CRISTIAN FURĂU

EGON DICZFALUSY

90 years for HUMANITY *through* SCIENCE

Empathy, Science, Hope

„Vasile Goldiș" University Press
Arad, 2010

EGON DICZFALUSY

90 years for HUMANITY

through

SCIENCE

"Fate il bene e fatelo bene"

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Descrierea CIP a Bibliotecii Naţionale a României
FURĂU, CRISTIAN
Egon Diczfalusy: 90 years for humanity through science/ Furău Cristian-George. – Arad: „Vssile Goldiş" University Press, 2010
Bibliogr.
ISBN 978-973-664-428-3

61 (485) Diczfalusy, E
929 Diczfalusy, E.

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Instead of introduction

I have started this journey to find out who Professor Dr Egon Diczfalusy really is. I wanted to place him in a different light, other than the eminent man of science the scientific community knew him as, reflecting more his human qualities and the achievements of creating a school of life and science.

Once upon a time, on a rainy autumn day, a medical student attending a scientific workshop met a simple and modest smiling old man; he turned out to be the great Professor Emeritus Dr Egon Diczfalusy.

A year later, the same medical student and his father were invited to the first meeting of the Egon &Ann Diczfalusy Foundation held at Tisza Hotel in Szeged. They felt like Alice in Wonderland as they made acquaintance with great professors about whom they read in medical textbooks. By the time the Foundation had its next meeting, the young doctor became more and more familiar with Egon's world and friends.

Soon after that the West University "Vasile Goldiş" of Arad awarded Professor Diczfalusy the title Doctor Honoris Causa. Travelling together from Budapest to Arad the young doctor suggested to the professor a very informal meeting with his friends from Rotaract. For them it was so surprising to see the young spirit of the old man so open to them, speaking the languages of their choice, literally. The young doctor really felt he became Egon's friend as he joined him the next day for the Symposium in Serbia.

The next lesson Egon taught his younger friend was the connection between art and medicine. Going to Syracuse to see an ancient Greek drama we also went to the roots of the Medical School. As our flights needed a connection from Munich to Catania we had plenty of time for stories, with half-full glasses of German beer (always the optimistic approach) in front of us; and then the idea of writing a different kind of Egon's biography appeared to me. I shared the idea with him and we discussed it during the wonderful weekend spent in Syracuse in the great company of Professor Salvatore "Ninni" Mancuso's family. Those magnificent days for which I think we can't thank "Ninni" and Egon enough, we had the opportunity to see for the very first time a live and very good representation of ancient Greek drama, we spent wonderful moments in the middle of our 'adoptive Italian family' (many thanks), we got to taste the best Sicilian food and even better, to taste their best wines. On our return, I planned with Egon the trip to Rönninge for the interview.

The interview took place in Rönninge in *"a very old- around a hundred years old- big wooden house, of yellow color, situated close to the shore of the lake "Flaten")* " where Egon had been living for the last 60 years. He welcomed me with a great smile and kind words. The hours ran rapidly as they were filled with memories told in his modest, simple and open-minded own way. We had small breaks to relax, walking in the forests where he used to walk almost daily with his wife Ann and to enjoy the great meals we cooked together… Thinking back it seems that there, in the backyard of his wooden house, time just stops now and then. Maybe that's the secret of his youth.

Having the records and the papers he gave me in my hands I wondered how I could turn them into a biographical book that Egon would

like to offer as a present to his friends. That's the reason I contacted more of Egon's friends to help me with my little project, so that in the end we would have a book written by friends for other friends and for the younger generation. I felt that the generation I belong to, the young generation of today, should have the opportunity to know a bit more about the outstanding Medicine's Superstar who lives among us, so much for the others, so modest, so simple …

I tried to comprise in the book the very complex personality Egon is. Therefore I presented the well-known professor, the eminent scientist, the man devoted to his family, the man passionate of art, the creator of a global school through the eyes of his friends.

Professor Diczfalusy is a well-known member of the international scientific community. The reprints of the "Identification of the human feto-placental unit" and the "Laudatio" written by Professor Giuseppe "Pino" Benagiano for the 80th birthday of Egon are best suited to present his international reputation. I was very pleased to include the words of acknowledgement addressed by the rector of the West University "Vasile Goldiş" from Arad.

Professor Diczfalusy's scientific activity is well illustrated in his overwhelming Curriculum Vitae. Part of his great research activity is presented in the series of republished articles that cover his work in the field of steroid hormones, reproductive health and aging problems. Revealing some other scientific research we republished the story of Limoncello Society.

The words and characterization of Egon by former students and close friends reflect the global dimension of the school he created.

How could one better present Egon than through his own words - the words of wisdom from Rönninge that you can find on the enclosed DVD with representative pictures from his life.

I would like to thank very much all of the people that helped me in this project. Many thanks especially to Egon who was always there with encouragements, good advices and support, to Wanda - Egon's step daughter who worked incredibly to gather all the emails of the former students, to Professor Giuseppe "Pino" Benagiano, to all former students and friends of Professor Diczfalusy who managed to write a few words for this book. I would like especially to thank my family for all their support and help, my students who worked a lot at this project too, my friends in Rotaract Cetate Club Arad. Hopefully I didn't forget anyone.

I found that the journey to discover Egon led me back to myself, a better self!

I wish you an enjoyable lecture and to you Egon, with my best thoughts for health and all my best wishes, an honest "HAPPY 90th BIRTHDAY"!

Cristian Furău

Picture 2: I front of Egon's big yellow old house from Rönninge

Picture 3: Egon, "Nini" Mancuso's family, my father and me waiting for Euripide's "Aiax" to start

A life dedicated to science

To bind into a book the dynamic life of such a man is not a task to be taken lightly. Our subjectivity can alter reality, or on the contrary, by over reasoning, dismiss it. However, to talk about a man of action, words have the power to put in value truths which yield prestige to one's personality, and for this I appreciate the efforts of our young colleague, Dr. Cristian Furău, whose task is to coordinate such a breathtaking biography.

The West University "Vasile Goldiş" of Arad – which bears the name of the prominent Romanian patriot - Vasile Goldiş – an institution of higher learning which has celebrated this year 20 years of existence and, in its short journey has become an active component in the European Community in both science and academic learning; member with full rights of the European University Association and the European Federation of European Schools. The efficiency of the university's employees has been recognized by its peers in the Romanian educational system, and there is no surprise that our university has received the highest grade a university in the Romanian system can get.

The West University "Vasile Goldiş" of Arad which I represent with pride and of which I am the founding rector, has had the honor of according the distinction of Doctor Honoris Causa to the distinguished Professor Dr. Egon Diczfalusy on the 2nd of December 2009, one day after we, Romanians celebrated our 91st National Day.

Besides his prodigious scientific activity, Professor Egon Diczfalusy has conducted a laborious didactic activity and a remarking humanitarian service. In other words, to make a short résumé of the professor's activity, I would like to mention that he is a professor of the Karolinska Institute,

author of many scientific works recognized internationally (over 630 titles), the creator of the concept of feto-placental unit, ex-director of four WHO research programs, counselor in the WHO program on aging and reproductive health, Doctor Honoris Causa in many prestigious universities and member of honor in many societies and scientific associations. For his scientific activity he has been rewarded with numerous distinctions and awards.

The interesting monography named, *„Egon Diczfalusy- 90 years for humanity through science"* of our Assistant Dr. Cristian Furău brings an eulogy to one of the greatest medical personalities to whom the University "Vasile Goldiş" of Arad has ever awarded the distinction of Doctor Honoris Causa; to the esteemed professor, Dr. Egon Diczfalusy.

It is a work of intricately elaborated and of tremendous prestige, extremely useful through its content, addressing to a large range of readers, from various fields of activity, which were touched by the personality of professor Diczfalusy. It is a great honor for our university to bring this homage to the distinguished Man, Egon Diczfalusy is, who, on the 19th of September this year, will celebrate the 90th anniversary.

The work elaborated entirely in English, in a concise and clear way, its rich content and the multitude of sources, takes the matter and deals with it in an elevated manner through competence in redacting and assembling the data, trying by this to surprise with the sheer complexity of Professor Diczfalusy's personality.

Professor Dr Aurel Ardelean,
Member of the Medical Science Academy of Romania,
Rector of West University "Vasile Goldiş" from Arad

Laudatio (2000)
PRESENTING PROFESSOR EGON DICZFALUSY ON HIS 80TH BIRTHDAY

Giuseppe Benagiano
Director general, Istituto Superiore di Sanità, Roma, Italy

To retrace the life and many accomplishments of Egon Diczfalusy on the occasion of his 80th birthday is at the same time a very simple and an almost impossible task. It is simple because I have been associated with him for more than thirty even years and because I am talking to his closest friends and associates: most of you have known him for years, have shared good and perhaps even bad moments with him and are well acquainted with the milestones in his life. It is a very complex, almost impossible endeavour, since his personality has so many facets that it is very hard to do him justice outlining all of them, even when you have know him for so long.

For this reason, while - on the one hand - I cannot avoid sketching the composite picture of the man and scientist, of the person dedicated to the progress of humanity and to its liberation from all kinds of evils - on the other - I must try to give you a sort of "inside" look into the more personal aspects of his life. In order to do so, I am afraid that, in presenting him, instead of a stuffy *Laudatio*, I will provide you with a slightly irreverent, although most affectionate description of a man who played such a unique role in my life to be second only to my own father.

Egon Diczfalusy's life can probably be said to comprehend four major epochs: the first, starting in his native Hungary and ending in 1946

when he, later followed by his mother, moved to Sweden. The second, comprising his *heroic* days in Sweden - the most productive part of his life - when the *foeto-placental unit* was discovered and carefully researched. The third, spanning over some 25 years and beginning when he, together with Alex Kessler and with the help of the Ford Foundation, conceived the *WHO Expanded Programme of Research in Human Reproduction*, which later developed into the *Special Programme of Research, Development and Research Training in Human Reproduction* (the mythical HRP), co-sponsored by UNDP, UNFPA, WHO and the World Bank; and the fourth reaching to our days when, as he himself aged, he turned his attention to an ageing humanity, quickly becoming - typical of the way he proceeds - a real expert.

Of his "first life", which I will name the Hungarian time, we all know but little; however, some of the stories we heard in Stockholm some thirty-five years ago, provide intriguing flashes into the life of the Hungarian military establishment to which his family belonged, thanks to his father, a General, pictured with Egon's grandfather and uncle in Figure 2. It was the time in between the two World Wars, a special period for Hungary, that of independence from Austria.

Two little anecdotes, which he told us many years ago and which I hope he still remembers: the first is the story of an officer who took the Orient-Express, all the way to Istanbul to buy flowers for the Lady he had just met and fell in love with; the second is the story of another officer who became a regular guest at lunch, each day for a month in his paternal house, the reason being that he had spent all his salary to celebrate in a café - inviting all those who happened to be present - his beautiful new love!

It was this cultural milieu which helped forge his personality in his native Miskolc, a small city in north eastern Hungary nd subsequently during his medical school days at the Semmelweiss University in Szeged in the very south of Hungary, where he graduated with *summa cum laude*. An italian proverb recites "if you start well, you are already mid-way...".

Less than 2 years later he moved to Sweden where the second phase of his life started, the one I will call the Swedish time. His first teacher in his new homeland, back in 1946 was a Nobel laureate, Prof. Hans Von Euler, whom Egon knew for a very specific reason, as I will tell you in a minute.

