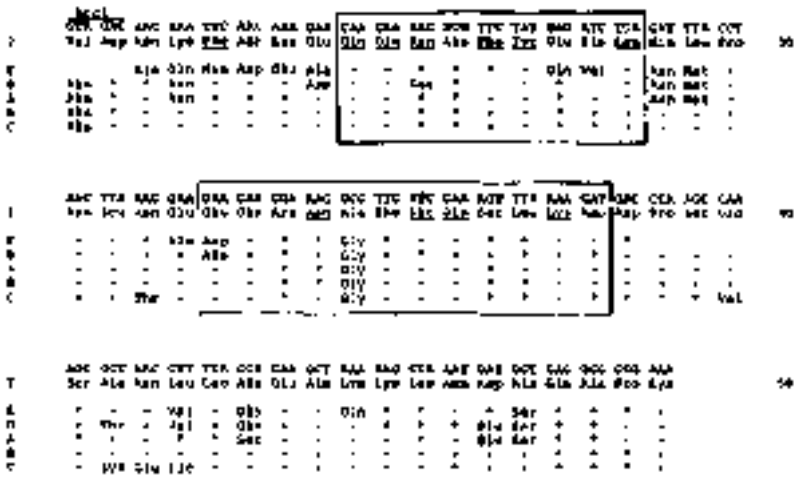

Fig. 1. A comparison of the different IgG-binding domains of staphylococcal protein A and the synthetic domain E, D, A, B and C used for different domains in protein A. *T* is the synthetic IgG-binding domain. Amino acid differences are shown. – = represents a deletion and . = represents the same residue as in domain E. The amino acid numbering of the *T* domain are counted in the following IgG (Fc-fragment) (Uhlén *et al.*, 1984) numbering. The two α-helices are boxed.

duty be introduced by site-directed mutagenesis to flank the gene fragment in the preferences, without altering the amino acid sequence, or to neutralize the charges.

For the SPA molecule, the sequence of the junction between all five IgG binding domains is Ala Asp Asn Lys Ala Asp (Uhlén *et al.*, 1984b), with the border defined at the C-terminal side of the arginine sensitive lysine residue (Sjödahl, 1977; Moks *et al.*, 1986). Unfortunately, this sequence cannot be maintained with any of the genes listed in Table II. Two alternative strategies remain. The first is to introduce a non-palindromic site further into the sequence of the IgG-binding domain where an amino acid sequence, corresponding to a site in Table II can be found. The second is to allow an arginine residue to be retained, instead of replaced to furnish a site. We decided to change an alanine residue in the junction to a valine residue. The substituted residue lies outside the α-helical regions involved in binding to IgG (Uhlén *et al.*, 1984b, Figure 1) and is thus unlikely to interfere with the binding. As a result of the resulting amino acid sequence, the synthetic protein A region will contain a valine – aspartic acid dipeptide sequence, which can be encoded by a unique palindromic AccI site (GTATAC). By reproducing the AccI site on both sides of the IgG-binding fragment a polymerization step is formed.

In Figure 1 a comparison of the five (E, D, A, B and C) homologous regions of the native SPA molecule and the synthetic IgG-binding domain, fragment Z, is given. The design of the sequence of fragment Z will be described below. The two α-helices involved in the IgG-binding are boxed and the 11 residues that are proposed from structural data to interact with IgG (Deisenhofer, 1981) are underlined. Where amino acid differences occur between the synthetic and natural domains, these are shown as well as silent mutations (*). Figure 1 shows that regions A, B and C are highly homologous, with no amino acid differences within the two α-helices. Region D is slightly more divergent with three amino acid substitutions in the α-helical region. Finally, region E is strikingly different, in particular within the N-terminal segment. The five right residues have been substituted by six others compared to the other regions (Figure 1). Despite these differences region E was recently shown to bind IgG (Moks *et al.*, 1986).

The multimeric readout, present in the domains E, D and A (Figure 1), make protein A sensitive to cyanogen bromide treatment (Uhlén and Nilsson, 1985). In addition, the asparagine – glycine sequence present in all domains (E, D, A, B and C) makes the protein sensitive to hydroxylamine treatment (Uhlén and Nilsson, 1985). We decided to design a synthetic IgG-binding fragment lacking both the methionine residue and the asparagine – glycine sequence. These changes would facilitate the purification of peptides by gene fusions in which specific chemical cleavages are used to obtain the desired product (Uhlén and Nilsson, 1985). The synthetic fragment was based on the sequence of domain B, which lacks methionine residues (Figure 1) and is thus cyanogen bromide resistant. There are however two more important reasons to choose this domain. First, domain B is chosen as a hypothetical consensus sequence of the IgG-binding domains (Moks *et al.*, 1986, Figure 1). Second, the structure of the protein A – IgG-complex is based on crystallographic studies using the domain D (Deisenhofer, 1981).

The proposed change in the asparagine – glycine dipeptide sequence at position 28 – 29 (Figure 1) is very critical since these residues are situated in one of the two α-helices responsible for Fc binding, the sequence being conserved in all five domains. As the domain B – IgG complex has been crystallized and the three dimensional structure solved (Deisenhofer, 1981), we decided to employ computer aided graphics, using the program package FRODO (Jones, 1978, 1982; Pett, 1986), to investigate the feasibility of changing the asparagine – glycine dipeptide se-

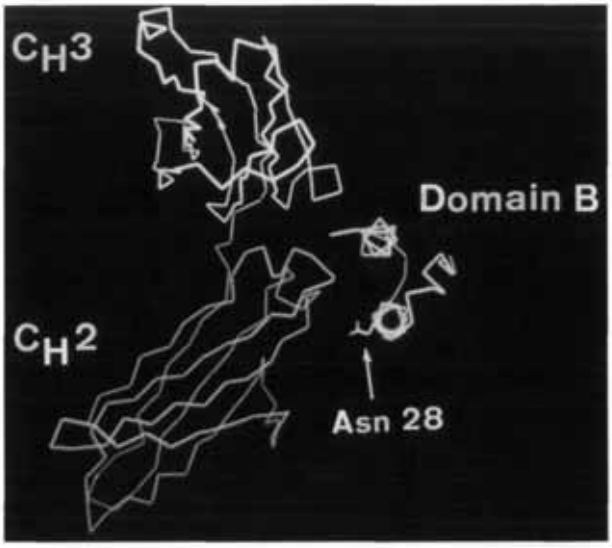

Fig. 2. A computer graphic representation of domain B of staphylococcal protein A bound to IgG. The C_H2 domain of IgG is shown in red and the C_H3 domain in yellow. The α-carbons of the protein A domain are connected in green. The side chain of the asparagine residue of the asparagine–glycine dipeptide sequence is shown.

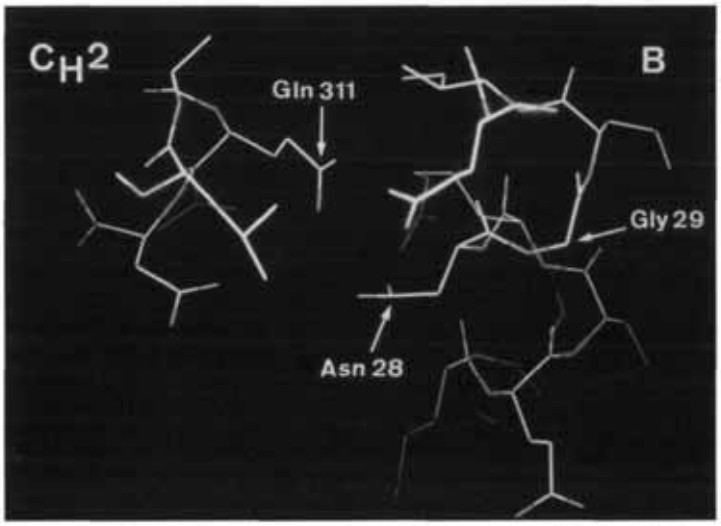

Fig. 3. A computer graphic representation of the region around the asparagine–glycine dipepide sequence. Asn 28 shows the side chain of the asparagine residue. Gly 29 shows the glycine residue. Gln 311 shows a glutamine residue of IgG. The colours represent oxygen (red), nitrogen (blue) and carbon atoms (yellow/green), respectively.

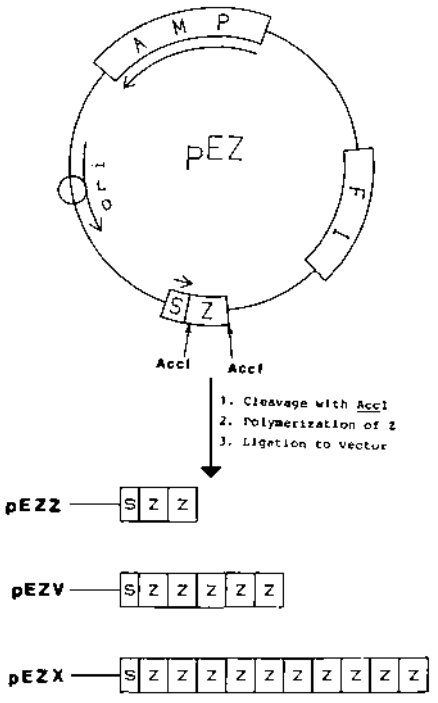

Fig. 4. Polymerization of the Z fragment by the use of the non-palindromic *Acc*I sites. The construction of the plasmid pEZ is described in Materials and methods. AMP represents the *bla* gene encoding β-lactamase. F1 represents the origin of replication from phage f1 (Boyer and Roulland-Dussoix, 1969) and *ori* is the colE1 origin of replication. *S* represents the signal sequence. The direction of transcription is shown by an arrow.

quence without interfering with IgG binding. Figure 2 shows the backbone of α-carbons of the protein A domain (green), the C_H2 domain (red) and the C_H3 domain (yellow). In addition, the side-chain of the asparagine residue in the unique asparagine–glycine dipeptide sequence is shown. As indicated in Figure 1, this amino acid is involved in the interaction with IgG. As shown in Figure 2, the protein A domain contains two antiparallel α-helices which are both involved in the binding to IgG at the site located between C_H2 and C_H3. The binding, with a dissociation constant of 2×10^{-8} M (Langone, 1982), involves interactions between 11 residues of the protein A domain and 10 residues of the Fc molecule (Deisenhofer, 1981).

A more detailed view of this interaction can be seen in Figure 3, in which all the side-chains of the residues in this area are shown. The asparagine side chain of SPA (Asn 28) forms a hydrogen bond to the side chain of a glutamine (Glu 311) in the Fc-fragment. This strongly suggests that substitution of this asparagine residue in domain B would be likely to affect the IgG interaction. In contrast, the neighbouring glycine residue (Gly 29), unusually placed in an α-helix, does not seem to be involved in the IgG interaction. With computer-graphic simulation, a methyl group was added to the glycine converting it into an alanine. This side chain substitution was found to be sterically acceptable to the structure (not shown). A comparison of the α-helix-forming properties of glycine and alanine suggests that the α-helix formation might even be enhanced. A mutation to an even larger side chain was not considered because we wished to make the minimum change in the structure. The computer-graphic analysis therefore suggests that the asparagine–glycine sequence in protein A may be changed to asparagine–alanine, to yield a hydroxylamine-resistant protein A-like domain.

The comparisons of domain Z to the five IgG-binding domains of native protein A reveals the following essential features (Figure 1). First, being based on fragment B it lacks methionine residues. Second, the glycine residue at position 29 has been substituted by an alanine to change the asparagine–glycine sequence. Finally, a non-palindromic *Acc*I site has been introduced in the 5′-end of the the fragment to enable polymerization of the fragment. This site introduces a valine residue instead of an alanine residue in position 1. The *Acc*I site is also present in the 3′-end of the synthesized Z fragment (not shown in Figure 1), thus encoding two additional amino acid residues.

Synthesis and cloning of fragment Z

Using overlapping synthetic oligonucleotides, fragment Z was assembled as described in Material and methods. We have earlier shown that the first six amino acids of domain E, which also are the N-terminal amino acids in mature protein A (Uhlén *et al.*, 1984a), differ from the other domains and are necessary for correct processing of the signal peptide (Abrahmsén *et al.*, 1984). Therefore codons for these six amino acids were also synthesized and included upstream of the first *Acc*I site of fragment Z. Plasmid pEZ was constructed containing the SPA promoter in front of the gene encoding the native SPA signal sequence followed by six residues of region E fused to the synthetic fragment Z (Figure 4) (for details see Materials and methods). The gene fusion terminates in a TAA stop codon downstream from the second *Acc*I site, to yield a mature gene product Z with the predicted mol. wt of 9393.

The polymerization of the Z fragment was performed as described in Figure 4. The plasmid pEZ was digested with *Acc*I and both fragments (Z fragment and vector fragment) were isolated. The Z fragment was ligated for 15 min prior to the addition of vector fragment. The non-palindromic *Acc*I ensures head-to-tail ligations due to the two non-identical sticky ends. Thus, the Z fragments will always be orientated in the transcription direction of the vector. After transformation to *E. coli* HB101, clones containing constructions with two or more Z fragments can be distinguished from those containing one or zero, as precipitation halos are formed around the colonies on agar plates containing 1% canine serum. Although the structure of these precipitates is not known in detail (Inganäs and Johansson, 1981), the phenomenon is technically very useful. Restriction analysis indeed showed multiplicities of 2–10 Z fragments of plasmid DNA isolated from different halo-forming clones. The multiplicities 2, 5 and 10 were selected for protein analysis and the plasmids were designated pEZZ, pEZV and pEZX respectively (Figure 4).