He soon moved to *Kvinnokliniken*, the Department of Women's diseases, under the direction of Prof. Axel Westman (See Figure 4b), where in 1953, he completed his thesis on *Chorionic Gonadotropin and Oetrogens in the Human Placenta* (1), to become *Dozent* (the equivalent of Ph.D.) and at the same time, Associate Professor of Experimental Endocrinology. From there on the main focus of his scientific work remained the hormones involved in human reproduction.

When I met Egon Diczfalusy, in 1963, he had just been awarded one of the largest Ford Foundation Grants in Reproductive Endocrinology of those days: one half million dollars to study *steroid biogenesis and metabolism in the human foetus, placenta and maternal organism*.

Through hard work and fighting difficult conditions (a foreigner has always difficulties in adjusting to a new country) he had become the *Laborator*, or Head, of the *Hormonlaboratoriet* at the *Karolinska Sjukhuset*, or Karolinska Hospital.

When, in 1978, Egon recalled the early days of Reproductive endocrinology in giving the Sir Henry Dale Lecture, he called this period

the merry post-war days. There is one sentence in this Lecture that I must recall for you, because it contains a lot of insight into his early life. Egon wrote: *How did I become a reproductive endocrinologist? By the Hungarian approach. As a second year medical student at the University of Szeged, Hungary, I was working in the Department of Pathology and Bacteriology of Professor Gyorgyi Iványovics and my first task was to repeat a study by the Nobel laureate, Hans von Euler and his co-workers in Stockholm, who found transaminase activity in suspensions of yeast and* E. coli *bacilli. I just could not confirm their findings, and my Professor felt that I must publish this. This negative report was my first publication; it was probably instrumental in bringing me to Stockholm after the war, when I had the privilege to work as Professor's Hans von Euler's assistant during the years 1946-1947* (2).

As you can see everything fits!

The old *Hormonlaboratoriet*, as well as the new one, havebecome famous for the number of young (and not so young) visiting scientists who trained there from all over the world: Diczfalusy's own *curriculum* states that some 150 scientists trained at Karolinska under him, a good half coming from developing countries. A couple of dozens, including two of his Italian graduate students, eventually became Chairmen of University Departments scattered in the four corners of the world.

Those were hectic days, hard work, but also great satisfaction. I remember the first time we all went to an International Meeting in Hamburg, an *Acta Endocrinologica* Congress. One of my friends from Italy had the venture to be the first of the group to give his paper (3); at the end he went to see Egon to receive his comments and all Egon said was: *Sergio, you better improve your English, if you want to ever present a paper again*;

no kidding! With this preamble, I still remember that I did not sleep that night; I swore to myself I would learn English and learn it well and, to achieve this, I even married an English-speaking lady!

I want to take this unique opportunity to reveal a little secret kept for 35 years. You may laugh at it, but at the time this little joke was considered "desacrating". The inside of the laboratory's toilet bore a small sign that read: *Efter toalet besöket, alltid twätta händerna*, that is *After visiting the toilet always remember to wash your hands*. But one terrible day the "h" in the word "*händerna*", *"the hands*", had been covered with medical white tape and the word now read "*änderna*", that is "*your ass*". In reality it was plural (your asses), bad swedish, perhaps, but, although perfectly understandable! Egon was furious and wanted to know who had dared to be so impertinent: Well Egon, I am afraid it was your demure, studious-looking, young Italian pupil, Pino Benagiano. I hope you are now ready to pardon me.

As I have mentioned, Diczfalusy's scientific production started in 1942 when he was a second year medical student (4); his first paper dealing with steroids appeared in 1948 (5). Today his production amounts to more than 620 publications; he is the editor of some 30 books and has been the series editor of the *Karolinska Symposia on Research Methods in Reproductive Endocrinology*, published between 1969 and 1975 and distributed to ten thousand people and of *Vitamines and Hormones* between 1970 and 1982. In this prolific activity, the years of the definition of the foeto-placental unit and of its meaning where certainly the most productive and Diczfalusy's papers became a landmark in *Acta Endocrinologica*. To symbolize this epoch I wish to remember one paper, *OEstriol metabolism at midpregnancy* (6), for two reasons: first, because my name appears next to

his and, second, because it deals with the metabolism of the one steroid that really requires the foeto-placental unit to be synthesized: OEstriol.

Notwithstanding the vast number of papers produced in those days, he personally typed each first draft on a little typewriter in his office, re-writing each sentence until he was satisfied that *all the data had been put on paper*.

In spite of being very exigent, he really cared for all of us; he was sort of jealous of letting us go our own ways. When - after receiving a good offer from the Population Council in New York - I decided to leave, he summoned me to his office and blunt1y asked me: *are you going because you will get a better salary, or because you like the type of research better, or because you will be able to do better politicking?* And I smiled innocently and said: "But of course all the three Sir!". I left because I had to leave, but I left my heart there (if you want the whole truth, both in terms of scientific interest and in terms of sentimental attachment). For these two reasons I kept in close contact with him, the laboratory and .... Sverige. So did many others and the *Swedish Mafia* that he created there, is - to this day - a solid group of friend scientists who learned at a severe and demanding school and who spread this new creed all over the world.

The "third life" of Egon Diczalusy, which I will call the Swiss connection, brought him the world dimension and perspective for which he is famous today: I am sure the seven languages he can speak easily helped him in dealing with WHO; however it is his personality, his knowledge and his dedication that made him indispensable to four consecutive Human Reproduction Programme Directors there, as *Senior Consultant*. Since then he has been on every major Task Force Steering Committee, while at the same time heading the WHO Collaborating Centre in Research and

Research Training in Stockholm, travelling on behalf of the Organization more than any other person I know and participating in more conferences and Workshops than probably any one in this audience.

During 25 years his advice to the Programme has been invaluable and his knowledge of the work carried out by it unsurpassed. For this reason when Josè Barzelatto decided that the Programme's first 15 years should be celebrated with a document summarizing the many accomplishments made, he turned to Egon Diczfalusy, who took the task with enthusiasm and thoroughness. I will only quote one sentence which summarizes his view on the role of international cooperation: "*A modern interpretation of history is said to be based on the analysis of the history of ideas. The history of the second part of the twentieth century represents an entirely new departure in this respect; for the first time in the history of mankind the policies emerging from World Conferences organized by the various Specialized Agencies of the United Nations broadened the views and perceptions of many Member States (including donor governments) and significantly influenced their policies*" (7).

It is also thanks to his indefatigable efforts that we were able to celebrate the Programme's 25th birthday in 1997 in Szeged, his home city (8). Summarizing these first 25 years was in fact my last duty as Director of the Special Programme, although, I had actually formally resigned three months earlier to return to Rome. In opening the celebrations, Egon challenged all of us by saying: *I myself represent the future. Yes, you heard right, I said the future. I represent the future of the past. As Paul Valéry puts it: "Are you not the future of all memories stored within you? The future of the past"* (9). And I want this to be the motto of this Symposium: we are here to celebrate above all the future of the past.

The celebration of the 25th anniversary of HRP was uniquely important to me, not only as my swan's song as HRP director, but also because László Kóvacs and his collaborators concocted a special celebration for my 60th birthday. I can only dream of what the celebration for my 80th birthday will be with Egon giving the *Laudatio*, although, with my 5 bypass, I should - as Egon often says - minimize my expectations: we shall see!

His work with WHO brought him more and more often to Geneva, where he sort of took-up residence in my apartment. Those were pleasant and busy days and we travelled together many times, often fighting over being late for planes: I remember once when we went to Palo Alto, renting a car at S. Francisco Airport. About two and a half hours before the plane's departure he became uneasy and wanted to leave. I reassured him that by taking the Junipero Sierra Freeway and speeding-up a little bit we could finish our business and still be at the airport half an hour before departure, but he was not satisfied. At the end I gave-up and we left, arriving at the Airport fairly early (at least by my standards); so I told him: "do you see? we are here 40 minutes before departure"; he looked at me scornfully and said: "don't you know that the gate opens 50 minutes before departure?". In this sense Egon should have married my wife and together they could happily go and....as I say, open-up the Airport in the morning.

As a little song of the Wolf Cubs goes: "Everything ends in this world" and even the HRP days ended for Egon. When he turned 75, Dario Sanvincenti the Director of Personnel informed me that he was forced to stop the Consultancy agreement with Egon Diczfalusy. This, however, would not preclude his working as an Advisor, and - of course - that's exactly what he did. With one caveat: he started advising the WHO Programme on Ageing, as well as the Rockefeller Foundation.

In a way, I am glad Sanvincenti did what he did, because it helped this "young-75 years old" to start a new *carrière* and the fourth and present phase in his life, which I will call the World time, not because he wasn't in the world arena before; rather, because he now is truly the epitomy of a world citizen.

As a matter of fact, I was among the first to benefit from this new phase of his life: FIGO, the International Federation of Gynecology and Obstetrics, had asked me to write a chapter for the IInd World Report on Women's Health and gave me *women's longevity* as the theme (10). I was buried in work and had no way to amass all the evidence, all the data necessary to write something meaningful. So I turned to Egon who, basically, sent me a ready to print manuscript to which I had little to add, except my name, of course.

I will only quote a couple of sentences from his latest papers, because they are recent and because his thinking in this field is still evolving. I will tell you more about it when we meet in ten years' time!

Last years, in a main article in the IJGO he wrote: "*The wind of new realities is blowing with increasing strength. It is up to us to decide whether we prefer protective windscreens or new types of windmills*" (11). Clearly, Egon has never cherished protective windscreens; he has always been out where "the action is".

His life has been dedicated to achieve what - and, again I quote from one of his latest papers - Arnold Toynbee remarked: "*Our time is the first since the dawn of civilization in which people have dared to think it practicable to make the benefits of civilization available to the whole human race*" (12). Indeed, as a faithful pupil, I have been saying that the true challenge for public health in the 21st century is not to apply the knowledge

arising from sequencing the human genome or from other frontier discoveries; rather it is to provide to the entire human race the kind of health care already available today to a privileged small minority.

The real challenge is - to use the Latin sentence with which he closed another leading article published this year - to do so "*in nomine dignitatis, scientiae et charitatis*" (13), *in the name of human dignity, science and love*.

Well, I was given only 30 minutes and therefore must finish this *Laudatio*. I can only do so apologizing for not having been able to do Egon justice, to provide you with a full picture of the man we are honouring here today; but that would have - of course - been simply impossible. I do hope, though, to have given you an idea not only of his achievements, of his dedication to the world of science, as well as to human suffering; not only an idea of how much he cared for his pupils, as well as for every couple on the earth needing help to cope with their family problems; but also an idea of the man behind the scientist, of the master and the friend of a lifetime. And allow me to paraphrase what the American Magazine Life said about the Romans in a famous series published over 20 years ago (14), and say: I tried to give you an idea of the man who shaped and forever changed my life.

There is, however, one very *last* (but certainly not *least*) mention I must make; in Italy we say: behind every great man there is a great woman. Nothing could be more true: Ann's influence on Egon has been enormous; more often than not, trying to calm him down, and God only knows if he needed it! She was the point of reference to always go back to; caring for children and grand children; withstanding drama and even tragedy, but

always there, whenever she was needed. Thanks Ann, for taking on the impossible job: to be his wife!

And it is with affection and pride that I conclude by way of a simple statement by Leonardo da Vinci: *tristo è quel discepolo che non supera il suo maestro*, that is: *Wicked is the disciple who does not excel over his master*. Well, Egon, *old fellah*, this statement has always been a thorn in my flesh. Here I have miserably failed; as a matter of fact, we all failed, since no one among us has been able to excel over you.

On my part I, I wish you the classic "one hundred of these birthdays" and at the same time I can only swear that I will continue to try, but with little hope, to at least come close to my friend and mentor, Egon Diczfalusy, Professor Emeritus at Karolinska Institutet in Stockholm.

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LAUDATIO (2010)
Egon Diczfalusy and the Identification of the Human Feto-Placental

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In September 2000 I was invited to Berlin where Schering AG had organised a Conference to celebrate Professor Egon Diczfalusy's 80th birthday and – in the best German tradition – they asked me to give a ***Laudatio*** in his honour [01]. I remember that, at the time I told the audience that I had already volunteered to give his 100th birthday *Laudatio* again in Berlin. Although history moved on and Schering AG no longer exists, having been absorbed into Bayer AG, I seem to be half way to maintain my promise, having now been asked to recount Diczfalusy's life achievements on the occasion of his 90th birthday, on 19 September 2010. I accepted with pleasure as I feel that an uninterrupted association and close friendship with him during almost fifty years gives me a unique opportunity to provide a picture of the man, the scientist, the humanitarian, the visionary, the philosopher, with a degree of accuracy quenched only by the bias of affection.

At the same time, to be meaningful a *Laudatio* must be focused and, for this reason, after a brief account of his early personal life, I shall concentrate on a description of the work that gave Egon Diczfalusy international fame: the definition of the concept of the Human Feto-

placental Unit. This will be followed by a few words on his many and important subsequent achievements.

Egon Diczfalusy was born in Miskolc, a small city in north-eastern Hungary on 19 September 1920; his father was a member of the new Hungarian army, created after the dissolution of the Austro-Hungarian Empire and eventually became a General. When he was 16 his family moved to the southern city of Szeged where he graduated *summa cum laude*, in Medicine from the Semmelweis University. As a second year medical student in that University he worked as an intern in the Department of Pathology and Bacteriology where his research project consisted in duplicating a study carried out at the *Stockholms Högskolan* (the name with which the University of Stockholm was known in those days) by the group led Professor Hans von Euler, a Nobel laureate. This group had published that suspensions of *E. coli* possessed transaminase activity. Intriguingly, young Diczfalusy could not confirm these findings, and this negative report became his first publication [02]. After the war, the issue of transaminase activity in bacteria was instrumental in stimulating him to go to Stockholm where, during almost two years, he worked as Professor's Hans von Euler's assistant.

In 1947 Prof. Axel Westman, head of the Department of Obstetrics and Gynaecology at the Karolinska Hospital in Stockholm offered him a full-time position in the Hormone Laboratory of his clinic. This event changed his scientific career and life with the focus shifting to reproduction, a field he remained firmly attached to until the present days. In 1953, he completed his thesis on *Chorionic Gonadotropin and Œstrogens in the Human Placenta* [03] and, from there on, the main

focus of his scientific work remained the hormones involved in human reproduction.

By 1948 Egon Diczfalusy had become the head of the Hormone Laboratory and, over the following decade, under his leadership the laboratory became one of the leading institution in the world on steroid hormone biogenesis and metabolism, to the point that in 1963 he received one of the largest Ford Foundation Grants in Reproductive Endocrinology of those days: one half million dollars to study *Steroid biogenesis and metabolism in the human fetus, placenta and maternal organism.*

I joined the "Stockholm Group" in 1964, right after the Grant was awarded and became one of the first Ford Foundation Fellows in Reproductive Endocrinology. This gave me the opportunity to be part of team that investigated and unravelled the intricacies of the Human Feto-placental Unit, defining the new concept. It is for this reason that I am able to provide a first-hand report of those incredible days.

Everything started from the recognition that the fetus and the placenta exert endocrine functions, a discovery that goes back to the beginning of the XXth century, [04]. The classical concept postulated that, during gestation, a "temporary endocrine organ", the placenta, carried out most of the greatly increased steroid biosynthesis at mid-gestation. At the same time, it was Diczfalusy who, much later, recognised the close inter-relationships between the fetus and the placenta [05]. He was the first to understand that the situation was much more complicated and that certain steroids present in the urine of pregnant women represent mainly metabolites of placental origin, whereas others seem mainly of

maternal production, with others yet resulting from a joint activity by the fetus and the placenta

Starting from these premises, Diczfalusy and his group developed a new concept: the creation during pregnancy of a functional unit made-up of an incomplete steroidogenetic organ (***the placenta***), interposed between a complete steroid metabolic system (***the maternal organism***) and a second incomplete system (***the fetus***). The unique characteristic of the latter is its ability to compensate for the deficiencies of placental enzyme systems.

In 1964, Diczfalusy enunciated the new concept [06]: the placenta and the fetus both lack certain enzymes which are essential for steroidogenesis; however, the enzymes that the placenta lacks, are present in the fetus and, *vice versa*, those absent from the fetal organism are active in the placenta. Thus, the integration of the two compartments allows the elaboration of most, if not all, biologically active steroids. It must be stressed that we still do not have full information on the situation at term, although – even in this case – Diczfalusy and other groups have collected important data indicating that the situation at term does not differ from the one at mid-pregnancy [07-10],.

Diczfalusy's work dealt with the full range of metabolic pathways leading to biologically active steroid hormones, starting with de-novo synthesis. His group was able to demonstrate that – contrary to earlier results [11-12] – when ^{14}C-labelled sodium acetate, is perfused into isolated placentas, little, if any, labelled cholesterol can be isolated from the placental tissue [13] and that all steroids isolated from the placenta and perfusates were devoid of any 14C-label [14]. Finally, when complete feto-placental units were perfused with ^{14}C-labelled sodium

acetate, no ^{14}C-labelled cholesterol was isolated from the placentas, although an abundant conversion to cholesterol was demonstrated in several fetal tissues [15].

In contradistinction to the placental situation, the group of Diczfalusy demonstrated that, following perfusion of ^{14}C-labelled sodium acetate into pre-viable human fetuses, considerable incorporation of acetate occurred, not only into cholesterol, [15] but also into a variety of steroids [13, 16]. The liver and adrenals formed large amounts of cholesterol and cholesterol was isolated from all perfusates, indicating that the mid-gestation fetus utilises part of the newly synthesized cholesterol for its own needs, while it secretes some of it to the placenta.

The Stockholm group also extensively investigated the side chain cleavage leading from cholesterol to pregnenolone and discovered that this metabolic step can take place in several fetal tissues [16, 17] and is especially active in the placenta [18], although the subsequent step leading from pregnenolone to progesterone, is extremely limited in the fetal organism, occurring mostly, if not exclusively, in the placenta [18] from circulating cholesterol, mainly of maternal origin [19]. The placenta then secretes pregnenolone both to the mother and to the fetus, where it is extensively metabolised by various tissues [18, 20]. The placental contribution to fetal pregnenolone synthesis is however limited: the major part of fetal pregnenolone is formed by de novo synthesis and immediately sulphurylated; [17, 21]. Worth of mention is the fact that the placenta does not sulphurylate pregnenolone, although it is capable of hydrolysing it. The quantitatively most important metabolic product of pregnenolone sulphate in the fetal organism is dehydroepiandrosterone sulphate (DEAS) formed by the adrenals [21].

A unique feature of the work of Diczfalusy and his group is the study of the fate of progesterone reaching the adrenals; they discovered that this typical pregnancy steroid undergoes a variety of hydroxylation reactions and, in this way, fetal adrenals can produce, all the biologically important corticosteroids, including deoxy-corticosterone, corticosterone, cortisol, and aldosterone [18, 22, 23]. Since the placenta is not capable of carrying out these hydroxylation reactions [20], it follows that the placental contribution to corticosteroid synthesis is restricted to the conversion of the hydroxylated 3ß-hydroxy-Δ^5-steroids of fetal origin to the corresponding α-ß-unsaturated 3-ketosteroids. Indeed, Diczfalusy's group has shown that the fetal adrenals can convert pregnenolone to 21-hydroxypregnenolone and that the perfused placenta can convert large quantities of the latter to deoxycorticosterone [24]. Many of the corticosteroids are rapidly sulphurylated by the fetus at carbon atom 21; these sulphates however are not metabolised to any major extent by fetal tissues [25].

Another very important discovery made by the Stockholm group deals with the metabolic fate of DHEAS, the most important steroid secreted by fetal adrenals. They started with the observation that a series of androgens are produced by the fetus and the placenta, including androstenedione and testosterone [16, 17, 26, 278] although by different metabolic pathways [28, 29]. In the fetal liver and gastro-intestinal tract, androstenedione and testosterone are freely interconverted, whereas in all other tissues more testosterone is converted to androstenedione than vice versa [30]. Fetal adrenals transform androstenedione and tetosterone into 11ß-hydroxylated forms and also convert testosterone to testosterone sulphate [30]. Androstenedione and testosterone are

actively metabolised by fetal tissues and testosterone is rapidly sulphurylated [31]; this steroid is then transferred to the mother without hydrolysis [32].

The bulk of the large quantities of DHEAS circulating in the fetus reaches the placenta with the umbilical circulation and is rapidly converted to oestrone and oestradiol (but – strikingly – not to oestriol) through a series of reactions involving first the hydrolysis of DHEAS. One pathway involves the direct conversion of DHEA to androstenedione, followed by aromatisation to oestrone [18]; another involves the direct conversion of testosterone to oestradiol [31]. Thus, DHEAS of fetal and maternal origin represents the most important precursor of placental oestrone, and oestradiol [33, 34]. Aromatisation can also take place, although in a limited fashion, in the fetal liver [35].

I have already mentioned that the placenta is unable to produce oestriol, quantitatively the most important oestrogen during the human pregnancy, accounting for more than 90% of the total oestrogen urinary excretion. Diczfalusy and his group have proven that, whereas the placenta possesses a very active aromatising system, it lacks the ability to carry out hydroxylation at Carbon atom 16 of the steroid ring D, the critical reaction leading to oestriol [36]. In further investigating this issue, the Stockholm group identified two separate pathways for the formation of oestriol, named the “neutral” and the “phenolic” pathways. They differ because in the former, 16α-hydroxylation precedes aromatisation, whereas in the latter aromatisation is followed by 16α-hydroxylation. The "neutral" pathway can be considered a typical feto-placental activity: in it, DHEAS from fetal adrenals is hydroxylated in position 16α-, mainly in the fetal liver; the 16α-DHEAS formed in this

way is then converted to oestriol by the placenta following hydrolysis and the formation of an intermediary product, l6α-DHEAS [18, 38].

The "phenolic" pathway, on the other hand, represents a materno-placental activity: in this case, oestrone and oestradiol of placental origin are hydroxylated in position 16α- mainly in the maternal liver [18, 38]. Interestingly, the fetal liver is also capable of synthesising oestriol directly from oestradiol [39].

The investigation of the complex mechanisms involved in oestriol formation and its further metabolism to 15α-hydroxy-oestriol [40, 41], will always be linked to the work of Egon Diczfalusy: he – for the first time – pointed out that oestriol synthesis involves a process of high substrate and organ specificity, as well as enzymatic mechanisms active in the fetal and maternal adrenals (formation of DHEAS), in the liver (16α-hydroxylation of DHEAS and oestrone sulphate) and in the p1acenta (aromatisation of DHEAS and of 16-α-DHEAS).

Whereas this intense work and the major results obtained would have been sufficient to fulfil most people's dreams, for Egon Diczfalusy this was only a phase in his life.