Expression of different multiplicities of fragment Z

The plasmid vectors pEZ, pEZZ, pEZV and pEZX (Figure 4) contain the gene encoding 1–10 Z domains inserted after the promoter and signal sequence of staphylococcal protein A. *E. coli* HB101 cells harbouring the different plasmids were grown in

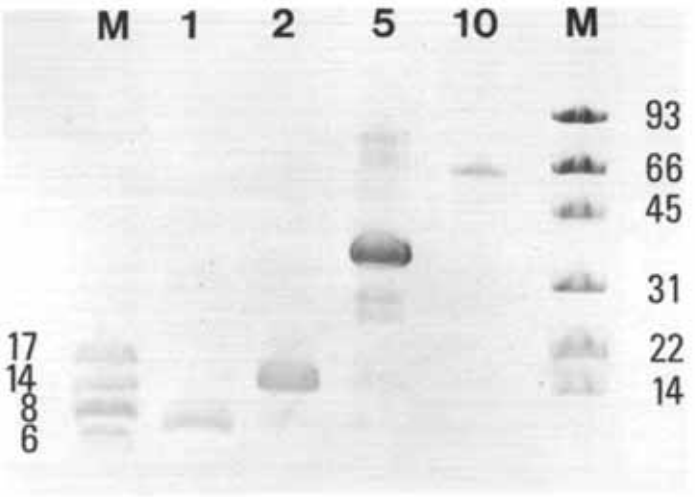

Fig. 5. SDS–PAGE of IgG affinity purified proteins from the growth medium of *E. coli* harbouring the different plasmids pEZ, pEZZ, pEZV and pEZX. The numbers refer to the number of Z domains in the gene product. Lanes M are marker proteins with the sizes shown as mol. wt $\times 10^{-3}$.

a 3 l lab bench fermentor (Chemoferm, Sweden) using standard conditions (Abrahmsén *et al.*, 1986). We have earlier found that protein A fragments expressed in *E. coli* from similar vectors were secreted to the growth medium of the host cells (Abrahmsén *et al.*, 1986). Therefore, the culture medium was recovered by centrifugation and subsequently subjected to IgG affinity chromatography. After elution and lyophilization, the bound material was analyzed by SDS–PAGE (Figure 5). The observed mol. wts are in good agreement with the values calculated from the deduced amino acid sequences of the mature proteins: 9393, 16 019, 35 887 and 69 000 daltons, respectively.

Discussion

In this paper, we have demonstrated a strategy to perform protein engineering of the IgG-binding region of SPA. The system is based on a synthetic fragment Z, which was polymerized to different multiplicities using non-palindromic *Acc*I sites. The asparagine–glycine dipeptide sequence and the methionine residue of SPA were removed to ensure resistance to hydroxylamine and cyanogen bromide respectively. The methionine was replaced simply by designing the new IgG-binding domain, Z, using the amino acid sequence of domain B (Figure 1). The asparagine–glycine dipeptide sequence was replaced by asparagine–alanine, based on results from computer-graphic analysis (Figures 2 and 3). In addition, an alanine residue outside the helical structures (residue 1 in Figure 1) was changed to a valine residue to furnish a non-palindromic *Acc*I site.

These amino acid replacements make it relevant to compare domains B and Z in terms of their relative affinity to IgG. Several observations support a binding of comparable or identical strength. First, precipitation halos around *E. coli* colonies can be used for qualitative measurements (Moks *et al.*, 1986). The halo produced by ZZ is identical in strength to the one produced by the gene product from regions EB but stronger than for domains EE (not shown), which has been suggested to have a lower affinity for IgG than EB (Moks *et al.*, 1986). Second, the binding strength between protein A fragments and IgG can be determined by the pH elution profile from IgG columns (Lindmark *et al.*, 1983). Domain Z and domain B are indeed eluted from IgG affinity columns at the same characteristic pH (not shown), which is slightly lower than the elution pH for domain E. IgG-binding competition studies using radioactively labelled domains B and Z are needed for a more exact comparison between the two domains.

The described concept for engineering of repetitive structures utilizes non-palindromic restriction sites, introduced at both ends of the DNA fragment encoding the repetitive unit to be engineered. After mutagenesis the fragment may be polymerized to any multiplicity by a ligation in which the non-palindromic site ensures a head-to-tail ligation (Harley and Gregori, 1981). This method is suitable for engineering of any repetitive structure, such as collagen (Fuller and Boedtker, 1981), streptococcal protein G (Guss *et al.*, 1986), streptococcal M protein (Hoolingshead, 1986), Balbiani rings (Höög and Wieslander, 1984), pro-α-mating factor (Kurjan and Herskowitz, 1982), transplantation antigens (Ohno *et al.*, 1982), fibronectin (Hirano *et al.*, 1983) or rabbit skeletal tropomyosin (Fischetti and Manjula, 1982). The method is limited by the need to find a non-palindromic site to be introduced in the junction of two repeats without altering the amino acid sequence. To enable polymerization of the protein A gene fragment none of the restriction sites listed in Table I could be utilized without altering the amino acid sequence in the junction. An alanine residue was instead changed to a valine residue to furnish a non-palindromic *Acc*I site (GTAGAC). Even though this change does not seem to interfere with the IgG binding, we cannot rule out effects on some of the other reported biological functions of protein A (Sjöquist and Stahlenheim, 1969; Cowan *et al.*, 1979; Sjödahl and Möller,

1979; Romagnani *et al.*, 1980; Inganäs and Johansson, 1981; Catalona *et al.*, 1981).

The polymerization concept was used to polymerize Z fragments to multiplicities of 2, 5 and 10. These gene products were all expressed and secreted in *E. coli* hosts. The constructions containing five (pEZV) and 10 (pEZX) Z fragments were not stable in recA$^+$ strains. In addition, the pEZX construction was susceptible to homologous recombinations at low frequency even in the recA$^-$ host (not shown). The question arises whether the high frequency of silent mutations found in the native protein A gene (Löfdahl *et al.*, 1983; Figure 1) reflects a selection pressure to avoid homologous recombinations of the five regions encoding the IgG-binding domains. Although homologous recombination is a well-known phenomenon in Gram positive bacteria (Michel *et al.*, 1982), the protein A gene does not seem to recombine in *S. aureus*. This is supported by an interesting finding by Guss *et al.* (1985) that an isolate of a *S. aureus* strain, expressing a truncated protein A containing only two functional IgG-binding domains, was not derived from recombination of the protein A gene, but instead results from a point mutation giving a stop codon.

We have earlier described the construction and use of fusion vectors based on the IgG-binding domains of staphylococcal protein A (Uhlén, 1983; Nilsson *et al.*, 1985a,b). The IgG-binding activity of the SPA 'affinity tail' allows for a rapid recovery of fusion products using IgG affinity chromatography. It is now possible to construct novel fusion vectors based on fragment Z. Such vectors will encode an improved IgG-binding protein with regard to resistance to hydroxylamine and cyanogen bromide treatments but having the same structure and function as the native molecule. These site-specific chemical cleavage methods may potentially be used for the purification of various peptides lacking methionine residues or asparagine–glycine dipeptides. A unique cleavage at the linker between the protein A moiety and the peptide would facilitate the recovery of the desired peptide simply by a second passage through the affinity column after cleavage, in which both uncleaved hybrid protein and intact 'affinity tail' will bind.

The Z fragment concept for the purification of peptides has recently been used to produce native human IGF-I (T.Moks, unpublished observations). Fusion protein (ZZ-IGF-I) was purified by IgG affinity chromatography, and subsequently cleaved by hydroxylamine followed by a second passage through the column. A pure native IGF-I with high specific biological activity was obtained by this procedure.

Acknowledgements

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□ □ □

EXCMO. SR. DR. ISMAIL SERAGELDIN

Discurso de presentación

Dra. Rosalía Arteaga Serrano
Ex – Presidenta y Vicepresidenta Constitucional de la República del Ecuador
Miembro del directorio Biblioteca de Alejandría

Miembro de Honor de la Real Academia Europea de Doctores

Deseo acercar a la audiencia a los pensamientos del Dr. Ismail Serageldin, el nuevo miembro de la Real Academia Europea de Doctores, con las palabras que aparecen como introducción a su página web en el internet, donde habla sobre su credo: "El mundo es mi hogar. La humanidad es mi familia. La no violencia es mi credo. Paz, justicia, equidad y dignidad para todos es mi propósito. Participación, racionalidad, tolerancia, diálogo, aprendizaje y comprensión son mis medios. Con manos extendidas doy la bienvenida a todos aquellos que comparten esta creencia....»

Hay expresiones que suelen sonar a lugar común porque se las dice repetitivamente, pero es necesario apelar a ellas, cuando se las siente de verdad y brotan del reconocimiento de la persona de quien se habla, como en este caso en el que debo decir que me siento profundamente honrada por el encargo recibido de parte del Ilustre Presidente de la Real Academia de Doctores de Europa, Alfredo Rocafort, de pronunciar este discurso para recibir como su miembro al

Excelentísimo director de la Biblioteca de Alejandría, Ismail Serageldin, con quien tengo el placer de compartir el consejo directivo de este centro de cultura, de pensamiento, que irradia su luz desde la hermosa y mítica ciudad egipcia.

Hablar de Ismail Serageldin, es tocar la personalidad de un hombre al que podríamos calificar de renacentista y con el don de la simultaneidad, dada la vastedad y la diversidad de sus conocimientos, de sus intereses, de los temas que ha tratado, de su rica vida de servicio público, docente y también en organismos internacionales, de lecturas intensas, de escritos y ponencias magistrales, que circulan alrededor del globo, de su privilegiada mente que no se cansa de crear espacios, de sugerir temáticas, de pergueñar asuntos complejos con palabras impregnadas de belleza, para conmover las conciencias, para sugerir caminos y hacer planteamientos que apunten a soluciones.

De entre la enorme pléyade de temas que Serageldin toca en sus escritos, en sus discursos, en sus ponencias, resulta difícil escoger un tema o unos temas para comentarlos; él mismo, para esta sesión solemne, ha escogido hablar sobre la ciencia médica, desde su visión como analista e historiador, como alejandrino, egipcio y musulmán que ama sus orígenes, que se siente orgulloso de ellos, que hurga en los documentos, en los pergaminos y papiros para extraer la esencia del saber que quiere comunicar, como heredero de una civilización que sirve de base para muchos de los conocimientos de los que hoy nos sentimos tan orgullosos en el mundo de la ciencia, de la tecnología, de la innovación, en el que nos ha tocado vivir.

El doctor Ismail Serageldin realizó sus estudios en las Universidades de Cairo y de Harvard, y enseñó también en estas universidades, profesor del Collégue de France y de Wageningen University en los Países Bajos, tiene una larga trayectoria de trabajo en el Banco Mundial, hasta llegar a la Vicepresidencia de este organismo multilateral de crédito.

En el año 2000 retornó a su país, para encabezar un ambicioso proyecto, la construcción de la nueva sede de la Biblioteca de Alejandría, de la que es su director fundador hasta la presente fecha. La Biblioteca fue inaugurada en el año 2002.

Es miembro de un número de boards en diferentes países y continentes, se destaca su actuación como cochair del Nizami Ganjavi International Center en Azerbaijan.

Ha publicado más de 100 libros y más de 500 artículos y artículos especializados, y ha recibido 34 doctorados honoris causa en diversas universidades y centros de educación superior. Ha dirigido y conducido programas de televisión, relacionados con la divulgación de la ciencia y la cultura.

Sus numerosos libros encierran un saber enciclopédico, de entre ellos quiero referirme a uno que me causó un gran impacto por el contenido y por la forma en que Ismail traza y construye su pensamiento, se trata de una colección de artículos, de reflexiones, titulado «Essays for our Time», en edición de 2016, en la que demuestra su vocación por la cultura universal, hace una interpretación del tiempo en el que nos ha tocado vivir.

En este mundo de incertidumbre, hunde sus raíces en lo que él es, en lo que de valedero tiene el mundo musulmán, el árabe, para la humanidad, con un canto de alabanza a su cultura, a su aporte, a la riqueza de las tradiciones y conocimientos aportados por su gente.

En estos capítulos cortos, resalta un lenguaje poético, sencillo, no complicado, yo diría que vital, rico en antecedentes y conciso en ideas, resalta el sentido común, que no suele ser tan común como nos imaginamos, aporta con algunas soluciones, no todas por supuesto, todavía está en construcción el cómo contribuir a generar un nuevo orden mundial, se esbozan sistemas híbridos de gobierno y se apuntala un «deber ser», del Sur Global como él le llama, tal vez una alternativa al terrorismo global.

Serageldin en sus escritos se nos manifiesta como un promotor de paz, de sustentabilidad, defensor del estado de derecho, el verdadero «pegamento» de la sociedad. Creo que traza una filosofía de vida cuando menciona reiterativamente la «ética junto con estética», con un «núcleo común que trasciende fronteras políticas», porque como lo manifiesta, « no debe existir ninguna excusa para transgredir los derechos humanos básicos de los individuos, sin importar las circunstancias».

Las 5 metas fundamentales que Serageldin menciona son: «Paz, libertad, justicia, equidad y sustentabilidad», en los cuales debe construirse el principio de Gobernabilidad, sustento de la democracia que requiere basarse en «democracia, pluralismo, participación y estado de derecho".

Hay otro aspecto que quiero resaltar en el pensamiento de nuestro nuevo académico y es la necesidad de construir confianza y tolerancia entre los diferentes sectores, basado en los viejos pensadores árabes de la época dorada, en principios tan hermosos como el de que « la justicia debe ser templada con misericordia". O cuando habla de que « Sustentabilidad es dejar a las generaciones futuras tantas, si no más, oportunidades como hemos tenido nosotros mismos».

En las páginas del pequeño libro al que he querido referirme en esta ceremonia en la que recibimos en el seno de la Academia a Ismail Serageldin, así como en su discurso de ingreso, hay referencias múltiples a pensadores tanto de occidente como de oriente, a manera de desfile profético de lo que debe ser ese pensamiento pluralista, tolerante, que reconozca al otro y en el otro se reconozca a sí mismo.