In the seventies, a second major development occurred in his life: it was 1971 and Alexander Kessler, a young, bright and determined American scientist working in the World Health Organisation (WHO) as head of a small Unit, conceived the idea of a research programme in human reproduction and invited Diczfalusy to join as senior Consultant of the newly established "*WHO Expanded Programme of Research, Development and Research training in Human Reproduction*". Over the next 25 years, he became involved with every Task Force Steering

Committee, while at the same time heading the WHO Collaborating Centre in Research and Research Training in Human Reproduction established by the Programme in Stockholm. This took him to the four corners of the World where he participated in literally hundreds of Congresses, Conferences and Workshops.

Eventually the Programme grew to become a “Special Programme”, co-sponsored by 4 international entities, UNDP (the United Nation Development Programme), UNFPA (the United Nations Population Fund), WHO and the World Bank [42] and Diczfalusy continued to be the intellect behind the various activities. When, in 1995, as Director of the co-sponsored Programme, I was told by the WHO’s Personnel Division Director that Diczfalusy could no longer act as a Consultant because of his age, the "young-75 years old" visionary started a new career and devoted his energies to an Ageing World. He became convinced that the future must deconstruct the deterministic worldview of past centuries and replace it with a “science-driven anthropocentric worldview” [43].

He lectured extensively on a topic that attracted the attention of new generations, which he summarised with the words: “To many grand parents for too few grand children”. He also continued to battle on behalf of all older women of the world, their plight, their needs, their aspirations [44].

Finally, at age 85, he met what seems to be the last challenge of his life: the creation of a Foundation dedicated to his legacy and that of his late wife: his original idea was simple, while at the same time revolutionary: breaking the historical barriers existing between the countries of Eastern Europe and in particular those in the Balkan peninsula. Having been

born in Hungary he had direct experience of the consequences of the breaking down of the Austro-Hungarian Empire, of the problems created by the IInd World war and half a century of communist rule. To him, bringing closer the countries neighbouring Hungary would create a long-lasting legacy for his name, through an achievement that many have considered impossible, at least in the short term.

Egon Diczfalusy believes that – at the Academic level, but not only at that level – the very survival of the Eastern European region may depend on close collaboration between all countries in that area of the world.

In concluding, what lesson can young scientists draw from learning the achievements of such an outstanding scientist and humanitarian?

A first important point is that made a few years ago by Paul G. McDonough [45], who – after summarizing his view of Joseph W. Goldzieher, one of the founding fathers of hormonal contraception – invited readers to go to the web (MedLine, PubMed, Scopus, etc.) "to retrieve the most important years of Reproductive Endocrinology". He concluded that from this search "you will be dismayed to realize how precious and perishable fundamental scientific knowledge can be, but you will be rewarded to learn that studies of the past can provide important scientific information for the future". A second major point can be made by quoting a great writer of the past century who said: "The past is never dead; it is not even past" [46].

My personal conclusion is that we have a duty to remain active and productive at all stages of our life: the focus of our attention can and, perhaps, even should change. What must remain, as Egon Diczfalusy

taught us, is the strength to meet the challenge, to continue to fight for humanity and especially for the under-privileged among humans.

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Reprinted from: J Reproduktionsmed Endokrinol 2010; 7 (Special Issue 1)

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HONORARY MEMBER OF THE FOLLOWING SOCIETIES :

- Association Medica Argentina
- Société Royal Belge de Gynécologie et d´0bstétrique, Bruxelles.
- La Sociedad Chilena de Obstetricia y Ginecologia, Santiago, Chile.
- Sociedad Ecuatoriana de Ginecologia y Obstetricia, Quito, Ecuador.
- La Società Italiana di Ostetricia e Ginecologia, Roma, Italy.
- Sociedad Peruana de Endocrinologia, Lima, Peru.
- Sociedade de Ginecologia e Obstetricia do Brazil, Rio de Janeiro.
- La Sociedad Mexicana de Nutrición y Endocrinologia, Mexico, D.F.
  (miembro honorario estranjero)
- Die Schweizerische Gesellschaft für Obstetrik und Gynäkologie, Bern, Switzerland
- The Canadian Fertility and Andrology Society, Montreal, Canada
- The Hungarian Society of Endocrinology and Metabolism, Budapest.
- The Hungarian Society of Obstetricians and Gynaecologists, Budapest, Hungary
- The European Society of Human Reproduction and Embryology

(ESHRE), Bruxelles, Belgium
- The Society for the Advancement of Contraception, Manchester, U.K.
- The Romanian Society of Obstetrics and Gynaecology, Timisoara, Romania.
- The Indian Society for the Study of Reproduction and Fertility, New Delhi, India
- The Obstetrical Society of Philadelphia (Pennsylvania) USA.
- The Pacific Coast Fertility Society, Los Angeles, Calif. USA.
- Union Professionelle Internationale des Gynécologues et Obstétriciens (UPIGO), Luxembourg.
- La Sociedad Vallecuana de Obstetricia y Ginecologia, Cali, Colombia
- La Sociedad Mexicana de Nutrición y Endocrinología A.C., Mexico
(socio honorario, 1997).
- The International Society for the Study of the Aging Male (ISSAM)
(2006)

FOREIGN CORRESPONDING MEMBER

- The Royal Society of Medicine, London, UK. (Affiliate)
- Die Deutsche Gesellschaft für Endokrinologie, Heidelberg, Germany.
- Sociedad Cubana de Obstetricia y Ginecologia, La Habana, Cuba.
- Sociedad Cubana de Endocrinologia y Enfermedades Metabolicas, La Habana, Cuba.

H O N O U R S

1970 The Ruth Gray Medal, Northwestern University, Evanston, Ill. USA.

1976 The Gold Medal of the Barren Foundation, Chicago, Ill. USA.

1976 The Medal of the American Gynecological Society, New York.

1978 The Sir Henry Dale Medal of the Society of Endocrinology, London.

1978 Prix d´Honneur (Hors Concours).The Medal of the Académie Internationale de Lutèce, Paris, France.

1979 The J.C.Roussel Medal of the "Tables rondes, Roussel Uclaf" to the members of the scientific Committee, on the 10th anniversary Paris, France.

1981 The Award of the Korean Society of Fertility and Sterility, Seoul, Republic of Korea.

1982 Award of the Shanta S.Rao Memorial Oration Trust, Institute for Research in Reproduction, Bombay, India.

1983 The Medal of the Central Drug Research Institute for delivering the A.B.Kar Memorial Oration, Lucknow, India.

1985 The Semmelweis Medal of the Hungarian Society of Obstetricians and Gynaecologists.

1985 The Ferenc Szontágh Medal of the University of Szeged, Hungary.

1985 The Medal of the King Edward Medical College, Lahore, Pakistan.

1986 Acknowledgement of faithful services, Kingdom of Sweden ("För nit och redlighet i Rikets tjänst")

1987 The Giulio Andreotti Medal of the Fiuggi Foundation, Rome, Italy

1988 The Award of the Doerenkamp-Zbinden Foundation, Chur, Switzerland.

1988 The Special Distinction of the Institute of Health Research, Chulalongkorn University, Bangkok, for initiating human reproduction research in Thailand

1990 The Sir James Simpson Award of the University of Edinburgh, Scotland.

1990 The Medal of the Albert Szent-Györgyi Medical University, Szeged, Hungary.

1991 The *Pro Universitate* Medal of the Medical University of Debrecen, Hungary.

1991 The Distinguished Scientist Award of the American Fertility Society, Orlando, Florida, USA.

1993 The King Rama VIII Medal of the Medical Faculty, Chulalongkorn University, Bangkok, Thailand.

1993 The Albert Szent-Györgyi Commemorative Medal of the Albert Szent-Györgyi Medical University, Szeged, Hungary.

1994 The Medal of the Romanian Society of Obstetrics and Gynaecology, Timisoara, Romania.

1994 The King Rama V. Gold Medal of the Chulalongkorn University, Bangkok, Thailand.

1994 The Medal of the Obstetrical Society of Philadelphia. Pennsylvania, USA

1994 The Gregory Pincus Award, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, USA.

1994 The Medal of the Indian Society for the Study of Reproduction and Fertility, New Delhi, India.

1995 The Special Award of the WHO-CCR in Szeged, Hungary on the occasion of his 75th birthday.

1995 The Special Award on Population, World Academy of Art and Science, Minneapolis, MN, USA.

1995 The Prince Mahidol Award, Bangkok, Thailand.

1996 The special case given by the Prime Minister of Thailand,

Mr. B. Silpa-Archa, on the occasion of receiving the Prince Mahidol Award, Bangkok, Thailand.

1996 The Buckle of the Mahidol University, Bangkok, Thailand, "presented to Dr. Egon Diczfalusy in honor of his conferment of the 1995 Prince Mahidol Award for Medical Science and in recognition of his significant contributions to the field of medical science and his service to humanity. Prof.Athasit Vejjajiva, President of Mahidol University"

1996 The plate of the Department of Obstetrics & Gynecology, Faculty of Medicine, Chulalongkorn University: "Congratulation on receiving the Prince Mahidol Award to Professor Egon Diczfalusy, January 31st 1996".

1996 Lecture Award of the Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, on the occasion of receiving the Prince Mahidol Award on 30 January 1996, Bangkok, Thailand.

1996 The Centennial Medal of the Hungarian Society of Obstetrics and Gynaecology, Budapest, Hungary.

1996 The Certificate of Appreciation of the Director General of the World Health Organization for special contributions to the UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction over the period 1971 - 1996, Geneva, Switzerland.

1997 The Honorable Guest Lecture Award of the Faculty of Medicine, Chulalongkorn University, Bangkok, on the occasion of the 50th Anniversary of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

1997 The FIGO "Certificate of Recognition for Outstanding Contribution to the Promotion of Women´s Health" given on the occasion of the XV World Congress of Gynecology and Obstetrics, 3-8 August 1997, Copenhagen, Denmark.

1997 The IFFS - FIGO " Certificate of Honour" for delivering the Hubert De Watteville Memorial Lecture on the occasion of the XVth

FIGO World Congress, Copenhagen, Denmark, August 1997.

1997 The Award of the Mexican Society of Nutrition an Endocrinology for delivering a plenary lecture at the XIV PanAmerican Congress of Endocrinology ,Cancun, Quintana Roo, Mexico.

2000 The Commemorative Árvay Medal of the Department of Obstetrics and Gynecology, University of Debrecen , Hungary.

2000 The CELSAM (El Centro Latinoamericano Salud y Mujer) "Recognition for scientific research in the field of contraception", Buenos Aires, Argentina, 10 November 2000.

2001 The 1901 -2001 Centenary Medal of Gedeon Richter Ltd. Budapest, Hungary for delivering a keynote lecture at its Centenary Scientific Meeting, Budapest, Hungary.

2001 The BERTARELLI FOUNDATION Award in Reproductive Health, Lausanne, Switzerland, 3 July, 2001.

SPECIAL MARKS OF DISTINCTION

1976 The Nagarjuna Plaque of the Central Drug Research Institute, Lucknow, India, on the occasion of its 25th anniversary Symposium

1978 The Memorial Plate of the 5th International Congress on Steroids for delivering the Gregory Pincus Memorial Oration, New Delhi.