Las palabras de Serageldin pueden parecer proféticas cuando dice en las líneas iniciales de un capítulo en el que se pregunta: «¿Qué está mal con el actual orden mundial?» y él mismo se responde: « no es el final de la historia como Fukuyama manifestó, tampoco es el choque de civilizaciones como Huntington creía… es mucho peor».

Y menciona «Los fanáticos están en todos lados desde Boko Haram en Nigeria a Al-Qaeda en el Sahara, y desde el Maghreb a las montañas de Afghanistan..." y requiere la necesidad de un pensamiento fresco, distinto, para el que provee escenarios y espacios tan maravillosos, tanto en Alejandría como en Baku o en los diversos foros en los que tiene presencia e influencia e interviene.

La necesidad de construir ese nuevo orden, sumado al amor por sus raíces, su cultura, el aporte árabe musulmán a la civilización, como en una especie de desesperado intento por hacer entender a una humanidad reacia, que ser árabe y musulmán no es ser terrorista o destructor de culturas, sino que es representar un legado de riqueza cultural y humanística de enormes proporciones.

Yo espero Ismail, que sus afanes sean coronados por el éxito, ciertamente su participación en esta academia europea es sintomática de la fuerza del diálogo, de las posibilidades de la coexistencia y de la imbricación de las culturas, de que un mundo mejor es posible y que los arquitectos de la construcción de este nuevo orden mundial basado en las razones de la ciencia y la conciencia, cuenta con representantes en esta misma sala, en este mismo auditorio, así como en muchos otros a los que su inteligencia y sus conocimientos le conducen.

El diálogo de las civilizaciones es posible, el diálogo interreligioso se impone, la apertura de las mentes es indispensable para dejar de lado las intolerancias, los desencuentros; por ello, como alguna vez conversábamos con el premio Nobel Richard Roberts, dejemos que la cordura prevalezca y el odio se desvanezca, hablemos mejor del sentido común.

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Dra. Rosalía Arteaga Serrano
Former President and Vice – president of the Republic of Ecuador
Member of the Boardwalk of Bibliotheca Alexandria
Honorary Member of the Royal European Academy of Doctors

I would like to introduce the audience to the thoughts of Dr. Ismail Serageldin, the new member of the Royal European Academy of doctors, with the words that appear as an introduction to his home page in the internet, where he talks about his creed: “The world is my home. Humanity is my family. Non-violence is my creed. Peace, justice, equality and dignity for all is my purpose. Engagement, rationality, tolerance, dialogue, learning and understanding are my means. With outstretched hands we welcome all those who share this beliefs....”

There are expressions that sound familiar because we repeat them constantly, but is necessary to appeal to them when they are really felt and when they burst forth from the acknowledgement of the person that we are talking about, as it is the case when I say I´m deeply honored for the commission given to me by the illustrious President of the Royal European Academy of Doctors, Alfredo Rocafort, of pronouncing this speech to receive his Excellently, the Director of the Library of Alexandria, Ismail Serageldin as a member of the Academy, and with whom I have the pleasure of sharing the Board of Trustees of this center of culture and thought that irradiates its light from the beautiful and mythical egyptian city.

To talk about Ismail Serageldin, is to touch a personality of a man who we could qualify as renaissance and a multitasking, given the vastness and diversity of his knowledge, interests, of the subjects he has discussed, his rich life in the public service, as a teacher and as member of international organizations, of

intense readings, writings and masterly lectures, that run around the globe; of his privileged mind that does not get weary of creating spaces, suggest thematics, of sketching complex issues with beautifully impregnated words, moving consciousness, drawing the path and approaches that lead towards solutions.

Among the privileged pleiad of topics that Serageldin touches in his writings, in his speeches, in his lectures, it can be difficult to choose one or two subjects to comment; himself on this solemn session, has chosen to talk about medical science, from his vision as an analyst and historian, as an alexandrine, egyptian and muslim that loves his origins, that is proud of them, that rummages documents, scrolls and papyri to extract the essence of the knowledge that he wants to communicate, as an inheritor of a civilization that is the foundation for most of the knowledge that make us proud in the world of science, technology and innovation that we live in.

Doctor Ismail Serageldin completed his studies in Cairo University and Harvard, and taught in these universities. He was also a professor in Collégue de France and Wageningen University in the Netherlands. He has a long trajectory in the World Bank, which led him to the vice-presidency of this multilateral credit organism.

In the year 2000 he returned to his country to lead an ambitious project, the construction of the new headquarters of the Library of Alexandria, opened in 2002, of which he is the founding director to this date.

He is member of a number of boards in different countries and continents, highlighting his performance as cochair of the Nizami Ganjavi International Center in Azerbaijan.

He has published more than 100 books and over 500 articles and technical papers and received 34 honorary doctorates from various universities and higher education centers. He has directed and conducted culture and science divulgation television shows.

His numerous books enclose encyclopedic wisdom, among them, I would like to refer to one that caused a profound impact on me, for the form and content through which Ismail traces and builds his thought, “Essays for our time” on its 2016 edition is the title of a collection of articles, of reflections, that demonstrates his vocation for universal culture, making an interpretation of the times that we have to live.

In this world of uncertainty, he sinks his roots on what he is, on what is valid from the muslim world, the arab, for humanity, with a chant of praise to his culture, to its contribution, to the richness of the knowledge and traditions contrived by its people.

In this short chapters, stands out a poetic, straightforward, simple language, vital and rich in background and concise in ideas, highlighting common sense, which is not as common as we would imagine, bringing if not all of the solutions, some of them. It's still under construction how to contribute to create a new world order, he sketches out hybrid government systems and braces a "must be", of a "Global South" as he calls it, and maybe an alternative to global terrorism.

Serageldin on his writings manifests himself as a promotor of peace, sustainability, defender of the rule of law, the true "glue" of society. I believe he lays out a philosophy of life when he mentions repeatedly "ethics along with esthetics" with a "common core that transcends political boundaries" because as he manifests "there should be no excuse for transgressing the basic Human Rights of individuals, no matter what the circumstances".

The five fundamental goals that Serageldin mentions are; "Peace, Freedom, Justice, Equality and Sustainability", on which the principle of Governance must be built as the sustentation of democracy, that requires "democratic pluralism, participation and the rule of law".

There is another aspect I would like to highlight in the thought of our new academy member and is the need of constructing trust and tolerance within the different sectors, based on the old Arab thinkers of the golden era, on such beautiful principles as "the justice must be tempered with mercy". Or when we state that "Sustainability is to leave future generations as many, if not more, opportunities as we have had ourselves".

In the pages of the little book I've been referring to, in this ceremony in which we receive Ismail Serageldin to this academy, as in his entering speech, there are multiple references to thinkers from the east and the west, as a prophetic procession of what a pluralist and tolerance thought that recognizes the other and in the other recognizes itself must be.

The words of Serageldin might sound prophetic when on the initial lines of a chapter he wonders: "What´s wrong with the current world order?" and he

answers himself: “it is not the end of history as Fukuyama claimed, nor is it the clash of civilizations as Huntington believed...it is much worse”.

And he mentions “The fanatics are everywhere from Boko Haram in Nigeria to Al-Qaeda in the Sahara and the Maghreb to the mountains of Afghanistan...” and requires a fresh and distinct thought, which he provides wonderful stages and spaces, both in Alexandria and Baku or in the different forums that he has presence, where we influences and intervenes.

The need for this lay out, this new world order, combined to the love for his roots, his culture, the arab-muslim contribution, as a desperate attempt to make the reluctant humanity understand that being arab and muslim is not being a terrorist or culture destructor, but a representative of a legacy of cultural and humanistic richness of immense proportions.

I hope Ismail, that your eagerness will be crowned by success; certainly your participation in this european academy is symptomatic of the strength of dialogue, of the possibilities of coexistence and the imbrication of cultures, that a better world is possible and that the architects of this new order based on the reason of science and conscience, count with representatives in this same room, in this same auditorium, as well as in many others where their intelligence and knowledge led them.

Dialogue between civilizations is possible, interreligious dialogue imposes itself, having open minds is essential for leaving aside intolerance, conflict. As I once talk with Nobel Prize Richard Roberts, let sanity prevail and hatred be vanished, let´s better talk about common sense.

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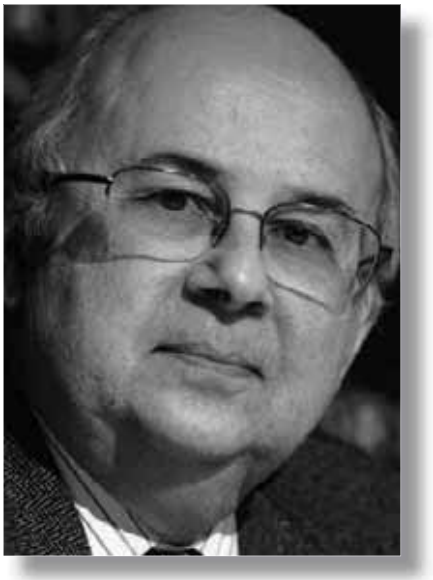

Discurso de ingreso

Dr. Ismail Serageldin
Director de la Biblioteca de Alejandría

Introduction: The Birth of Medicine

Excellencies, Ladies and Gentlemen,

It is with great humility that I accept the great honor you bestow upon me today. Thank you from the bottom of my heart.

In coming here, I reflected upon the nobility of the medical profession, healers and researchers all, and how proud I am to be included in your company. But I also looked back at my forbears and I am also proud of the legacy that I am heir to as I come to stand in the midst of this august company.

I come from Egypt where medicine was born and where the brain was given a name and its components studied. Egypt, home of the earliest students of physical disease and mental health, the healers of millennia ago, who wanted to assist their fellow humans out of their misery by the application of that special combination of art and science that medicine remains to this day.

Egypt has a unique time scale. The Stela of King Narmer – dating from over 5100 years ago – recorded the unification of northern and southern Egypt, thereby creating the longest continuous human society with a central government within boundaries that have remained approximately unchanged, and have been recognized as "the land of Egypt" ever since. The vastness of that time scale is worthy of reflection. When Alexander the great came to Egypt, there was more distance in time between Alexander and the pyramid builders than there is between Alexander and us today!

So from the mists of time, there emerges the majestic figure of the ancient Egyptian Polymath Imhotep, known for having been the architect/engineer of the stepped Pyramid of Saqqara, precursor to all the great Egyptian pyramids that were to follow. But Imhotep was also a medical doctor of great ability, and he was also deified by the ancients as the Egyptian God of Medicine. So it was in that land, as old as time itself, that something miraculous happened: Imhotep was the first recorded case of advancement based on intellectual merit rather than by birth or conquest. I would invite all who believe in the virtue of a merit based system to reflect on the time scale and on the significance of that advancement based on merit.

Imhotep who flourished almost 5000 years ago, is considered to be the author of a medical treatise which was handed down through the generations, and survives in a copy– the so-called Edwin Smith papyrus – which is between 3500 and 4000 years old. That document is remarkable for containing anatomical observations, diagnoses of ailments, and recommendations for cures, all of which are based on empirical observation and devoid of magical interpretations.

Thus, Imhotep should deservedly be considered the father of Medicine for having established that humans can study and understand both trauma and disease and can intervene to deal with it by surgery and herbs. It was under his tutelage that the exquisite combination of art and science that medicine represents was born. The study and understanding of both trauma and disease and the designation of treatment by surgery or herbs – the science part – was added to the skill of talking with the patient, the agility in handling the broken limbs and the dexterity of suturing – the art part. It is also interesting to note that in this earliest catalogue of interventions, the recommendations also include the cases where it is recommended not to intervene

However, the Edwin Smith Papyrus is also remarkable for something else. It is the first time that the word 'brain' appears in any language. Further, the papy-

rus describes realistic anatomical, physiological and pathological observations. It contains the first known descriptions of the cranial structures, the meninges, the external surface of the brain, the cerebrospinal fluid, and the intracranial pulsations.

The procedures described in the Edwin Smith papyrus demonstrate that the Egyptian level of knowledge of medicine surpassed that of Hippocrates, who lived 1000 years later than the papyrus, not to mention the original and much older text from which it was copied.

But that is not to say of course that the ancient Egyptians did not also believe in magic and resorted to incantations and other such formulae to complement what their empirical studies and clinical observations had allowed them to diagnose as diseases and what to recommend as treatments. Again, in another very famous ancient medical Papyrus – the so-called Ebers Papyrus – we have a much larger document, and it is considered the single most voluminous record of ancient Egyptian medicine known. The scroll contains some 700 remedies and magical formulas. But it is striking also for reflecting evidence of a long tradition of empiricism. For example, the Ebers papyrus suggested treatment for asthma to be a mixture of herbs heated on a brick so that the sufferer could inhale their fumes.

Also worthy of note is that the ancient Egyptians conceived of mental and physical diseases in much the same way. Disorders such as depression and dementia are covered not as spiritual conditions to be treated by magic only, but also as diseases to be treated by Doctors. These Mental disorders are detailed in a section or chapter of the Ebers Papyrus called the Book of Hearts. The descriptions of these disorders suggest that there was serious empirical observation that had gone in the study of these diseases.

Alexandria: From Greece to Rome and Beyond

Leaving aside the evolution of medicine in non-western cultures such as Asia and particularly China – which deserves a separate lecture – we can trace the next chapters of the evolution of medicine to the Golden Greeks who flourished in the first millennium BC and who still dazzle us with their philosophy, science and art.

In ancient Greece, Asclepius was the god of medicine, combining religion and mythology. The staff of Asclepius, a snake-entwined staff, remains a symbol of medicine today.