1986 The Plaque of the Institute of Health Research, Medical Faculty, Chulalongkorn University, Bangkok, Thailand

1987 The Plaque of the Department of Obstetrics and Gynaecology, Medical Faculty, Chulalongkorn University, Bangkok, Thailand

1988 The Plaque of the Indonesian National Family Planning Coordinating Board (BKKBN), Jakarta, Indonesia

1988 The Plaque of the Faculty of Medicine, Diponegro University, Semarang, Indonesia

1990 The Plaque of the Society for the Advancement of Contraception (SAC), Manchester, UK with lifetime membership

1991 The case given by the XIIIth Asian and Oceanic Congress of Obstetrics and Gynaecology, Bangkok, Thailand for delivering a Plenary Lecture.

1992 The Plaque of the Chulalongkorn University, Bangkok, Thailand

1992 The Plaque of the Royal Institute of Thailand, Bangkok,

1997 The Commemorative plate of the50th anniversary of Chulalongkorn University, Faculty of Medicine

1998 The Plate of Recognition by the Department of Obstetrics and Gynecology to commemorate the 50th anniversary of WHO and the 25th anniversary of the WHO CCR, Chulalongkorn University, Bangkok, Thailand.

2000 The Plate of Recognition by the Department of Obstetrics and Gynecology, Faculty of Medicine, in connection with the Symposium on Reproductive Health and Our Common Future, Chulalongkorn University, Bangkok, Thailand

2002 The Plaque of the Universidad Nacional Mayor de San Marcos, Lima, Peru.

CONSULTANCIES AND MEMBERSHIPS

- Member, Expert Advisory Panel on Human Reproduction, World Health Organization, Geneva, Switzerland (1972 – 2007)
- Consultant, Human Reproduction Program, The Ford Foundation, New York, NY, USA (1962 - 1978)
- Member, Specialist Panel on Systemic Contraception, International Planned Parenthood Federation (IPPF), London, UK (1965 - 1969)
- Member, Scientific Advisory Committee, International Institute for the Study of Human Reproduction, Columbia University, New York, N.Y. USA (1960 -1963)
- Chairman, International Organizing Committee, Karolinska Symposia on Research Methods in Reproductive Endocrinology, Karolinska Institutet, Stockholm, Sweden (1969 - 1975)
- Member, Program Committee, III. International Pharmacological Cong-ress, Saô Paolo, Brazil (1966)
- Member, Program Committee, V. and VII. World Congress on Fertility and Sterility (Stockholm, Sweden, 1966; London, UK, 1974)
- Member, International Organizing Committee, II. and III. International Congress on Hormonal Steroids (Milano, Italy, 1966; Hamburg, Germany, 1970)
- Member, Program Committee, III. International Congress of Endocrinol-ogy, Mexico City, Mexico (1968)
- Member, Scientific Advisory Committee, Departamento de Investigación Cientifica, Instituto Mexicano del Seguro Social, Mexico City, Mexico, (1970 - 1978)
- Member of the Jury, Prix Roussel, Paris, France (1972 - 1978)
- Member of the Jury, Organon Family Planning Scholarship, Oss,

Holland (1986)
- Member of the Jury, Premio Internazionale Fiuggi, Roma, Italy (1987)
- Member, The ESHRE Capri Workshop Group, (European Society of Human Reproduction and Embryology) (since 1986 -)
- Member, Delegation of the Kingdom of Sweden at the United Nations Population Conference, Mexico City, Mexico (1984)
- Member, International Scientific Advisory Committee, National Centre for Research in Reproduction, Nairobi, Kenya (1991 - 1997)
- Member, Advisory Group, Second Contraceptive Technology Revolution, The Rockefeller Foundation, New York, N.Y. (1994 - 2002)
- Member, Strategic Advisory Board, Consortium for Industrial Collaboration in Contraceptive Research (CICCR), CONRAD - Program, Arlington, VA, USA (1995-2002)
- Member, Advisory Board, The Institute of Medicine, Law and Bioethics of the Universities of Liverpool and Manchester, UK (since 1995 -)
- Member, International Advisory Committee, 9th World Congress on Human Reproduction, Philadelphia, Pennsylvania, USA, 1996.
- President, International Advisory Committee, The 5th World Congress on the Aging Male, Salzburg, Austria (2006).

S E R I E S E D I T O R

- Karolinska Symposia on Research Methods in Reproductive Endocrinology, Karolinska Institute, Stockholm, Sweden (1969 - 1975)

- Vitamins and Hormones, Academic Press, New York, N.Y., USA (1970 - 1982)

EDITORIAL BOARD MEMBERSHIPS

- Acta Endocrinologica, Copenhagen, Denmark (1955 - 1965)
- Contraception, Los Altos, Calif. - New York, N.Y. (since 1971 -)
- Clinical Endocrinology, London, UK (until 1984)
- Current Topics in Experimental Endocrinology, New York, N.Y. (until 1985)
- International Journal of Fertility
- Excerpta Medica, Section: Endocrinology, Amsterdam, The Netherlands
- European Journal of Obstetrics and Gynaecology (Advisory Board), Amsterdam, The Netherlands
- Clinica e Investigación en Ginecologia y Obstetricia, Barcelona, Spain
- Argentine Journal of Gynecology and Obstetrics (Consultative Committee) Buenos Aires, Argentina
- Hungarian Journal of Obstetrics and Gynaecology (Foreign Editorial Board), Budapest, Hungary
- Fertility Control Reviews, Bussum, The Netherlands
- The Journal of Reproductive Medicine (Corresponding Editor), Beijing, The People´s Republic of China

P U B L I C A T I O N S

Some 630 papers and chapters in books (see Annex 1);
editor for some 30 books.

SOCIETY MEMBERSHIP

- Endocrine Society, USA
- The New York Academy of Sciences, New York, N.Y.
- Society for Endocrinology, London, UK
- Swedish Endocrine Society
- International society for the study of the aging male
- Society for Contraception and Reproductive Health, Arlington, D.F. USA

HONORARY AND INAUGURAL LECTURES

1955 - Invited Lecture: 3. Symposion der Deutschen Gesellschaft für Endocrinologie, Bonn, W. Germany
1956 - Opening Lecture: Second Acta Endocrinologica Congress, Oslo, Norway
1960 - Invited Lecture, 17th Laurentian Hormone Conference, Mont Tremblant, Quebec, Canada
1961 - Invited Lecture: I. Coloquio Europeo de Endocrinología Playa de Aro, Spain
1965 - The Barone Lecture, University of Pittsburgh, PA, USA
1968 - The Baxter-Flint Lecture of the Canadian Society of Clinical Investigators, Toronto, Canada
1969 - The Second Ernst Laqueur Memorial Lecture of the Dutch Endocrine Society, Amsterdam, The Netherlands
- The Ayerst Lecture of the American Fertility Society, Miami Florida, USA
1970 - The Ruth Gray Memorial Lecture, Evanston Hospital, North-western University, Chicago, Ill., USA
1973 - Congress Lecture, VII. World Congress of Gynecology and Obstetrics, Moscow, USSR
1974 - Opening Address, IV. International Congress on Hormonal Steroids, Mexico City, Mexico
1975 - Congress Lecture, 19th Assembly of the Japan Medical Association, Kyoto, Japan
- The Gomez-Mont Memorial Lecture of the Mexican Society of Nutrition and Endocrinology, Acapulco, Mexico
1976 - The Sune Genell Memorial Lecture, South-Swedish Society of Gynecology, Lund, Sweden
1977 - The First Ortho Lecture, University of Sheffield, Sheffield, UK
- The Third Robert W.Greenblatt Lecture, University of Augusta, Georgia, USA
1978 - The Sir Henry Dale Lecture of the Society for Endocrinology, London, UK
- The Gregory Pincus Memorial Lecture, V. International Congress on Hormonal Steroids, New Delhi, India
1980 - Inaugural Lecture as an Honorary Member of the Hungarian Society of Endocrinology and Metabolism, Budapest, Hungary

1982 - The Second Shanta Rao Memorial Oration, Institute for Research in Reproduction (Indian Council of Medical Research), Bombay, India
1982 - Keynote Address, IV. International Congress on the Menopause, Lake Buena Vista, Florida, USA
1983 - A.B.Kar Memorial Lecture, Central Drug Research Institute, Lucknow, India
1985 - The Ferenc Szontágh Memorial Lecture, University of Szeged, Szeged, Hungary
- Invited Lecture, 18th Annual Meeting of the Spanish Society of Gynecology, Granada, Spain
- Invited Lecture, 125th Anniversary Celebration of the King Edward Medical College, Lahore, Pakistan
1986 - The Uwe Goebelsmann Memorial Lecture, University of Münster, Münster, Germany
- Keynote Address, Schering Silver Jubilee Symposium for the Pill, Berlin
- Inaugural Lecture as an Honorary Member of the Hungarian Society of Obstetrics and Gynaecology, Szeged, Hungary
- Invited Lecture, First International Symposium, Japan Family Planning Association, Tokyo, Japan
1987 - Keynote Address: World Health Organization Symposium on Safety Requirements for Contraceptive Steroids, WH0, Geneva, Switzerland
- Inaugural Lecture as Doctor Honoris Causa, Albert Szent-Györgyi Medical University, Szeged, Hungary.
1988 - Keynote Address: International Conference on Reproductive Endocrinology, Beijing, People´s Republic of China
- Plenary Lecture: International Symposium of the Indonesian Society of Andrology, Semarang, Central Java, Indonesia
- Keynote Address: Jubilee Conference of the Zhordania Institute of Human Reproduction, Tbilissi, Georgian SSR
1989 - Keynote Address: Symposium on Contraception Research to celebrate the 10th Anniversary of the National Research Institute for Family Planning, Beijing, the People´s Republic of China
1990 - The Sir James Simpson Lecture, University of Edinburgh, Edinburgh, Scotland
- Plenary Lecture: Symposium on Advances in Fertility Regulation, National Academy of Medicine, Mexico City,

Mexico
- Keynote Address; International Symposium on Contraception, University of Heidelberg, Heidelberg, Germany
- Plenary Lecture: 16th National Congress of Science and Technology, Bangkok, Thailand
- Conference Lecture: USSR Ministry of Public Health, All Union Conference on Environmental Health, Chernovtsy, Ukrainian SSR
- Keynote Address: International Conference on Frontiers in Reproductive Physiology, All India Institute of Medical Sciences, New Delhi, India
- Invited Lecture, Second Congress of Asian and Oceanian Physiological Societies, New Delhi, India

1991 - Plenary Lecture: 13th Asian and Oceanic Congress of Obstetrics and Gynecology, Bangkok, Thailand
- The Joseph Price Oration of the American Gynecological and Obstetrical Society (AGOS), La Costa, Carlsbad, California, USA
- Keynote Address: The 3rd European Winter Conference in Gynecology and Obstetrics, Madonna di Campiglio, Italy
- Plenary Lecture (the ICI Lectureship), Annual Meeting of the American Fertility Society, Orlando, Florida, USA
- The 9th Schering Lecture, AG Schering, Berlin, Germany
- Keynote Address: Third International Symposium on the Pregnant Uterus, The University of Debrecen, Debrecen, Hungary

1992 - The CD Christian Distinguished Guest Lecture of the Society for Gynecologic Investigation, San Antonio, Texas, USA
- Invited Lecture presented to the Royal Institute of Thailand, Bangkok, Thailand
- Special Lecture, World Health Organization, on the occasion of the 20th Anniversary of the Special Programme of Research, Development and Research Training in Human Reproduction, WH0, Genève, Switzerland

1992 - Keynote Address: International Symposium on Infertility and Fertility, Airlangga University, Surabaya, Indonesia
- Inaugural Lecture: Second International Conference on Advances in Reproductive Research in Man and Animals, National Centre for Research on Reproduction, Nairobi, Kenya

- Opening Lecture: 8th Meeting of the European Society for the Study of Human Reproduction and Embryology, The Hague, The Netherlands
- Keynote Address: 33rd Congress of the Japan Society of Maternal Health, Hamamatsu, Japan
- Opening Lecture, 20th Anniversary Celebration of the World Health Organization´s Special Programme of Research, Development and Research Training in Human Reproduction, Szeged, Hungary
- Keynote Address: First Asian and Oceanic Congress of Andrology, Nanjing, The People´s Republic of China

1993
- Plenary Lecture, XIVth World Congress on Fertility and Sterility, Caracas, Venezuela
- Main Lecture, VIIIth World Congress on Human Reproduction, Bali, Indonesia
- Inaugural Address (Festvortrag); "Dreiländertagung" of The Austrian, German and Swiss Societies of Fertility, Sterility and Family Planning, Lugano, Switzerland
- Main Lecture: VIIth International Congress on the Menopause, Stockholm, Sweden
- Plenary Lecture: 20th International Symposium on Controlled Release of Bioactive Materials, Washington, DC, USA
- Keynote Address: 50th Anniversary Celebration of the Medical Faculty, University of Pretoria, Pretoria, Republic of South Africa
- Invited Lecture: Centennial Celebration of the birth of Albert Szent-Györgyi. Albert Szent-Györgyi Medical University, Szeged, Hungary
- Plenary Lecture: International Symposium on Perinatal Nutrition and Brain Development, Troina, Sicily, Italy
- Invited Lecture: XIVth Latinamerican Congress of Obstetrics and Gynecology, Panama City, Panama

1994
- Keynote Address: International Symposium of the Portuguese Society of Obstetrics and Gynecology, Lisbon, Portugal
- Keynote Address: 150th Anniversary celebration, Gesellschaft für Geburtshilfe und Gynäkologie in Berlin, Berlin, Germany
- Plenary Lecture: 130th Anniversary Celebration of the

Academia Nacional de Medicina, Mexico City, Mexico
- Keynote Address: Joint Meeting of the Romanian and Hungarian Gynaecological Societies, Oradea, Romania
- Valedictory Lecture: International Symposium, All India Institute of Medical Sciences, New Delhi, India
- Invited Lecture: *Techno-Indochina.* The International Congress on Science and Technology for Cordial Relationship with Neighbouring Countries, Organized by Thailand´s National Science and Technology Development Agency, The Ministry of Science, Technology and Environment and the Committee on Science and Technology of the House of Representatives of Thailand, Bangkok, Thailand

1995 - Plenary Lecture: XVth Asian and Oceanic Congress of Obstetrics and Gynecology, Bali, Indonesia
- Keynote Address: WH0- AIIMS International Symposium on Male Contraception: present and future. New Delhi, India

1996 - Inaugural Lecture as a Prince Mahidol Awardee, Mahidol University, Bangkok, Thailand
- Plenary Lecture: 30th Congress of the Federation of Scandinavian Societies of Obstetrics and Gynecology, Stockholm, Sweden
- The Gedeon Richter Memorial Lecture: XI. Congress of The European Association of Gynaecologists and Obstetricians (EAGO), Budapest, Hungary

1997 - Plenary Lecture, Medical Congress in Commemoration of the 50th Anniversary of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
- President´s Introductory Lecture, International Workshop on Hormone Replacement in the Ageing Male, Weimar, Germany
- The IFFS-FIGO Hubert de Watteville Memorial Lecture, XV FIGO World Congress of Gynecology and Obstetrics, Copenhagen, Denmark

1997 - Congress Lecture, IV. European Congress on the Menopause, Vienna, Austria
- Keynote Address, Symposium to celebrate the 25th anniversary of the World Health Organization´s Special Programme of Research, Development and Research Training in Human Reproduction, Szeged, Hungary

- Congress Lecture, VII Congreso Nacional de Ginecología y Obstetricia, Juriquilla, Querétaro, Mexico.
- Plenary Lecture, XIV Pan American Congress of Endocrinology, Cancún, Quintana Roo, Mexico.

1998
- Honorary Opening Lecture: 1st Asian-European Congress on the Menopause. Bangkok, Thailand
- Invited Lecture presented at Chulalongkorn University to commemorate the 50th anniversary of WHO and the 25th anniversary of the WHO-CCR at Chulalongkorn University, Bangkok, Thailand.
- Opening Lecture, the First World Congress on the Aging Male, Geneva, Switzerland.
- Opening Lecture, 3rd International Symposium on Women´s Health and Menopause, Florence, Italy.
- Opening Lecture, 5th Congress of the European Society of Contraception, Prague, Czech Republic

1999
- Inaugural Lecture, as Academician, Hungarian Academy of Sciences, Budapest, Hungary
- Opening Lecture, Joint Meeting of the Bavarian and Austrian Gynecological Societies, München, Germany
- Plenary Lecture, 11th International Meeting of Gynaecological Oncology, Budapest, Hungary
- Plenary Lecture, 9th World Congress on the Menopause, Yokohama, Japan
- Presidents Introductory Lecture, 2nd International Workshop on the Ageing Male, Weimar, Germany
- Introductory Lecture, 30th Anniversary Workshop of the International Health Foundation on Hormones and Ageing, Hoevelaken, The Netherlands
- Valedictory Lecture: Conference of the Rockefeller Foundation on Public/Private-Sector Collaboration in Contraceptive Research and Development, Bellagio , Italy

HONORARY AND INAUGURAL LECTURES FROM THE YEAR 2000

2000 - Plenary Opening Lecture, the Second World Congress on The Aging Male, Geneva, Switzerland.
- Plenary Opening Lecture, International Symposium on Reproductive Health and Our Common Future, Bangkok, Thailand.
- Inaugural Lecture for the S.Shan Ratnam Professorship in Obstetrics & Gynaecology, The National University of Singapore, Singapore.
- Keynote Address, First International Conference on Ageing and Health, IFMSA (the International Federation of Medical Students' Associations), Porto, Portugal.
- Keynote Address, Millennial World Congress of Hungarian- speaking physicians (Magyar Orvosok Millenniumi Világtalálkozója) Szeged, Hungary.
- Concluding Response. Symposium on the occasion of the 80th Birthday of Prof. Egon Diczfalusy, Ernst Schering Research Foundation, Berlin, Germany.
- Opening Lecture: Workshop organized by the Society of Obstetricians and Gynaecologists of Canada on the occasion of 40th anniversary of the contraceptive pill, Montréal and Toronto, Canada
- Keynote Address, Centro Latinoamericano Salud y Mujer, Commemoration of the 40th anniversary of the contraceptive pill, Buenos Aires, Argentina.
- Opening Lecture: Schering Symposium organized in collaboration with the Associación Médica Argentina, Buenos Aires, Argentina

2001 - Keynote Lecture, Postgraduate Course on the Physiopathology of Human Reproduction, Segrate, (Milano), Italy.
- Keynote Lecture: Gedeon Richter Ltd Centenary Scientific Meeting, 1901 – 2001, Budapest, Hungary
- Opening Lecture, Symposium investigación y Salud. Retos y necesidades en el siglo XXI. México, D.F.
- Keynote Address: Schering Symposium organized in collaboration

with the Federation of Mexican Societies of Obstetrics and Gynecology, Mexico City, Mexico

- Keynote Address: Schering Symposium organized in collaboration with the Brazilian Society of Obstetrics and Gynecology, Sao Paolo, Brazil

2001 - Opening Lecture: Symposium on Estrogen Replacement; are all estrogens equal? Bali, Indonesia

- Keynote Address: 33rd International Congress of Pathophysiology of Pregnancy, Cluj-Napoca, Romania
- Keynote Address at the Symposium organized by the International Health Foundation, The Netherlands 2002
- Opening Plenary Lecture, Third World Congress on The Aging Male, Berlin, Germany
- Congress Lecture, 7th Congress of the European Society of Contraception, Genova, Italy
- Plenary Lecture, 10th World Congress on the Menopause Berlin, Germany
- Invited Special Lecture, Sociedad Ecuatoriana de Ginecologia y Obstetricia, Quito, Ecuador.
- Keynote Address: Schering Symposium organized in collaboration with the Universidad Nacional Mayor de San Marcos, Lima, Peru.
- Opening Lecture: the 34th International Congress on Pathophysiology of Pregnancy, Balatonfüred, Lake Balaton, Hungary.

2006 - Opening Lecture, Schering Workshop in collaboration with The Italian Society for Reproduction and the Faculties of Medicine and Surgery, University of Milano, Italy

- Keynote Lecture, First Pantarhei Symposium, Sparrendaal, The Netherlands
- Opening Keynote Lecture, the 5th World Congress on The Aging Male, Salzburg, Austria
- Lettura Magistrale , Le Facoltà Mediche delle Università Romane, 17 Maggio 2006 Roma, Italy.
- Keynote Lecture 29th Postgraduate Course on the Physio-pathology of Human reproduction, Segrate (Milano) Italy.
- Lettura Magistrale, Corso di Aggiornamento “Le pause ormonali” Ospedale San Giovanni Calibita Fatebenefratelli,

Isola Tiberina, Roma, Italy.

- Lettura Magistrale, Corso di Aggiornamento, Roma, Italy
- Valedictory Speech on the occasion of the retirement of Prof. Britt-Marie Landgren, Stockholm, Sweden 2007
- Opening Lecture: First Meeting of the Symposia Partisco-Romana, Szeged, Hungary.
- Valedictory Lecture: AMPPA Meeting on Male Fertility Control – where are we? – Gross-Ziethen, Germany.

2008 - Specially Invited Guest Lecture given at the 52nd Annual Congress of the Serbian Society for Obstetrics & Gynaecology, Beograd, Serbia

- Opening Remarks, International Symposium, Timisoara, organized by the Romanian Academy, the Romanian Societies of Obstetrics and Gynecology and Assisted Human Reproduction and the University of Timisoara, Romania.
- Opening Lecture given at the first meeting of the Task Force of the E.& A. Diczfalusy Foundation on the Prevention of Cardiological Complications in Women, Väsby Gård, Sweden.
- Keynote Address Symposium on The Ageing Male organized by the Swedish Medical Association to celebrate its 200th anniversary, Malmö, Sweden.
- Opening Lecture, meeting of the Rectors and Deans of Medical Faculties of the Universities of Novi Sad, Szeged and Timisoara,(Szeged, Hungary).
- Keynote Address: Annual meeting of the IPPF, Hungarian Branch, (XXXIII. Congress of the Hungarian Scientific Society for the Protection of the Family and Women, Gyula, Hungary.

2009 - Honorary Chairman at the Symposium on Medical Science, Humanism and Our Common Future organized in Novi Sad, Serbia, by the Serbian Academy of Sciences and Arts, Branch in Novi Sad, and the Academy of Medical Sciences of the Serbian Medical Association, Branch of Vojvodina, with the collaboration of the Berlin – Brandenburg Academy of Sciences and Humanities and the Hungarian Academy of Sciences Regional Committee, Szeged.