Asclepius and his daughters represent the best about being and remaining healthy. His daughters are:

- Hygieia ("Hygiene", the goddess/personification of health, cleanliness, and sanitation),
- Iaso (the goddess of recuperation from illness),
- Aceso (the goddess of the healing process),
- Aglæa/Ægle (the goddess of beauty, splendor, glory, magnificence, and adornment), and
- Panacea (the goddess of universal remedy).

What a lovely household.

But it is from the most famous practicing physician of the Age of Pericles, Hippocrates of Kos, who was born in 460 BC, and died in 370 BC, that we trace the ethical oath that all doctors repeat to this day.

But that brings us back to the land of Egypt. For less than half a century after the death of Hippocrates, Alexander the Great (356 BC- 323 BC), pupil of Aristotle, would conquer the known world of his time and found the city of Alexandria. His successors the Ptolemies would turn it into the intellectual capital of the world.

So between the decline of splendid Greece and the rise of mighty Rome, there is the glorious period of the dazzling new capital of learning and knowledge on the shores of the Mediterranean: Hellenistic Alexandria, with its magnificent lighthouse and its famous ancient library. Alexandrian medicine would bring the best of the Egyptian and Greek traditions together to create a very important school of medicine.

Herophilus, who was one of the greatest figures in Alexandrian medicine and who established his own school of medicine, was a pioneer of functional physiology, and produced a very large amount of anatomical writings. He correctly identified that it is the brain that is the controlling organ of the body, and not the heart as Aristotle had said. He carried out pioneering work on the anatomy of the brain and nervous system, and is credited with the identification of the dura mater and pia mater, two of the brain's membranes; and with tracing the connections between the spinal cord, nerves, and the brain.

So important was the Alexandrian school, that even long after it had started its gradual decline, Galen, the famous Roman physician, came to study for a while in Alexandria before practicing in Rome. His teachings and writings – which incorporated much of the Alexandrian Knowledge – survived well into the sixteenth century and formed the basis of more modern medical practices during the Renaissance.

The Golden Ages and the Dark Ages:

Europe was to sink into the so-called dark ages of the medieval period where learning remained confined to a few beacons among the monasteries with an emergence of some universities and learned societies in the later middle ages. But in the east, the golden Ages of Islam were to flourish.

The sun of Islam burst out of Arabia and soon covered the world from Andalusia in Spain through Morocco to Egypt and eastward to parts of India while stretching north to Central Asia and south to Sudan and eastern and western Africa. The Arabs who carried Islam into the world were very soon a minority among Muslims of all ethnicities and races. In those vast lands, under a largely tolerant and open system of governance, Science would flourish. In fact, after the destruction of the ancient Library of Alexandria, and the murder of Hypathia at the hands of a zealot Christian mob in 415 CE, it was in the early 9th century that much of the knowledge of the ancient world was re-collected in Baghdad's House of Wisdom and translated into Arabic.

Indeed, throughout the dark ages it was the Muslims who held up the torch of rationality and reason, while Europe was in the throes of bigotry and intolerance. And here all of you, scholars and medical practitioners alike may be interested in this amazing story:

We are at the beginning of the ninth century and the Abbasid Empire stretches from Morocco to India, and from central Asia to the Sudan. The capital is Baghdad. The new Caliph Al-Maamoun, son of the legendary Caliph Haroun Al-Rasheed of Arabian Nights fame, would give a big push to the project of the House of Wisdom. He actually offered that anyone who would translate an ancient manuscript into Arabic would receive its weight in gold. Soon, from all over the vast empire manuscripts were being collected and translated. Soon the Vizier, Al-Maamoun's minister of Finance, said that: "The scholars are cheating: they are using big letters and thick paper in order to increase the gold they will receive". To which Al-Maamoun replied: "let them be, for what they give

us is infinitely more valuable than the gold we give them"…. All scholars must aspire to have rulers with such priorities!

As a result of that enormous program of translation Arabic became the language of knowledge and science within less than a century. But the program also helped gather the remnants of all the copies of manuscripts from the Great Ancient Library of Alexandria that remained anywhere in the vast Abbasid empire. These were gathered back into the house of Wisdom in Baghdad as their owners rushed to have them translated and get their weight in gold. The Arabic translations were copied many times over and they traveled far and wide in the Empire and found their way to Europe through Spain and Sicily and other points.

Ah! But not all societies in the middle ages were so sympathetic to learning and books. Baghdad, with its fabulous House of Wisdom, was destroyed by Hulagu and his Mongol armies in 1258 CE. But the talents of Muslim scientists would still shine on for another 400 years as they had in the preceding 400 years, spread throughout the lands under Muslim control from Andalusia to India and from Central Asia to Sudan.

In Cairo, we can name Ibn al-Haytham (965-1040), Latinized as Alhazen, who was born in Basra but practiced in Egypt. He is the true founder of the modern empirical scientific method. Listen to how Ibn Al-Haytham described that method, centuries before Bacon, Descartes and Galileo, and how he laid down the rules of the empirical approach, describing how the scientific method should operate through observation, measurement, experiment and conclusion:

> "We start by observing reality … We then proceed by increasing our research and measurement, subjecting premises to criticism, and being cautious in drawing conclusions… In all we do, our purpose should be … the search for truth, not support of opinions".

From the land of Spain, we can name Abū al-Qāsim Al-Zahrawi (936-1013), Latinized as Abulcasis, a brilliant Arab Muslim physician and surgeon who designed many surgical instruments that have proven very useful over the centuries, as has his book *Al-Tasrif*.

From Persia, or modern day Iran, we can cite Abū Bakr al-Rāzī (854-925), Latinized as Razes, was a Persian polymath, physician, alchemist, philosopher, and

a major contributor to medicine. And also from Persia, we have Ibn Sina (980-1037), Latinized as Avicenna, who was one of the greatest figures of the middle ages, another polymath who produced much in many fields. He is said to have written 450 work of which around 250 have survived, including 150 on philosophy and 40 on medicine. Of his medical works, *Al-Qanun*, or the Canon would remain one of the most influential medical references throughout the middle ages.

But getting back to Egypt, we must also cite the great Ibn al-Nafis (1213-1288), a physician mostly famous for being the first to describe the pulmonary circulation of the blood, but whom I admire mostly for his advocacy of openness to unusual and contrarian views, and accepting opinions only subject to evidence and argument. Listen to the voice of Ibn Al-Nafis (13th C) on accepting the contrarian view, subject only to the test of evidence and rational analysis.

> "When hearing something unusual, do not preemptively reject it, for that would be folly. Indeed, horrible things may be true, and familiar and praised things may prove to be lies."

This was the practice in these golden years of Islam, while Europe mostly suffered in the dark ages, the inquisition was still to come, and Galileo, four centuries after ibn Al Nafis would be put on trial in 1633.

Likewise, you all know the difficulties that confronted Darwin and the supporters of the theory of evolution in western societies even as late as the 19th century. Well, listen to the words of one of the most respected scientists of islam, father of sociology, and important historian, judge and diplomat, listen to how Ibn Khaldun reflected on the world and arrived at his own theory of evolution in the 14th century, some 500 years before Darwin and he was not attacked nor vilified for his having sidestepped the story of Adam and Eve :

> One should then take a look at the world of creation. It started out from the minerals and progressed, in an ingenious, gradual manner, to plants and animals. The last stage of minerals is connected with the first stage of plants, such as herbs and seedless plants. The last stage of plants, such as palms and vines, is connected with the first stage of animals, such as snails and shellfish which have only the power of touch.
>
> The word 'connection' with regard to these created things means that the last stage of each group is fully prepared to become the first stage of the newest group.

> The animal world then widens, its species become numerous, and, in a gradual process of creation, it finally leads to man, who is able to think and reflect. The higher stage of man is reached from the world of power, in which both sagacity and perception are found, but which has not reached the stage of actual reflection and thinking. At this point we come to the first stage of man.
>
> This is as far as our (physical) observation extends.
>
> -- Ibn Khaldun (1332 – 1406)

This is the Muslim tradition that must be revived if the Arab World, Muslim and non-Muslim alike, will indeed join the ranks of the advanced societies of our time. Rejecting politicized religiosity, and reviving these traditions would promote the values of science in our societies… but that is for another discussion another day. Suffice to say that after the early years of the Ottoman Empire it quickly became an ossified structure and the gradual decline of the Muslim and Arab lands was to begin, while the torch would now pass to Europe.

A Special Case: Mental Diseases:

Excellencies, ladies and gentlemen,

I would like to add one more aspect to this discussion of Medieval Medicine as it was practiced in the Muslim empires of the time, namely, how mental disorders were addressed.

We can cite among the most famous authors who wrote on mental disorders and/or proposed treatments during this period: Al-Balkhi, Al-Razi, Al-Farabi, Ibn-Sina, Al-Majusi, Abu al-Qasim al-Zahrawi, and Ibn Rushd (Averroes). They wrote about fear and anxiety, anger and aggression, sadness and depression, and obsessions. Arab medical texts from this period contain detailed discussions of melancholia, mania, hallucinations, delusions, and other mental disorders. They were concerned with the links between the brain and disorders, while they also searched for spiritual/mystical meaning of the disorders.

Mental disorder was generally connected to loss of reason. And in the Islamic tradition, the mentally ill were considered incapable of running their own affairs, but fully deserving of humane treatment and protection.

Muslims built the first psychiatric treatment hospital in the World. Within the first century of Islam, by order of the Umayyad Caliph Al-Walid ibn Abd al-Malik, such a hospital was founded in Baghdad in 705. Insane asylums were built in Fes in the early 8th century, Cairo in 800 and in Damascus and Aleppo in 1270. Insane patients were treated with baths, drugs, music and activities. In fact by some accounts, the physicians of the Islamic world would invent and use a variety of treatments, including occupational therapy, music therapy, as well as medication.

In the centuries to come, Latin translations of many scientific Islamic texts would play a major role in Europe. But these Muslim progressive concepts to dealing with mental disorders would not come to Europe until the 19th century. By that time, the eastern lands of Islam had fallen behind, and Europe had become the dominant civilization on the planet.

Before the 19th century, conditions in the so-called lunatic asylums of the West were basically beastly places of confinement and mistreatment, not to say outright abuse. Some of the more famous inmates of such facilities as Charenton, included the Marquis de Sade, whose visions were romanticized in such literary works as Peter Wiess' play and Peter Brooks' film Marat/Sade and Kaufmann's film Quills.

Improvement finally came in the West from the beginning of the 19th century when under the impetus of great reformers like Phillipe Pinel (1745- 1826) in France and William Tuke (1732-1822) in the United Kingdom, both advocates of Moral treatment of the insane, brought about a more humane and scientific outlook. Very gradually throughout the 19th and 20th centuries, western concepts of mental health and the humane and scientific treatment of patients suffering from mental disorders would evolve towards its contemporary configurations.

In the meantime, Medicine also improved in the west by such breakthroughs as the introduction of anesthesia, nursing and better understanding through the work of such giants as Pasteur and Koch who gave us the germ theory of disease, Darwin who gave us evolution and Freud who invited us to look into the inner self. They and others helped pave the way for the giant strides that western medicine made in the 20th century, a time when Europe was practically ruling the world, and medicine and mental health would transform themselves in the 20th century.

Europe Ascendant, America Dominant

Following the European Renaissance and the scientific revolution, Europe would rule the world. The values of the Enlightenment would appear mostly from the 17th and the 18th century and bring their fruits in the American and French revolutions and the subsequent reforms that would cover England and the rest of Europe. The 19th century would see the full emergence of the modern state, whose seeds were found in the treaty of Westphalia.

I will not go over that history which you know only too well, and which covers the establishment of your profession, with its many disciplines and its evolving standards of practice. Rather let me skip to a few observations about the challenges of the new Century. The emergence of the new genetics, the much expanded understanding of the human body, and the new technologies that will allow nano-scale interventions, all herald a new golden age of medicine for the treatment of all diseases, much expanded life expectancies, and healthier lives for all. But that rapidly expanding knowledge base also reminds us to be humble, for it also underlines how much more we still have to learn.

But from those who treat the individuals, we need to build bridges to those who want to treat entire societies. For when I see the failures of our political discourse, the shallowness of our vaunted new domains of social connectivity, and the complete collapse of once-vibrant societies, I can only hope that other professions will be as effective as medical doctors in developing their domains of knowledge and insights, so that we will be able to better understand and cope with the transformations that we are living through, and that we will also be able to better diagnose the social pathologies that societies like our own are being subjected to.

In Egypt, as in other parts of the Muslim and Arab Worlds, we the intellectuals who produce art and science must hold up mirrors to ourselves and to our societies and ask why is it that our societies have become such fertile ground for extremism and violence? We must overcome fear and open windows onto the rest of the world and seek out different and more open relations with the "other". We must promote pluralism, dialogue and understanding, and cherish diversity and the enrichment it brings. We must help move the values of our societies to embrace not only the new technologies but also a vision of a more desirable future.

Thank you.

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Trabajos aportados por el nuevo Académico de Honor

THE VALUES OF SCIENCE

Dr. Ismail Serageldin

Director de la Biblioteca de Alejandría

In Egypt and Tunisia, ordinary citizens have toppled autocrats; elsewhere in the Arab World, they still battle dictators, armed with little more than their belief in freedom, human rights, and democracy. What sort of society comes after the revolution? Many fear that the idealism of the revolutionary democrats will only pave the way for theological autocrats who preach an intolerant doctrine. But fighting extremism is best done not by censorship or autocracy but by embracing pluralism and defeating ideas with ideas. And here, science has much to say, particularly about the values that are needed for societies to be truly open and democratic, because these are the values of science.

As the British scientist Jacob Bronowski observed more than half a century ago, the enterprise of science requires the adoption of certain values that are adhered to by its practitioners with exceptional rigor. These values also provide the basis for enhancing human capabilities and human welfare. Truth and honor are of the utmost importance. Any scientist who manufactures data risks being ostracized indefinitely from the scientific community, and he or she jeopardizes the credibility of science for the larger society. A scientist may err in interpreting data, but no one can accept the fabrication of data. What other fields of human activity can rival this level of commitment to absolute truth? Teamwork has become essential in most fields of science, and it requires that all the members of the team receive the recognition they deserve. Contributions are also cumulative, and each should be recognized for his or her contribution. It is a sentiment well captured in Isaac Newton's famous statement that “if I have seen farther than most, it is because I have stood on the shoulders of giants.”

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Science requires the freedom to enquire, to challenge, to think, to imagine the unimagined. It cannot function within the arbitrary limits of convention, nor can it flourish if it is forced to shy away from challenging the accepted. Science advances by overthrowing an existing paradigm, or at least substantially expanding or modifying it. Thus there is a certain constructive subversiveness built into the scientific enterprise, as a new generation of scientists makes its own contribution. Our respect and admiration for Newton are not diminished by the achievements of Albert Einstein. We can admire both. This constant renewal and advancement of our scientific understanding is a central feature of the scientific enterprise. It requires a tolerant engagement with the contrarian view that is grounded in disputes arbitrated by the rules of evidence and rationality.

Science demands rationality and promotes civility in discourse. Ad hominem attacks are not accepted. Science treats all humans equally. Scientists are concerned with the content of the scientific work, not with the person who produced it. Science is open to all, regardless of nationality, race, religion, or sex. These values of science are universal values worth defending, not just to promote the pursuit of science but to produce a better and more humane society.

The new Arab societies we are building must be open pluralistic societies that are producers of knowledge and new opportunities. Our youth have sparked our revolution, just as other young people have transformed societies, reinvented business enterprise, and redefined our scientific understanding of the world we live in. Today, as they lead the rebuilding of our societies, they must embrace the values of science. Together, all armed with these values, we can think of the unborn, remember the forgotten, give hope to the forlorn, include the excluded, reach out to the unreached, and by our actions from this day onward lay the foundation for better tomorrows.

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ANCIENT ALEXANDRIA AND THE DAWN OF MEDICAL SCIENCE

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THE ANCIENT WORLD AND THE BIRTH OF MEDICINE

From the banks of the Nile to the shores of the Mediterranean, it is in the land of Egypt where medicine probably started. This is the story of a great period in the history of medicine. But let us start at the beginning. Imhotep, who flourished about 5,000 years ago, is the first person whose name is recorded not for being a king or a conqueror, but for the way he contributed to knowledge[1]. He was a statesman: he advised Pharaoh Zoser. He was an engineer: he built the stepped pyramid of Saqqara, precursor to the many great pyramids to come. But above all he was a physician of talent, who launched the first true medical revolution: that disease was not something to be dealt with by magic, but by science: observation, diagnosis, and treatment. Egyptians would later deify him as the god of medicine.

The great tradition of ancient Egyptian medicine was maintained for millennia. The Edwin Smith papyrus and the Ebers papyrus both speak of exquisite knowledge and understanding.

The Edwin Smith papyrus is the earliest known medical document, written around 1600 BCE, but is thought to be based on material from as early as 3000 BCE. It is an ancient textbook on trauma surgery. It mentions trepanation.

It describes anatomical observations and the examination, diagnosis, treatment, and prognosis of numerous injuries in exquisite detail. It gives the first descriptions of the cranial sutures, the meninges, the external surface of the brain, the cerebrospinal fluid, and the intracranial pulsations (Figure 1).

Other documents, like the Ebers Papyrus, give details showing an in-depth understanding of how the body works. It defines the heart as the center of the blood supply, with vessels attached for every member of the body.

Mental disorders such as depression and dementia are covered, and the descriptions suggest that mental and physical diseases were considered in the same way. The document also has chapters on contraception, diagnosis of pregnancy and other gynecological matters, intestinal disease and parasites, eye and skin problems, and the surgical treatment of abscesses and tumors, bone-setting and burns. Dentistry is also covered, and we have evidence of false teeth existing in Egypt some 4,000 years ago (Figure 2).

In a number of ways, this ancient Egyptian knowledge was superior to the later Greek knowledge that would flourish in the first millennium BCE. But civilizations wax and wane, and Egypt was in a slow decline. It was finally conquered by the Persians in 525 BCE, while Greek civilization, already well-established from the time of the Minoan and Mycenaean Civilizations, would take over the torch of knowledge and enter a golden period whose achievements continue to dazzle the world to this day.

The Greeks erected a philosophical, artistic and scientific culture that was to rival the best that the world has seen before or since. Medicine would be an important part of this edifice of knowledge, as it

[1]Women also enter our records early on, shortly after Imhotep: Merit Ptah in Egypt, sometimes called the first astronomer, and En Hedu Anna in Mesopotamia – daughter of Sargon of Akkad and priestess of the Moon God.

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was in the age of Pericles, largely through the work of Hippocrates of Cos (c. 460 BCE – c. 370 BCE) that medicine was recognized as an independent field of science, separate from either magic or the other sciences. It was a profession which women were originally forbidden from practicing[2]. As usually happens with pivotal figures in history, Hippocrates would be a summation of the best of what preceded him, a major contributor himself, and the founder of a school of disciples who followed his teachings. His contributions are many, but he is most famous for the Hippocratic Oath[3] still in use in many parts of the western world.

Like Imhotep before him, Hippocrates believed that diseases were caused naturally, not because of superstition and gods, arguing that disease was not a punishment inflicted by the gods but rather the product of environmental factors, diet, and living habits. Indeed in the entirety of the Hippocratic Corpus there is not a single mention of an illness or a cure due to mystical factors. However, Hippocrates did work with many convictions that were based on what is now known to be incorrect knowledge of anatomy and physiology, such as his theory of Humors[4].

But Rome would soon rule the world, and Roman medicine reaches its apex with Galen (CE 129 – 216)[5], on whom we will have more to say later.

But between the decline of splendid Greece and the rise of mighty Rome, there is a glorious period, where in the ancient land of Egypt Alexander the Great would found a new capital of learning and knowledge on the shores of the Mediterranean: Hellenistic Alexandria.

THE GLORY OF ALEXANDRIA

To the ancient land of Egypt, 2300 years ago, Alexander the Great, Aristotle's pupil, brought his dream of culture and conquest, of uniting the world and launching a new era. Alexander selected the site for a new capital: Alexandria. His successors in Egypt, the Ptolemies, built Alexandria, and made it the intellectual capital of the world. Its lighthouse, the Pharos, was considered one of the seven wonders of the ancient world. But a greater legacy was the Ancient Library of Alexandria. Launched in 288 BCE by Ptolemy I (Soter) under the guidance of Demetrius of Phaleron, the Mouseion, or temple to the muses, was part academy, and part research center, in addition to

[2]In late 4th C BCE Athens the Physician Agnodice was put on trial for pretending to be a man to practice medicine, which was formally illegal. Her women patients (many of whom were wives of important men) saved her and had the law repealed.

[3]A modern translation (Wikipedia, accessed 18 10 2013) would read as follows:
I swear by Apollo, the healer, Asclepius, Hygieia, and Panacea, and I take to witness all the gods, all the goddesses, to keep according to my ability and my judgment, the following Oath and agreement:

To consider dear to me, as my parents, him who taught me this art; to live in common with him and, if necessary, to share my goods with him; To look upon his children as my own brothers, to teach them this art; and that by my teaching, I will impart a knowledge of this art to my own sons, and to my teacher's sons, and to disciples bound by an indenture and oath according to the medical laws, and no others.

I will prescribe regimens for the good of my patients according to my ability and my judgment and never do harm to anyone.

I will give no deadly medicine to any one if asked, nor suggest any such counsel; and similarly I will not give a woman a pessary to cause an abortion.

But I will preserve the purity of my life and my arts.

I will not cut for stone, even for patients in whom the disease is manifest; I will leave this operation to be performed by practitioners, specialists in this art.

In every house where I come I will enter only for the good of my patients, keeping myself far from all intentional ill-doing and all seduction and especially from the pleasures of love with women or men, be they free or slaves.

All that may come to my knowledge in the exercise of my profession or in daily commerce with men, which ought not to be spread abroad, I will keep secret and will never reveal.

If I keep this oath faithfully, may I enjoy my life and practice my art, respected by all humanity and in all times; but if I swerve from it or violate it, may the reverse be my life.

[4]Hippocrates assumed that an excess or deficiency of any of four distinct bodily fluids in a person directly influences their temperament and health. These four fluids are black bile, yellow bile, phlegm, and blood. Each was posited to correspond to one of the traditional four temperaments: melancholic (analytical and thoughtful), choleric (ambitious and leader-like), phlegmatic (relaxed and quiet), and sanguine (pleasure-seeking and sociable). From Hippocrates onward, this theory was adopted by Greek, Roman and Persian physicians. It became the most commonly held view of the human body among European physicians until the advent of modern medical research in the nineteenth century.

[5]Claudius Galenus better known as Galen of Pergamum was the most accomplished of all medical practitioners of antiquity. He would be the personal physician of two emperors, and contributed greatly to the understanding of anatomy, physiology, pathology, pharmacology, and neurology, as well as philosophy and logic.

Figure 1. The Edwin Smith papyrus, the world's oldest surviving surgical document. Written in hieratic script in ancient Egypt around 1600 B.C., the text describes anatomical observations and the examination, diagnosis, treatment, and prognosis of 48 types of medical problems in exquisite detail. Plate 6 and 7 of the papyrus, pictured here, discuss facial trauma. Reproduced under a US Public Domain license. Source: Wikipedia http://en.wikipedia.org/wiki/File:Edwin_Smith_Papyrus_v2.jpg.

the great ancient library. The great thinkers of the age, scientists, mathematicians, poets from all cultures came to study and exchange ideas. Greek was reinforced as the language of science and knowledge in that period.

700,000 scrolls, the equivalent of more than 100,000 modern printed books, filled the shelves. Girls and boys studied regularly at the Ancient Library which was open to scholars from all cultures. And at the Library an explosion of science would amaze the world. It was there that:

- Aristarchus was the first person to state that the earth revolves around the sun, a full 1800 years before Copernicus;
- Eratosthenes proved that the earth was spherical and calculated its circumference with amazing accuracy, 1700 years before Columbus sailed on his epic voyage;
- Callimachus the poet described the texts in the library organized by subject and author, becoming the father of library science;
- Euclid wrote his elements of geometry, the basic text studied in schools all over the world even now;
- Herophilus identified the brain as the controlling organ of the body and launched a new era of medicine;
- Manetho chronicled the pharaohs and organized Egyptian history into the dynasties we use to this day.

They and many others were all members of that amazing community of scholars, which mapped the heavens, organized the calendar, established the foundations of science and pushed the boundaries of our knowledge as they unleashed the human mind on myriad quests. They opened up the cultures of the world, established a true dialogue of civilizations, promoted rationality, tolerance and understanding and organized universal knowledge. For over six centuries the ancient Library of Alexandria epitomized the zenith of learning. To this day it symbolizes the noblest aspirations of the human mind, global ecumenism, and the greatest achievements of the intellect. The library completely disappeared over sixteen hundred years ago, but it continues to inspire scientists and scholars everywhere (Figure 3).

Figure 2. Ebers Papyrus by Einsamer Schütze derivative work via Wikimedia Commons http://commons.wikimedia.org/wiki/File%3APEbers_c41-bc.jpg.

ALEXANDRIAN MEDICINE 288 BCE–300 CE

A famous medical school was established in old Alexandria during the third century BCE. Although mainly Greek in essence, and following the Hippocratic teachings, it was heavily affected by the medical practices of ancient Egypt. Anatomy was particularly advanced due to the possibility of dissecting the human body. The most important Alexandrian physicians were Herophilus and Erasistratus. Many graduates of this medical school traveled and practiced throughout the Mediterranean basin.

Galen, the famous Roman physician studied in Alexandria before practicing in Rome. His teachings and writings survived well into the sixteenth century and formed the basis of more modern medical practices during the Renaissance. These writings were conserved partly by Christian monks and partly by Arab and Jewish scholars of the middle ages. The medical school of Alexandria was still active until late in the 3rd century CE. However, it slipped slowly into oblivion after the fire of 391 CE, which also devastated the last remnants of its famous library.

Unfortunately, almost no work of Alexandrian medicine survived intact; thus the production of the Alexandrian doctors is largely lost in the gap between two great bodies of ancient medical writings: the Hippocratic Corpus and the writings of the Imperial period, particularly those of Galen. But Alexandria was the bridge between these two worlds, and Galen, was the last of the great medical specialists to

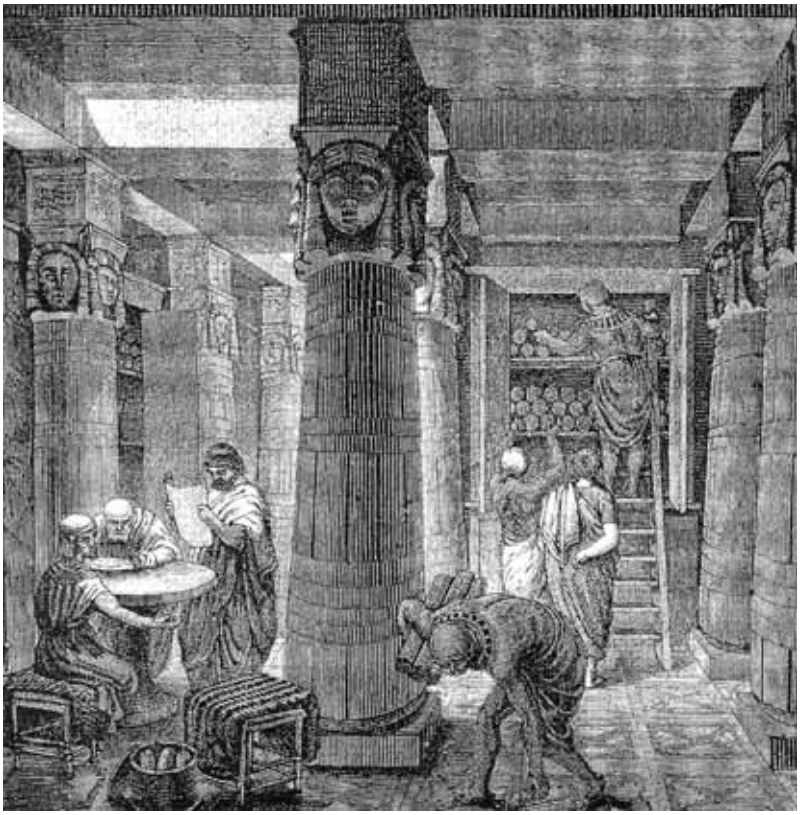

Figure 3. Artistic rendering of the Library of Alexandria, based on some archaeological evidence. Reproduced under a US public domain license, via Wikimedia http://commons.wikimedia.org/wiki/File:Ancientlibraryalex.jpg.

have drunk from the Pierian Spring of Alexandria[6]. The best of that school's writings would be incorporated into his work, and it was his work that would represent the summation of the medicine of antiquity.