- Honorary President at the Second Symposium Partisco-Romanum at the Hungarian Academy of Rome with a Special Lecture entitled "From Pleistocene to anthropocene: *quo vadimus?"*
- Invited Lecture , the Faculty of Medicine, University of Firenze, Italy:

"La Rivoluzione Demografica e il Nostro Futuro: Troppi Nonni per Pochi Nipoti"

- Honorary President at the 5th Good Clinical Practice Conference & Course organized by the Department of Obstetrics and Gynecology at the University of Szeged, and the Hungarian Clinical Trial Management Society, with the support of the Department of Reproductive Health and Research of the World Health Organization.
- Guest of Honour at the First Beograde Symposium of the Egon & Ann Diczfalusy Foundation, taking place within the framework of the 5th Congress of the Ob&Gyn Associations of Serbia, Montenegro and Republica Srpska in collaboration with the Hungarian and Romanian Societies of Obst.& Gyn., dedicated to the celebration of the 89th birthday of Prof. Egon Diczfalusy.

A couple of days in Rönninge - Egon's thoughts of wisdom

An original interview of Professor Egon Diczfalusy
before his 90th birthday anniversary

Anyone who had the opportunity of listening Professor Egon Diczfalusy lecturing was certainly fascinated by the professional approach of the thesis, but foremost delighted by his quotations and the human values he transmitted.

Interviewing the great man, Professor Emeritus Dr Egon Diczfalusy, is somewhat of a true challenge for anyone, as it was for me. During the three days spent with him gathering material for this book, it was more like Egon's listener was carried in a "words of wisdom's world" by the stories he heard. The DVD brings Egon's perspective closer and offers a representative image of the professor before his 90th birthday celebration.

Egon would describe himself now in a cynical way as being old, but remaining optimistic as he is known to be, he says that every age has its characteristics, its compensations and is always in a continuous change; he gives the example of the old Greek saying that '*everything is moving'*.

There were so many questions as we were caught up in the conversation, which I later grouped in chapters, the first being about his former students.

He admitted that having had so many former students so close represented a privilege got from fate and the list of 150 former students he showed me, meant not only 150 names, but 150 close friends who represent an extended family even now. Quoting his words: "*It was probably the*

greatest satisfaction to me to see how these boys and girls were struggling and then, some of them came up to the top, some of them didn't…but this is life. I felt that to teach the younger generation was at least as important for a scientist as bringing some valuable new information. Unless you can transfer that information, unless you can motivate the young generation you are not fulfilled."

Everything started when he "*got a few students from the neighbouring countries"*. He remembered: "*I got a grant from the Swedish Medical Council which enabled me to bring in 2 or 3 foreign students; then, the Ford Foundation heard that I was particularly interested in training and eventually provided me with a big grant or research and training. Young people believed my laboratory was the place to go because they could not only learn technology, but also be confronted with a kind of a philosophy of science"*. He stated: *"Obviously science is very personal. I established a very personal relation with all these boys and girls and even today, Cristi, 40 years later I still get Christmas cards from the old friend in Argentina wishing me all the best. I can tell you that this has enriched my life incredibly*".

When asked about how he had managed to keep them motivated throughout their journey of becoming scientists, he said that it wasn't difficult as "*it was very exciting for them to be suddenly in a truly international atmosphere; they had not had it before. In the lab there were almost a dozen nationalities or even more and they established close friendships that lasted a lifetime*". It all happened with the help of very good technicians about whom Egon remembered: "*I had an incredible luck having very high quality technical assistance. All this of course depended on personality…and in order to get friends you must be one of them. It was as*

simple as that…so I was very good friend with my technicians… they were very devoted, they were teaching all these people". In addition, about the atmosphere in the laboratory, he continued: "*They had a lot of respect from the beginning and they might have come from a country where there still was the system of 'herr professorissimo'. They could understand that we had no such things. We had an entirely different atmosphere, there were no big VIPs, no one had more prestige than the evidence presented by him in a given field, on a given occasion. If you kept this in mind then it was very easy to sort out all the lightweight champions, who lived for the prestige, being uncertain of themselves or those who lived for acquiring prestige because it was needed for their careers; that was easy to spot already on the young people's arrival. I didn't need to tell them they should work hard, they were working day and night; they were working like robots and sometimes I was worried about them being 16-17 hours in the lab.…that meant there was a lot of good will in the young when they came to learn, which was extremely important for their future career. If they had the proper environment, the proper scientific atmosphere then they would develop enthusiasm that would last for a very long time even when they got home where they sometimes would find a very difficult atmosphere, with the tyranny of the 'herr professorissimo' and this existed almost everywhere, perhaps less in this country or in the USA. It was very helpful that we could establish a free society*".

He then continued: "*First, there came some highly qualified boys who spent a year or two at Harvard or at Columbia, mostly Americans, or Belgians.…so they were high standard, but then the Ford Foundation wanted us to train people from developing countries. That was a big order. There came these boys and girls very enthusiastic, but with no research*

background; some of them had never kept a test tube in their hands. Everything was new for them. My technical assistants taught them under the guise of assisting…this was the secret of the operation".

Remembering his first students, he smiled and searched into his vast memory: "*The very first ones were, I think, two people from Finland: Lasse Sjöblom and Maya Lassila. They were joined by Eeva Axelson and Martha Halla and later by Herman Adlercreutz, who then became a great professor in Helsinki*". Laughing, he recalled: "*Lasse Sjöblom wrote a thesis, a part with me and finished it back in Turku. It was a major exercise to get a doctor's degree in that period. One worked three-four years for a thesis. I was invited as the opponent of the faculty… and there was the old academic tradition. I was seated at a table and the respondent was seated on the opposite side of this table and in between the two of us were 'the Custos' with a big sword. It came from the tradition that in earlier times it was usual the discussion often ended-up into a fight…it was a very nice thing. My memories are that I have never had such a glorious black-out in my life… and you know, I am not used to this. So, in the evening there was a big toast and beauty after everything was finished. The next morning I was trying to get somewhat sober. The telephone rang and it was the professor in chief asking: 'Where the hell are you?'; 'In bed'; 'Come at once at my house. We are waiting for you'…so they were waiting for me with a cognac session… and from there it went …terrible"*.

Asked if the pleasant work atmosphere and the friendly environment were easy to maintain, Egon said*: "It was a headache at the same time, because students came with maximum of expectation, so they would get something like a publication when they went home. You could never guarantee it. There were always anxiety and problems like 'Did I give them*

the proper project?' To most of them I did. In a few cases it was a failure and when it was a failure it hit me very hard because it affected so much their future career".

Always concerned about his students, he told me the treatment they received when working with him: *"Our routine was that when new fellows came, we invited them at the house; when they left we invited them back to our house with their wives, but we couldn't do much more for the wives. They had a really tough time in a foreign environment as the men were working and what we tried to do was to entice most of the wives to the work of their husbands'. Sometimes it was a glowing success, sometimes it didn't work out. For instance Ninni Mancuso came as a bachelor, like Pino Benagiano did. Then Ninni went home to Sicily and his family told him 'Now you get married to Franca'. So, Ninni came with Franca. Franca was a very nice girl and we had to find some occupational therapy for her, so she came to the lab and we had counter current distribution at that time, a very primitive machinery that others nonetheless did not have! You needed to shake those tubes to get separations…and Franca was so short that we had to make a pedestal for her to be able to shake them. And, so she did… ".*

Describing his younger collaborators the professor recalled: "*They were different, like people are different, but it was extremely rare the case when I got disappointed, because the good intention came up to the surface. They realized what I had told them, something I didn't realize myself then: 'Boys and girls, this is the happiest time of your life because you are here and you have no major concerns, as the concerns are ours. As soon as you go back to real life, you will find that you are no longer in a protected atmosphere, but there you are exposed to competition, to double crossing … so enjoy the protected environment here, make friends with the others and*

try to see the permanent value of trying to achieve something for the benefit of the race'. And usually it had an impact".

Although the atmosphere in the lab was a very peaceful one, Egon had to add: "*Human nature is human nature. And among 150 guys it was bound to happen. Some of them had a genetic attraction and others had not. Some of them became very good friends…and of course I was always concerned that there would be conflicts between the different nationalities in the lab, but there was a very good spirit, Africans, South-Americans – no problem. The only problem I had, it was with the Canadians, because there were two Canadians, one from Toronto- a British Canadian and the other from Montreal- French Canadian. Of course, everybody was in very good friendship but these two were always discussing about who was more impossible than the other. Finally, there was a 'big conflict'; one said that 'our queen said' and the other replied 'I'm not interested in your queen, sir'. There I had to say: 'Hey boys, take it easy! We have a king here and we don't bother about him' ".*

A little surprised by the question about the way his former collaborators described him, he honestly admitted: "*I have not the foggiest idea. Except one thing, you know, the story of the two mirrors situated side by side; in one of the mirrors you see yourself, in the other mirror the others see you. The difference between the two is usually significant…sometimes very significant."*

Then we got to the relationship of his children with his work: "*My kids were too small in the beginning, but then I remember that some of them came to the lab …first I brought Jonas, the gynaecologist, who is now in retirement, after that Ulf became interested... and later Bo, the younger boy. … I can only say that whenever there is a chance, one should try to use it,*

because it means so much for the children to have an early exposure to another country, other people and the opportunity to understand that life is not moving around a small place like the city you live in".

Many of my questions tried to reach the interesting aspects of the vast scientific life Egon had. Here are some very interesting stories and answers I received.

Talking about how a scientist looks like, Egon said: "*A scientist is a scientist. Many of them have a one-tracked mind, which means that as a human being you discover that there are major shortcomings, but there is something he can see that you don't…and that's the genius. We are exposed to the same things, but we perceive them differently*".

Probably for most of us, Professor Egon Diczfalusy is a role model. But who was his role model? Well: "*My first professor in Hungary was Professor Ivanovic, who was a young professor in Pathology and Bacteriology. He took me to his department and to social acquaintances at the age of 20 when I was a medical student. I was with him during the war, through very difficult times and then came the Russians and he escaped to Germany, but I didn't. He was certainly teaching me the basics of research. I remember that I have written my very first paper by myself. He told me: 'Try to write it up' … and encouraged me saying 'Look it is quite good. So don't worry, continue'. That's how it started. It is like…think of a violinist. He always has a teacher, a good teacher. Nobody is a self-made man, but sometimes the input of the older generation is more difficult to see and it varies of course, a great deal from individual to individual. Maybe, Cristi, the dividing line goes between those who question… and those who accept*". One of the qualities a scientist must have arose from this statement. Egon told me:" *Do I know this is correct, that it is really so, are there other*

explanations? And the truth is, there are always other explanations. As one of my teachers said: 'I measure the intelligence of a student by the number of explanations he can find to explain a given fact'". But, in his view, the most important quality is that identified some 150 years ago by the French scientist Claude Bernard:" *The best definition comes from Claude Bernard and he says: 'A scientist should have a robust faith, but still shouldn't believe. A scientist needs to believe in order to get new information, but at the same time, be careful not to over interpret what you find'. That's very often the case*". All in all: "*a scientist should be aware of his and of others' limitation, he should be aware that there is no such thing as the ultimate truth, there are only half truths in life…A scientist should know that he needs, more than anything else, good luck, and sometimes he will find something…Bacon said: 'Life is full of wonders if you have the eyes to see it'. Modesty and humility are very essential requisites for any scientist*".

Our discussion slipped then to his scientific research and, as usual, he started with a nice quotation:" *You may remember Churchill who said 'the longer you can look backwards, the further you can look forward'. It is good to look back…*" The story continued as followed: "*When I started to work with Professor Axel Westman it was not very long after the first estrogens and progesterone had been isolated by people I knew…The sex hormones were very much in the coming and I became one of those who were fascinated …so when I started, the only method to estimate estrogens was bioassay on castrated mice… The first fluorimetric method was in the late 1940s … when I started in the early 1950s there was really no good method… so I started first developing methods… it was only in 1956 when the first colorimetric method was worked out… and this was the first study ever, where you could estimate the foetal tissue estrogens… and so it*

started… all the other methods came with me…". It is amazing to understand how methodologies have developed, and when you listen to Egon, you feel fascinated and want to hear more and more. It is like he uses to say, you should have two glasses of wine rather than one.