Most of the Hellenistic writings were written by historians of medicine; they wrote both medical textbooks and commentaries on the Hippocratic Corpus, which was already lost by the time of Galen, whose voluminous writings and many interests and references remain one of the principle sources for understanding the importance of what was produced in Alexandria in the four centuries that preceded him.

The earliest figures in the history of the Alexandrian medicine were Philitas of Cos (b. 340 BCE), and Praxagoras of Cos (second half of the fourth century BCE) who was the teacher of the famous Herophilus and was an anatomist. He also provided a study of the diagnostic value of the pulse and the nature of its origin in the blood vascular system.

Aristotle's famous writings on anatomy found particular resonance in Ptolemaic Alexandria, where Herophilus of Chalcedon (c. 330–260 BCE) and Erasistratus of Iulis on Ceos (about 315–240 BCE) made extraordinary progress in anatomy and physiology. They were to found two important schools of medicine in the heyday of ancient Alexandria.

[6]In Greek mythology, the Pierian Spring of Macedonia was sacred to the Muses. As the metaphorical source of knowledge of art and science, it was popularized by a couplet in Alexander Pope's poem "An Essay on Criticism" (1709): "A little learning is a dang'rous thing;/Drink deep, or taste not the Pierian spring." (see Wikipedia at http://en.wikipedia.org/wiki/Pierian_Spring)—since the Ancient Library was founded as the temple to the muses, and by Alexander the great, originally from Macedonia, it is an apposite reference here.

Herophilus, who was one of the greatest figures in Alexandrian medicine and who established his own school of medicine, was a pioneer of functional physiology, and produced a very large amount of anatomical writings. He correctly identified that it is the brain that is the controlling organ of the body, and not the heart as Aristotle had said. He carried out pioneering work on the anatomy of the brain and nervous system, and is credited with the identification of the dura mater and pia mater, two of the brain's membranes; and with tracing the connections between the spinal cord, nerves, and the brain. Herophilus also studied the anatomy of eyes, neural anatomy, and the male and female reproductive system. He also distinguished between veins and arteries. In addition, He did a lot of work in functional physiology, and named parts of the human body such as the duodenum.

Erasistratus of Iulis on Ceos (about 315-240 BCE), initially a collaborator of Herophilus, argued that the body is composed of threefold web; elemental nerve, veins, and arteries. In his account of the heart and its function, he distinguished between pulmonary and systemic circulation. He said that veins distribute blood through the body, and that air enters the body and then is drawn by the lungs into the heart, where it is transformed into vital spirit, and is then pumped by the arteries throughout the body. He also placed great emphasis on the study of the pulse, and on the concept of body temperature.

Beyond these two giants, Herophilus and Erasistratus, there were many other eminent physicians in Alexandria, who by their collective work made Alexandria the major center of learning in medicine in the ancient world for about four centuries, until c. 162 CE, when Galen went to Rome and flourished there.

The School of Alexandrian Medicine boasted many other luminaries, such as Apollodorus of Alexandria (3rd century BCE), a physician whose works on botany, pharmacology and toxicology were renowned; as well as Callimachus of Bithynia (later 3rd century BCE) a physician who belonged to the Herophilian school. Callimachus was also interested in pharmacology and wrote important commentaries on works of the Hippocratic corpus.

Other luminaries included Hegetor (2nd cent BCE), an Alexandrian Herophilian physician, who was interested in pulse theory, and who treated the dislocation of the thigh bones on the basis of anatomical studies, rather than the empirical school's analogies. Chrysermus (fl. mid-1st cent. BCE), was another Alexandrian Herophilian physician, who developed a theory of pulse that differed from that of Herophilus and Erasistratus.

But not all the Alexandrian physicians were disciples of Herophilus or Erasistratus. There was an important empiricist school, with such luminaries as Serapion (or Sarapion) of Alexandria (fl. late-3rd cent. BCE) who established the Empiricist school or was its second head after Philinus of Cos, and Apollonius of Citium (c. 90–15 BCE), who advanced orthopedic surgery depending on Hippocratic texts, on which he wrote commentaries. Apollonius also wrote a substantial critique of fellow empiricist physician and surgeon Heraclides of Tarentum (fl. 85–65 BCE) who in addition to his Commentaries on Hippocrates, wrote four books on external and internal therapy, as well as a dietetic treatise, and some works in pharmacology. Of the works of Heraclides approximately 90 fragment and testimonia survive. Sostratus (who probably practiced in Alexandria after 30 BCE) was Alexandrian zoologist and surgeon, whose medical practices were chiefly in gynecology. Zopyrus of Alexandria (about the beginning of 1st cent. BCE) another surgeon, who invented an antidote for poisons and asked Mithridates, King of Pontus, to allow him to test it on criminals.

Alexandrian science, though much diminished, continued to exist even as Rome eclipsed it, and among the later physicians of note, we can mention Didymus of Alexandria (fl. in the 4th or 5th century) who was both physician and agriculturist, who is said to have produced an important medical treatise in eight books and an agricultural treatise in fifteen books, the latter being supposed to be one of the sources of the Byzantine work "Geoponica".

But the transition from Alexandria to Rome, which was to be completed by the career of Galen as imperial physician and great writer, was already presaged in the emergence of Pedanius Dioscorides (c. 40—90 CE), who was a Greek physician, pharmacologist and botanist, who practiced in Rome at the time of Nero, and produced the most important pharmacopeia of all of antiquity – *De Materia Medica* – a 5-volume encyclopedia about herbal medicine and related medicinal substances that was widely read for more than 1,500 years and which existed in Greek, Latin and Arabic, throughout the middle ages. It remains the most important reference for our understanding of the medicines used in antiquity and the Middle Ages.

GALEN AND THE ROMAN EMPIRE

Claudius Galenus, commonly known as Galen, is the fitting closure of Hellenistic medicine and the start of Imperial medicine. He is the most important and the most influential of the ancient Greek physicians,

Figure 4. Galen of Pergamon (Claudius Galenus, or in French, Claude Galien), the most famous medical researcher of classical antiquity. Lithograph by Pierre Roche Vigneron. (Paris: Lithograph by Gregoire et Deneux, ca. 1865). Reproduced from http://en.wikipedia.org/wiki/File:Galen_detail.jpg.

whose enormous mass of surviving treatises have had an extensive influence, for more than fourteen centuries, on the different branches of medical science. Few if any others, before or after, have had such an impact.

He was born (around 129 CE) at Pergamum, into a wealthy family and studied medicine there. At the age of twenty, he travelled to the major medical centers such as Smyrna and Corinth to improve his practical acquaintance with human anatomy, but still in a limited way, i.e. only the skeleton. Then he went to Alexandria where he absorbed all he could from the fading schools of the once splendid metropolis[7]. At the age of twenty eight (c. 157 CE), he returned from Alexandria to Pergamum, where he

[7]Galen absorbed and then added to the body of knowledge of his time. For example, although Herophilus and Erasistratus were the first to state the importance of the ventricles, it is Galen who creates a detailed anatomical and physiological description of the brain, the cranial nerves and the spinal cord.

became the surgeon at the School of Gladiators. In 162 CE he left for Rome where he acquired a reputation as a skilled practitioner, physician, lecturer and writer. He was to leave Rome for a brief while but returned in in 169 CE at the invitation of the Emperor Marcus Aurelius who wanted his services. He would be physician to the Emperors Marcus Aurelius (161–180 CE), Commodus (180–192 CE), Septimus Severus (193–212 CE). But he did not abandon his research and over the next two decades he would write and edit his many books. The date and place of his death is uncertain. While some say that he spent his last days at Pergamum in 199 CE Suidas says that Galen died at the age of seventy (i.e. c. 201 CE); however, Arabic biographers propose that he died in Sicily at age of 88 (i.e. 219 CE). (Figures 4 and 5).

The works of Galen alone form about half of the mass of the surviving Ancient Greek medical writings. He wrote no less than five hundred treatises, out of which eighty three are extant and acknowledged to be genuine, covering not only every aspect of medicine, but also ethics, philosophy, logic, and grammar.

Many of Galen's works reached the West, during the early Middle Ages, thanks to the Syriac and Arabic translations of Hunain ibn Ishaq and Hubaish ibn al-Hassan. His principal treatise is 'On Anatomical Procedures' in fifteen books, the last six of which —as well as about two thirds of the ninth— are now extant only in Arabic translation.

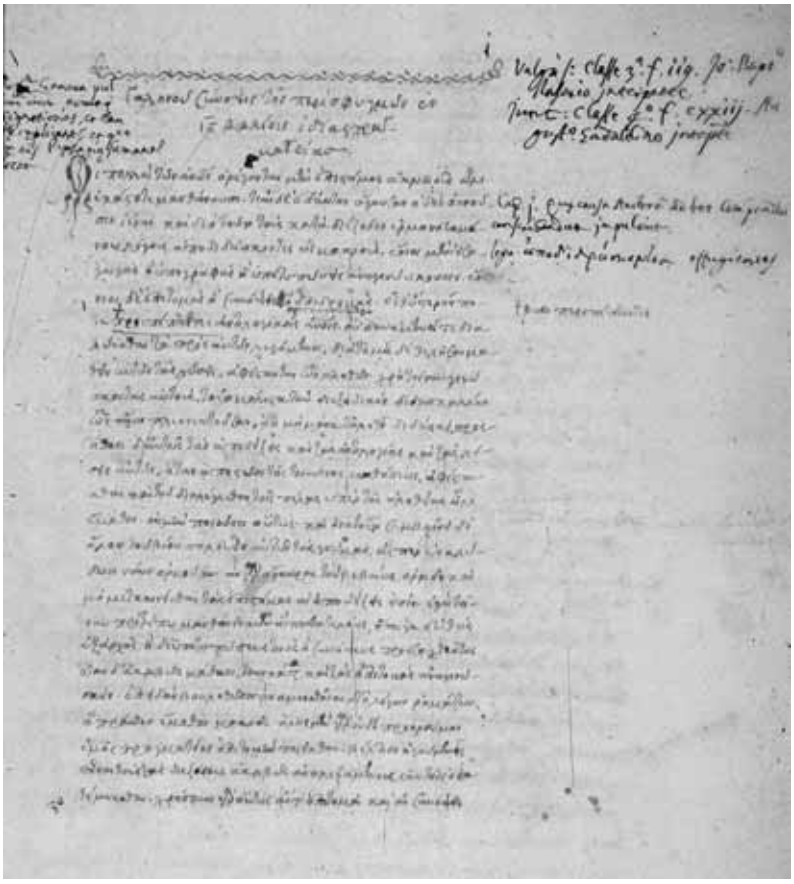

Figure 5. Galen. De pulsibus. (Manuscript; Venice, ca. 1550). This Greek manuscript of Galen's treatise on the pulse is interleaved with a Latin translation. Reproduced from http://en.wikipedia.org/wiki/File:Greek_doco1.jpg.

With the death of Galen, a brilliant chapter of the history of medical science comes to a close. The impetus he provided would carry on in the declining Roman Empire for several centuries. But Rome was waning, and the Dark Ages were setting in.

For the best part of the next thousand years, it would be the Arabs and Muslims who would carry the torch of science and learning as Europe would remain in the grip of the Dark Ages. Then with the Renaissance and the scientific revolution of the 16th and 17th centuries, the torch would pass once more to Europe. And it was in the West that the great journey of medicine would continue, a journey started so long ago by Imhotep who said that disease was not due to magic but rather a natural, physical process that could be studied and treated. A journey, where so much of the creative work in Greece, Alexandria and Rome would carry humanity forward a long distance, and constitute an incredible body of work, parts of which are still of relevance today.

EPILOGUE: THE NEW LIBRARY OF ALEXANDRIA

1600 years after the disappearance of the Library of Alexandria in 391 CE and the murder of Hypatia in 415 CE, the Library of Alexandria was reborn largely on the same spot where it was born some 23 centuries earlier. It was a bold imaginative idea, to recapture the spirit of the ancient library with the tools of the new millennium, a dream that seemed for many to be beyond attainment, but the dream was realized in a few short years.

Today, the library stands as a beautiful building that is like a large disk slanting towards the Mediterranean. Whether the disc is an echo of the sun disc, so important for ancient Egyptians, or the rising sun of knowledge slanted towards the endless sea with its unbounded horizons is better left to the imagination of visitors and viewers. The library is a vast enterprise that relinks with its past in many fields, including medicine, although much more modestly at present than what once was and could be again (Figure 6).

The Library is a hive of active institutions: Planetarium, Exploratorium, conference center, 19 museums and permanent art exhibits, four art galleries with changing exhibitions, advanced informatics, seven specialized libraries, the large library, a super computer and 15 research institutes, including a small research laboratory that connects researchers on the genetics of HCM in Egypt and Europe under the watchful eye of a modern day leader of medicine, Sir Magdi Yacoub. But the library does not house a formal school of medicine today, and perhaps it does not need to. Today, great scientists fly in for a seminar and leave, they do not have to reside years after perilous and long

Figure 6. The modern-day Bibliotheca Alexandrina from the Mediterranean side. Reproduced from http://en.wikipedia.org/wiki/File:CW_BibliotechaAlexandrina_Front.jpg – with thanks to Carsten Whimster.

journeys in small boats on the Mediterranean. Practitioners organize huge annual conferences to compare notes on their work and discuss the latest technologies and techniques. The new Library of Alexandria, which is host to many such activities in many fields, including a number in the field of medicine, is active in the promotion of knowledge and its dissemination, much as its ancient namesake did.

It is on the pages of learned journals and in such meetings as those organized by the Bibliotheca Alexandrina (BA), the new Library of Alexandria, that the future – including the future of medicine – is being imagined as it gets crafted in the many cutting-edge laboratories around the world. And like its ancient namesake, the New Library of Alexandria stands for values that we hold dear: rationality, liberty of inquiry, freedom of expression, pluralism, dialogue, learning and understanding. Anchored in the past, active in the present, embracing the future, the modern contemporary BA is just beginning its journey of rediscovery and the best is yet to come.

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SCIENCE IN MUSLIM COUNTRIES

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WITH MORE THAN A TRILLION DOLLARS IN CASH AND A POPULATION OF OVER A BILLION PEOPLE, the Muslim world should be poised for a remarkable scientific explosion. Yet despite some very high-profile projects in the Gulf, including the building of massive state-of-the-art facilities for research across all disciplines (and serious efforts elsewhere), the reality is that Muslim countries tend to spend less on scientific research itself, as distinct from buildings and equipment, as compared to other countries at the same income scale. Furthermore, even where funding for science has been available, the results in terms of output—research papers, citations, and patents—are disappointingly low. Why?

Throughout the Muslim world, we are witnessing an increasingly intolerant social milieu that is driven by self-appointed guardians of religious correctness, who inject their narrow interpretation of religion into all public debates. Rejecting rationality or evidentiary approaches, they increasingly force dissenting voices into silence and conformity with what they consider acceptable behavior. Of course, Muslim zealots are not alone in challenging the scientific enterprise; in the United States, battles over evolution and creationism continue to rage.

Yet it was our Muslim forefathers who first held up the torch of rationality, tolerance, and the advancement of knowledge throughout the dark ages of medieval Europe. Centuries before the European scholars Bacon, Descartes, and Galileo considered the scientific method, the great thinker Ibn Al-Haytham (10th century) laid down the rules of the empirical approach, describing how the scientific method should operate through observation, measurement, experiment, and conclusion, the purpose being to "search for truth, not support of opinions." Likewise, Ibn Al-Nafis (13th century) stressed the importance of accepting contrarian views, subject to the test of evidence and rational analysis.

This is the Muslim tradition that must be revived if current efforts are to bear the scientific fruit that a billion Muslims need and that the world has a right to expect of us. Rejecting politicized religiosity and reviving these traditions would promote the values of science in our societies.

There is a central core of universal values that any truly modern society must possess, and these are very much the values that science promotes: rationality, creativity, the search for truth, adherence to codes of behavior, and a certain constructive subversiveness. Science requires much more than money and projects. Science requires freedom: freedom to enquire, to chal-

lenge, to think, and to envision the unimagined. We must be able to question convention and arbitrate our disputes by the rules of evidence. It is the content of scientific work that matters, not the persons who produced it, regardless of the color of their skin, the god they choose to worship, the ethnic group they were born into, or their gender. These are the values of science, but even more, they are societal values worth defending, not just to promote the pursuit of science but to have a better and more humane society.

The future can be bright, but it requires a commitment to fight for the values of science and to reject obscurantism, fanaticism, and xenophobia. It requires that members of the scientific and academic communities in Muslim countries be willing to challenge accepted populist views and insist on creating the "space of freedom" necessary for the practice of science and the advancement of knowledge. We must engage with the media and the public and defend the values of science in our societies. These efforts will not be easy, but they constitute a major and necessary step toward liberating minds from the tyranny of intolerance, bigotry, and fear, and opening the doors to free inquiry, tolerance, and imagination.

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BIOTECHNOLOGY AND FOOD SECURITY IN THE 21ST CENTURY

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Biotechnology can contribute to future food security if it benefits sustainable small-farm agriculture in developing countries. Presently, agrobiotechnology research cites ethical, safety, and intellectual property rights issues. Protection of intellectual property rights encourages private sector investment in agrobiotechnology, but in developing countries the needs of smallholder farmers and environmental conservation are unlikely to attract private funds. Public investment will be needed, and new and imaginative public-private collaboration can make the gene revolution beneficial to developing countries. This is crucial for the well-being of today's hungry people and future generations.

The human family has achieved outstanding progress in the 20th century. Developing countries have covered as much ground over the past 35 years in challenging poverty, hunger, disease, and ignorance as the industrialized nations covered in more than a century. The developing countries have doubled school enrollments, halved infant mortality and adult illiteracy, reduced malnutrition by a third, and extended life expectancy at birth by 20 years (*1*).

One of the greatest achievements since the Second World War has been the phenomenal increase of research-based agricultural productivity that has fed millions and served as the basis of economic transformation in many poor countries, especially on the Indian subcontinent (*2*). This "Green Revolution" has avoided dire predictions of death and famine in Asia (*3*). Food production has instead outpaced population growth, mainly because of substantially higher yields and increased irrigated land area. Food availability per capita grew and prices fell.

However, much remains to be done despite these gains. Poverty continues to limit access to food, leaving hundreds of millions of people undernourished in developing countries (*4*). Increased population, income growth, and urbanization will drive sustained growth in food demand, with a doubling of food needs in developing countries possible over the next four decades (*5*). Will the world continue to provide the supplies to meet this demand?

A priori, biotechnology—one of many tools of agricultural research and development—could contribute to food security by helping to promote sustainable agriculture centered on smallholder farmers in developing countries. Yet, biotechnology is now a lightning rod for visceral debate, with opposing factions making strong claims of promise and peril (*6*).

The World on the Eve of the New Century

Today the world is marked by aggregate affluence, but also by economic uncertainties, poverty, hunger, and violent conflict. Averages mask or divert attention from inequalities within and among societies. The natural resources on which future progress depends are imperiled (*7*). Population growth adds about 86 million persons a year, mostly in the poorest countries (*8*). Poverty and environmental degradation go hand in hand, for it is the poor who suffer the consequences of desertification and live the misery of unsanitary conditions. Tackling these problems is closely related to the policies that will be followed in transforming agriculture in developing countries (*9*).

Despite some problems, the Green Revolution has been a great success. There are, however, questions about whether a new, "doubly green revolution"—environmentally

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sustainable as well as yield-increasing—could help food needs over the next two decades. This revolution will need the political will to remove policy distortions that discriminate against poor people, investments in rural health and education, as well as rural roads, credit institutions, and high-quality research, within which biotechnology will have an increasing role (*2*, *9*).

Feeding the World in the 21st Century

Nobel laureate Norman Borlaug estimates that to meet projected food demands by 2025, average cereal yield must increase by 80% over the 1990 average (*10*). Making this formidable task even more difficult is that, to ensure that food production is coupled with both poverty reduction and environmental conservation, it will be essential that this increase occur in the complex smallholder farming systems of the poorest countries (*11*).

That requires policies and actions to promote agriculture and rural development, an enabling regulatory framework, fair trade, flexible and responsive institutions, increased investments in health and education, especially for women, and access to credit, roads, marketing, and extension. Research is a necessary but not sufficient condition for sustainable agricultural development, just as food production is a necessary but not sufficient condition for food security (*9*). The transformation will require access to and ability to apply technological advances, since future growth in food production will have to come largely from agricultural intensification on existing land. Most land suited to agriculture is already in use. More efficient use of water, energy, and labor is also essential (*12*).

A Double Shift in the Agricultural Research Paradigm

Two shifts in the research paradigm are necessary. The first involves integration of crop-specific research, which has been so successful in the past, into a broader vision that includes sound management of natural resources, as well as the productivity and profitability of smallholder farming; promoting synergies among livestock, agroforestry, food and cash crop, and aquaculture production, all on a hectare or so; integrated management of soil, water, and nutrients; integrated pest management; attention to postharvest losses; and recognition of the socioeconomic realities of farmers, including gender issues. In many developing countries, women produce the bulk of food crops (*2*, *9*, *11*, *12*).

Doubling the yields of complex farming systems in an environmentally sound manner is a difficult challenge (*13*). It is even harder than pushing the yield frontier on a particular crop. But such daunting challenges advance science.

The second shift is to harness the genetic revolution. Cutting-edge work associated with genetic mapping, molecular markers, and biotechnology must be focused on benefiting poor people and the environment. It is vital to realize the promise of this revolution while avoiding the pitfalls.

Delivering on the Promise of Biotechnology for the Poor

The initial successes in plant genetic engineering marked a significant turning point in crop research. Particularly in the 1990s, there has been an upsurge of private sector investment in agricultural biotechnology. Some of the first products were plant strains capable of synthesizing an insecticidal protein encoded by a gene (Bt) isolated from the bacterium *Bacillus thuringiensis*. Bt cotton, maize, and other crops are now commercially grown. There are also crop varieties tolerant to or capable of degrading herbicides. Proponents stress the value of these crops in minimum-tillage soil conservation (*14*).

Over the last 3 years, there have been dramatic and continuing increases in the area planted to transgenic crops. From 2.8 million hectares in 1996, the area increased

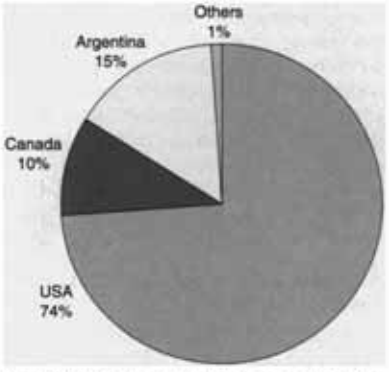

Fig. 1. Distribution of global area planted to transgenic crops [adapted from (*14*)].

nearly 10-fold to 11 million hectares in 1997 and rose to 27.8 million hectares in 1998. The United States alone accounted for 74% of the area planted to transgenics. Argentina was the only developing country with a significant transgenic hectarage (Fig. 1). The five principal transgenic crops were soybean, maize, cotton, rapeseed/canola, and potato (*14*, *15*).

Total transgenic crop sales grew more than sixfold, from U.S. $235 million in 1996 to $1.2 to $1.5 billion in 1998. The market is projected to increase to $3 billion or more in the year 2000, to $6 billion in 2005, and to $20 billion in 2010 (*15*). Consolidations in the form of acquisitions, mergers, and alliances continue to be a dominant feature of the biotechnology industry. Since 1996, more than 25 major acquisitions and alliances valued at $15 billion have taken place among agrobiotech, seed, and farm chemical firms (*16*).

This biotechnology revolution is very relevant to the problems of food security, poverty reduction, and environmental conservation in the developing world. But for many, it raises important questions relating to ethics, intellectual property rights, and biosafety (*17*). There have been widespread protests against the spread of agro-biotechnology. Some of the concerns come from scientists who fear that "novel" products will destroy agricultural diversity, thus changing agricultural patterns into unrecognizable and uncontrollable forms. Many protests have been made by civil society institutions on ethical or ecological grounds. The dominance of a highly concentrated private sector has raised fears of a new phase of comparative disadvantage and increased dependency in the developing world (*18*).

Also very much at issue are patenting and intellectual property rights. Supporters of patenting point out that if the private sector is to mobilize and invest large sums of money in agrobiotechnology R&D, it must protect and recoup what it has put in (*19*). On the other side of the argument is fear that patenting will lead to monopolization of knowledge, restricted access to germplasm, controls over the research process, selectivity in research focus, and increasing marginalization of the majority of the world's population (*20*).

These concerns cannot and must not be ignored. Effective regulatory mechanisms and safeguards need to be universal so that the impact of agrobiotechnology is both productive and benign. Every instrument of agricultural transformation should be mobilized in efforts to promote food security and help poor people.

Take the so-called Terminator Gene Technology. The Consultative Group on International Agricultural Research (CGIAR), which sponsors a global network of 16 international agricultural research centers, has announced that it will not release any germplasm that contains technologies that would prevent smallholder farmers from holding and replanting seeds (*21*). Instead, CGIAR centers are pursuing the apomictic gene to assist smallholders to replant hybrid varieties (*22*). But it is legitimate to study the sophisticated terminator technology, and learn from it, or seek out possible benign applications, such as a platform that would bond novel transgenes in desirable varieties, preventing their escape through unwanted gene flow.

Caution is warranted concerning the long-term effect of technologies, even if their application is benign. If terminator technologies were to become widespread, even if economically feasible and advantageous to the smallholder farmer, what would become of the constant introduction of variability that farmers bring to the plant gene pool? If there were large-scale agricultural homogeneity, what would the loss of local biodiversity mean? Would it destroy the environmental "early warning system" that enabled humans to recognize potential problems before they had major impacts on humans, as in the case of DDT (*23*)?

The answers to these questions are not yet known. Scientific research on such issues must be guided by ethical and safety principles, as well as respect for the private sector's need to earn a decent return. It is essential to harness the benefits of new technologies in sustainable ways for poverty eradication.

The promise that biotechnology holds for smallholder agriculture in the developing world is not yet realized. To do so will require addressing the issues of ethics, biosafety, and intellectual property rights. Potential risks remain the primary reason for slow acceptance of transgenic crops. To address such concerns, a global biosafety protocol is under negotiation.

More complex and contentious are intellectual property rights (IPR) issues. Application of intellectual property concepts to agriculture at-

tracts much debate because technological development in agriculture, particularly in developing countries, has been driven primarily by public investment. Most of the products of agricultural research, including those generated by CGIAR centers, are considered "public goods." The largest germplasm collections of important crops in the developing world are held in trust by these centers and remain in the public domain (*21, 23*).

Supporters of patenting argue that it enables and drives large private sector investments in biotechnology research. But the applications and their benefits are currently skewed to the markets of the rich and largely exclude the concerns of the poor. Released transgenic crop varieties are mainly suitable for North America. The growing gap between the developed and developing worlds in the rapidly evolving knowledge frontier is exacerbated by privatization of scientific research. An emerging "scientific apartheid" would further marginalize poor people.

This results in the ethical dilemma posed by conflict between two competing claims to just and fair treatment. Intellectual property protection and private sector participation in research are keys to continued technological innovations, but there is also a moral obligation to ensure that scientific research helps address the needs of poor people and safeguards the environment for future generations.

Toward New Public-Private Partnerships

The way out may lie in establishing more precise domains of intellectual property. Public goods should be left to the public, and private goods that stand at the pathway of achieving these public goods should also be treated differently from private goods that are produced by the private sector in direct relation to the end user. In the past, CGIAR research centers could access the knowledge generated by basic research and apply it to the problems of poor people, leaving the results available to all for free. Today this arrangement is seriously threatened or is no longer possible because of patenting of both processes and products.

Private sector companies should certainly have patents for the products that they develop and choose to sell. However, it becomes a serious concern if their patents prevent CGIAR centers or the national agricultural research systems (NARS) of developing countries from using the same basic scientific processes to develop products that would benefit poor people and that the private sector patent holders would not develop, precisely because of their public goods nature, that is, because the initial investment would likely not be recovered. There is an ethical question here, and not just a legal one. The answer lies not in abolition of patenting or discouraging private research. Rather, an imaginative approach is needed, one that recognizes the interest of poor people.

There are many areas of research with potential benefits to poor people that are not being carried out by the private sector. Tomatoes with a long shelf life are now available and profitable. Research remains to be done on drought-resistant millet, with a much less certain commercial payoff, but a high food security impact among poor Africans. In cases where the public sector has recognized its role and decided to invest in such "orphaned" areas of research, its work is hampered by inability to use freely basic but proprietary knowledge generated by private research.

For developing countries to benefit from biotechnology research, public sector institutions (CGIAR centers and the NARS) must develop new partnerships with the private sector and advanced research institutions, just as they develop in parallel partnerships with nongovernmental organizations and farmer associations. Some arrangements involving transfer of proprietary technologies by private companies to developing countries without royalties are already taking place. These usually involve cases where a developing country's use of the technology does not compete with use in targeted markets. For example, the Monsanto company has entered into agreements with Kenyan and Mexican agricultural research institutes on developing virus-resistant crops.

These partnerships seem to have worked well. However, they are few, highly bilateral, and components of philanthropic programs. New and more comprehensive collaboration with the private sector, while respecting IPR protection, is needed to access the process side of biotechnology for "public-goods" research in developing countries. Partnerships with legally binding agreements on sharing results have to be developed.

The international public research system has a critical role in ensuring that access to potential

benefits of new technologies are guaranteed for poor people and environmental conservation. There must be a recognition of the need for increased public involvement with biotechnology and for complementing private sector research, to ensure transparency and accountability and to promote a broad range of public goods research just as markets expand for results of private goods research. There is a need for win-win-win scenarios for all concerned actors, and for creative efforts to identify and put to work enabling mechanisms for the developing countries to benefit from the gene revolution. For the sake of today's poor, marginalized, and hungry people, and for future generations, we must not shirk this important challenge.

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ABOLISHING HUNGER

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The scientific community can play an essential role in providing the tools for humanity to satisfy its moral imperative to feed the hungry.

The first of the Millennium Development Goals, which were adopted by the world's leaders at the United Nations in 2000, was a promise to fight poverty and reduce the number of the hungry by half by 2015, from 850 million to 425 million hungry souls on this planet. Shame on us all! By 2008, the figure had actually risen to 950 million and is estimated to reach 1 billion in a few years.

It is inconceivable that there should be close to a billion people going hungry in a world as productive and interconnected as ours. In the 19th century, some people looked at slavery and said that it was monstrous and unconscionable; that it must be abolished. They were known as the abolitionists, and they were motivated not by economic self-interest but by moral outrage.

Today the condition of hunger in a world of plenty is equally monstrous and unconscionable, and it too must be abolished. We must become the new abolitionists. We must, with the same zeal and moral outrage, attack the complacency that would turn a blind eye to this silent holocaust, which causes some 40,000 hunger-related deaths every day.

As we celebrate the bicentennial of Abraham Lincoln, the founder of the U.S. National Academy of Sciences and the Great Emancipator, it behooves us to become these new abolitionists. Lincoln said that a house divided cannot stand and that a nation cannot survive half free and half slave. Today, I say a world divided cannot stand; humanity cannot continue living partly rich and mostly poor.

Our global goal should be that all people enjoy food security: reliable access to a sufficient quantity, quality, and diversity of food to sustain an active and healthy life. Most developed countries have achieved this goal through enormous advances in agricultural techniques, plant breeding, and engineering schemes for irrigation and drainage, and these advances are making a difference in developing countries as well. The Malthusian nightmare of famine checking population growth has been avoided. Global population has grown relentlessly, but many lagging societies have achieved a modicum of security that would have been unthinkable half a century ago. India, which could not feed 450 million people in 1960, is now able to provide the food energy for a billion people, plus a surplus, with essentially the same quantities of land and water.

Still, much more needs to be done. Achieving global food security will require progress in the following areas:

- Increasing production to expand the caloric output of food and feed at rates that will match or exceed the quantity and quality requirements of a growing population whose diets are changing because of rising incomes. This increase must be fast enough for prices to drop (increasing the accessibility of the available food to the world's poor) and be achieved by increasing the productivity of the small farmers in the less-developed countries so as to raise their incomes even as prices drop.
- Such productivity increases will require all available technology, including the use of biotechnology, an approach that every scientific body has deemed to be safe but is being bitterly fought by the organic food growers' lobby and various (mainly European) nongovernmental organizations.
- Climate change has increased the vulnerability of poor farmers in rain-fed areas and the populations who depend on them. Special attention must be given to the production of more drought-resistant, saline-resistant, and less-thirsty plants for the production of food and feed staples.
- Additional research is needed to develop techniques to decrease post-harvest losses, increase storability and transportability, and increase the nutritional content of popular foods through biofortification.
- Biofuels should not be allowed to compete for the same land and water that produce food for humans and feed for their livestock. We simply cannot burn the food of the poor to drive the cars of the rich. We need to develop a new generation of biofuels, using cellulosic grasses in rain-fed marginal lands, algae in the sea, or other renewable sources that do not divert food and feed products for fuel production.
- Because it is impractical to seek food self-sufficiency for every country, we need to maintain a fair international trading system that allows access to food and provides some damping of sudden spikes in the prices of internationally traded food and feed crops.
- The scientific, medical, and academic communities must lead a public education campaign about food security and sound eating habits. Just as we have a global antismoking campaign, we need a global healthy food initiative.
- And we need to convince governments to maintain buffer stocks and make available enough food for humanitarian assistance, which will inevitably continue to be needed in various hot spots around the world.

New technologies to the rescue

No single action is going to help us solve all the problems of world hunger. But several paths are open to us to achieve noticeable change within a five-year horizon. Many policy actions are already well understood and require only the will to pursue them. But there are a few more actions that will become effective only when combined with the development of new technologies that are almost within our grasp. Critical advances in the areas of land, water, plants, and aquatic resources will enable us to take a variety of actions that can help put us back on track to significantly reduce hunger in a few short years.

Land. Agriculture is the largest claimant of land from nature. Humans have slashed and burned millions of hectares of forest to clear land for farming. Sadly, because of poor stewardship, much of our farmland is losing topsoil, and prime lands are being degraded. Pressure is mounting to further expand agricultural acreage, which means further loss of biodiversity due to loss of habitat. We must resist such pressure and try to protect the tropical rainforests in Latin America, Africa, and Asia. This set of problems also calls for scientists to:

- Rapidly deploy systematic efforts to collect and classify all types of plant species and use DNA fingerprinting for taxonomic classification. Add these to the global seed/gene banks and find ways to store and share these resources.
- Use satellite imagery to classify soils and monitor soil conditions (including moisture) and launch early warning campaigns where needed.
- For the longer term, conduct more research to understand the organic nature of soil fertility, not just its chemical fertilizer needs.

Water. Water is life. Humans may need to consume a few liters of water per day for their survival and maybe another 50 to 100 liters for their well-being, but they consume on average about 2,700 liters per day for the food they consume: approximately one liter per calorie, and more for those whose diet is rich in animal proteins, especially red meat. At present, it takes about 1,200 tons of water to produce a ton of wheat, and 2,000 to 5,000 tons of water to produce a ton of rice. Rainfall is also likely to become more erratic in the tropical and subtropical zones where the vast majority of poor humanity lives. Floods alternating with droughts will devastate some of the poorest farmers, who do not have the wherewithal to withstand a bad season. We absolutely must produce “more crop per drop.” Some of what needs to be done can be accomplished with simple techniques such as land leveling and better management of irrigation and drainage, but we will also need plants that are better suited to the climate conditions we expect to see in the future. Much can be done with existing knowledge and techniques, but we will be even more successful if we make progress in four critical research areas:

- First, we know hardly anything about groundwater. New technologies can now map groundwater reservoirs with satellite imagery. It is imperative that an international mapping of locations and extent of water aquifers be undertaken. New analysis of groundwater potential is badly needed, as it is likely that as much as 10% of the world’s grain is grown with water withdrawals that exceed the recharge rate of the underground reservoirs on which they draw.
- Second, the effects of climate change are likely to be problematic, but global models are of little help to guide local action. Thus, it is necessary to develop regional modeling for local action. Scientists agree on the need for these models to complement the global models and to assist in the design of proper water strategies at the regional and local scales, where projects are ultimately designed.
- Third, we need to recycle and reuse water, especially for peri-urban agriculture that produces high-value fruits and vegetables. New technologies to reduce the cost of recycling must be moved rapidly from lab to market. Decision-makers can encourage accelerated private-sector development programs with promises of buy-back at reliable prices.

- Finally, the desalination of seawater, not in quantities capable of supporting all current agriculture, but adequate to support urban domestic and industrial use, as well as hydroponics and peri-urban agriculture, is possible and important.

Plants. Climate change is predicted to reduce yields unless we engineer plants specifically for the upcoming challenges. We will need a major transformation of existing plants to be more resistant to heat, salinity, and drought and to reach maturity during shorter growing seasons. Research can also improve the nutritional qualities of food crops, as was done to increase the vitamin A content of rice. More high-risk research also deserves support. For example, exploring the biochemical pathways in the mangrove that enable it to thrive in salty water could open the possibility of adding this capability to other plants.

Too much research has focused on the study of individual crops and the development of large monoculture facilities, and this has led to practices with significant environmental and social costs. Research support should be redirected to a massive push for plants that thrive in the tropics and subtropical areas and the arid and semiarid zones. We need to focus on the farming systems that are suited to the complex ecological systems of small farmers in poor countries.

This kind of research should be treated as an international public good, supported with public funding and with the results made freely available to the poor. Such an investment will reduce the need for humanitarian assistance later on.

Aquatic resources. In almost every aspect of food production, we are farmers, except in aquatic resources, where we are still hunter-gatherers. In the 19th century, hunters almost wiped out the buffaloes from the Great Plains of the United States. Today, we have overfished all the marine fisheries in the world, as we focused our efforts on developing ever more efficient and destructive hunting techniques. We now deploy huge factory ships that can stay at sea for months at a time, reducing some species to commercial extinction.

We need to invest in the nascent technologies of fish farming. There is some effort being made to promote the farming of tilapia, sometimes called the aquatic chicken. In addition, integrating some aquaculture into the standard cropping techniques of small farmers has proven to be ecologically and economically viable. The private sector has invested in some high-value products such as salmon and shrimp. But aquaculture is still in its infancy compared to other areas of food production. A massive international program is called for.

Marine organisms reproduce very quickly and in very large numbers, but the scientific farming of marine resources is almost nonexistent. Proper farming systems can be devised that will be able to provide cheap and healthy proteins for a growing population. About half the global population lives near the sea. Given the billions that have gone into subsidizing commercial fishing fleets, it is inconceivable that no priority has been given to this kind of highly promising research. Decisionmakers must address that need today.

Science has been able to eke out of the green plants a system of food production that is capable of supporting the planet's human population. It is not beyond the ken of scientists to ensure that the bounty of that production system is translated into food for the most needy and most vulnerable of the human family.

Science, technology, and innovation have produced an endless string of advances that have benefited humanity. It is time that we turn that ingenuity and creativity to address the severe ecological challenges ahead and to ensure that all people have that most basic of human rights, the right to food security.

Most of the necessary scientific knowledge already exists, and many of the technologies are on the verge of becoming deployable. It is possible to transform how we produce and distribute the bounty of this earth. It is possible to use our resources in a sustainable fashion. It is possible to abolish hunger in our lifetime, and we need to do so for our common humanity.

Ismail Serageldin (ismail.serageldin@bibalex.org) is the director of Egypt's Library of Alexandria and the former chairman of the Consultative Group on International Agricultural Research.

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