In his research unexpected help came from "*the Swedish law of abortion which was the first in the world where social conditions were included… and in Sweden there were about 6000 abortions every year. When the Medical Research Council heard that I had a method for measuring steroids, then, they said that we could have all this material from the abortions and if I were willing to start a project, the MRC would consider our application…I did it …and they provided the support, that was the prelude to even bigger support from the Ford Foundation* ". From this: "*The first step was that we collected fetal tissues from the products of abortions and then we developed a method for tissue analysis and to my great surprise, we found that, when we analyzed fetal liver tissue, virtually, all estrogens were in a conjugated form, so that meant it had already been modified by the fetus. Then, we introduced steroid tracers and published more than 100 papers fetal and placental metabolism. From there came the concept of fetal-placental unit so that neither the fetus, nor the placenta could produce all the steroids, but together they did everything …. I presented the new concept in the late 1960s and early 1970s*". This is how a great new concept in medicine has developed.

Not everyone was so happy about this: "*Of course there were some religious groups that disagreed…*" There were also counterarguments, some of them very funny in our times: "*When I presented in 1953 the first data on the fetal metabolism, the professor in Kiel said: 'How can the fetus be so active when he is so small?' …that was the argument*".

Most of the scientific community reacted positively, so that "*my laboratory became the Mecca of feto-placental research… That was thanks to the Swedish legislation and the support of the MRC, because I obviously was attacked by clerical circles and the MRC said that they had approved those studies and they thought they should go on…* "

Even now, Egon thinks about his research and what else there is still to be done in this field: "*One thing we surely missed was the understanding of the reason why nature was providing just the human species with that enormous steroid production during pregnancy, by the fetus. Even in studies concerning higher apes, steroids were much more limited; we couldn't find a good reason to what was behind it. The theories were very simplistic, the estrogens were needed to provide the uterus a better circulation, but still we couldn't provide a satisfactory answer. I am convinced that in the operations of nature, nothing is in vain… so there has to be a very compelling reason in human development why it became unique in this field…* ".

Having started from almost nothing efficient, Professor Diczfalusy had to develop good methods of measuring the hormones and proper interpretation of the data he obtained. That is why, he emphasized from the beginning "*the importance of methodology, because without sensitive and specific methods you are in the dark; and, that was the case, in the middle of the 20th century. Professor Halban said: 'When you measure something, that is the beginning of positive knowledge'* ". He remembered: "*I was interested in problems of biological standardization, developing bioassay methods for hormones, for which there were no good chemical methods, like gonadotrophins. There were very primitive essays in the 1930s, 1940s and even when we started, so we had to work from scratch …*"

Egon was still very grateful towards the Ford Foundation: "*They really changed my life. Because I was able to buy modern equipment for the laboratory, I could get some fellows from abroad. Without funds, it would have been impossible. I took over the laboratory when it was very poorly equipped, it had no modern equipment; there was nothing modern and I had to run the laboratory on a commercial basis. I got a small grant from MRC that helped me get some reputation; after that I was fortunate enough to meet a few people from the Ford Foundation and they came with a grant and announced I had already received the first 500000 $, which was a large sum of money for that time. My whole life was entirely changed, as well as my working conditions and possibilities to do something up to date. "*

The research conducted by Professor Diczfalusy in the field of steroid hormones inevitably lead to the field of contraception. Remembering Professor's Gregory Pincus visit to Stockholm, he smiled and said: "*I was much better known abroad than in my country*".

The large field of contraception as it is now was only beginning to rise then; attracting a good scientist into it, was a real challenge. "*There was, as difficult as it may be to understand now, an incredible shortage in research workers interested in human reproduction, not to mention contraception, contraception was a kind of very 'cheap' research, it was done by people who spoke about vaginal rings and condoms and it had a very low 'social' reputation. So, the Ford Foundation had to attract scientists of higher quality; they were very clever as they started telling you: 'Just do what you have to do in human reproduction. Don't worry, you find out how important contraception is only when you will be in it, then come back to us' - this was the hook- "*.

The very important role played by Professor Egon Diczfalusy in the field of contraception is better understood from the books presenting the symposia he had organized. One of the most important, as he remembers, was the one in Stockholm in 1983 that led to many other scientific developments. He recalls: "*The Swedish government allowed me to organize a symposium in 1983, when for the first and the last time in my life there were no financial restrictions. They provided me 1 million Swedish crowns and suggested that I invite the best people in the world, because we had to start the good way. We had the symposium that was opened by the Swedish minister of Health. We printed the book in 12000 copies and it was ready for the WHO Conference in Mexico. The Swedish government decided this was going to be their contribution to the international conference and they sent it to every participanting country*".

The work in the field of contraception had a major impact because of Egon's key position in the World's Health Organization (WHO) in Geneva. With his fantastic memory, he recounted the history of WHO: "*In the 1930s there was the League of Nations in Geneva… good place for ventilating ideas. Nothing happened, but there was fighting…they had a small group that was concerned about health issues, a small nucleus with little support; that was the precursor of WHO. After World War II they realized that there was a big need for world organization dedicated to health and in 1948, the United Nation established WHO and placed it in Geneva…*"

In the late 1960s he "*started collaborating in the late 1960s with Dr Alexander Kessler, who had a small unit with no money and one secretary. We decided to initiate a programme in human reproduction. Dr Kessler was not very well-known in the international scientific community, but I was, so that helped in getting support. So the new program of WHO, the 'Human*

Reproduction Programme', started. It was in 1968 or 1969 when I became chief advisor; 3 or 4 years later I become senior advisor…"

Many things could be told about Professor Diczfalusy's achievements in the WHO and an entire new book could be written on this, so here, I will just present a few interesting points touched during my interview. Egon said: "*What we could do in most countries was to bring qualified people and organize symposia and acquaint them with the state of art… and this was more important than we had thought at that time*." Playing down his very important contribution, he continued: "*If the spirit of the time is not right, you are talking to the walls, and then something happens, the 'spirit of the time' sets in when they discover what you had know for a decade… and then, there is action, movement*". "*It was the first time in human history that a programme was designed to reach each and every country; there was indeed a global approach to a major problem, although every country did it different nuances, and to accomplish this was very exciting… WHO could have had a big future, if it was to be kept apolitical which turned out to be very difficult due to the human nature, which spoils everything by politicking sooner or later". The Human Reproduction Programme was bringing news to isolated places in the world. In the 1970s I had been to India already, even 5 times a year; we had symposia also in Pakistan, Latin America; the fact that I spoke some Spanish helped. Suddenly, we had a global programme that was working...*" The reception of the Programme (identified with the acronym "HRP") was also very interesting: "*The communist countries were still closed, but we were able to enter; in the 1970s we organized a symposium in the USSR, together with the Minister of Health, for the first time ever… Most of those things were done for the first time…"* The financial aspects were not to be

neglected: "*Since we were bringing in some money, it was a very attractive way to create friendship for most of these people. For example, Cuba modified its national five-year health programme with an investment by the programme of 15000$"…" The Muslim countries were, of course, very difficult to tackle as they had problems accepting with the idea of gender equity; this was complicated work…*" In Africa:*" it was extremely difficult, because without the active collaboration of women this program could not survive. In Africa the general thinking around fertility was that one should not try to establish any contact with the woman by ways other than through her husband. The concept of women being in charge of their own fertility is a no go where men decide.*" In Asia:" *China had a policy of a one child family. The symposium organized with them was a great success. The Russians did as well. In a way, we succeeded in establishing work relations in countries where other programmes were officially received, but nothing further.*" India: "*they had their own family planning programme… in the south it worked, but in the north not very well.*"

As a conclusion "*In the years that followed, the WHO programme could bring information about family planning to countries where it used to be a vacuum, because of the church, or because of the politics.*

The latest area Egon worked in, remaining still very active is the aging process as a major concern of contemporary human society. Thus he mentioned: *"In 1984 I gave my first talk on aging at a congress on menopause … At first, I was very surprised, then I became more and more fascinated by the aging process, not at the individual level, but at the population level; how little we know about consequences of aging. In Europe, for instance, in the next 50 years there will be more people over 80 than under 15 years of age. How will such a society function? Nobody is*

willing, nor dares to talk about this! Life expectancy has increased a lot in the last decades; human race will prevail, but it would not do any harm to look into this a little in advance…It is obvious that there is progress, if you look back in time. What we need now, is to involve the younger generation more as they will shape our future. Do not believe that you will live forever! …"

Hopefully, I managed to draw a little sketch on what Professor Egon Diczfalusy meant for the scientific community. Proceeding from the professional career to the social life, I chose to make the transition using one of his hobbies which he enjoys while working as well - travelling- "*I have seen the world*".

Egon recalled some amusing happenings while travelling: "*We were invited to go to Latin America. So we were flying from Guatemala to Mexico and as we were having coffee, my wife looked out the window and she asked: 'Is it always like this?' 'Like what?', I replied. I saw the engine was burning 'No, it is not usually the case…'. After a moment, the captain announced that we encountered a small technical problem; I was surprised that for such a small technical problem, the plain was expected by so many fire-fighting cars back on the ground."*

"My most interesting travel was to China… We were allowed to fly only by Chinese Airlines, that had very old planes and no good communication between the cities we had to go to… except the telephone to our next location. We would get to the airport at 6 am and if we were lucky enough, the plain would take off during the afternoon".

Not neglecting the simple pleasures of life, Egon admitted "*The best food for my taste is probably the Chinese and the Japanese. From Europe I like the Italian and maybe the French cuisine*". Of course, a good meal is to

be served accompanied and helped by a glass of good wine: "*I used to be very fond of food and extremely good wines. The problem is that, now, I am physically handicapped to be able to enjoy food; I can still enjoy some liquids. If I have a bottle of wine I do not think it should be a cheap one, I'm too spoiled*". He also said:" *I like very much the writings of Bernard Shaw, I still admire Shakespeare and Garcia Marques*". Adding a good quotation, he cited William Faulkner: *'the past is never that. It is not even past" (Requiem for a dream)*. He still has time for his hobbies, although as he says: "*It is getting worse. Music is still one of the greatest, and art, paintings, galleries are still delighting me; even looking at an interesting piece of architecture, but it has been something that was instinctual. 'Without music life would be a mistake' Nietzsche said*". His favourite composers are: "*Mozart, J.S. Bach and then some modern like Bizet. I like opera very much opera, Verdi and also Puccini…"* Shocking news: "*When I am tired and if there is a very good football match, I enjoy watching it. Though, I have never played it…"*

Egon also developed a passion for languages: "*I think I am fluent in English, Italian, in German, in Swedish, Hungarian, and if I get sufficient exposure, in Spanish. I like to read in French, but I find it difficult to speak without too many mistakes. I enjoyed Latin in school as it was part of our education. I still like quotations in Latin, though it is not a current language.*" Asked on how he managed to learn Swedish, he admitted: *"I knew two words, which were stamped on my passport and they stated: 'Not valid'. Those were the first words I learned. The others came by the classical Hungarian approach, of the pickup method, which is very useful, but sometimes it may lead to major problems. I came to Sweden in 1946, met my wife in 1948 and she was, by mistake, a linguist. So she tried to*

teach the hopeless, to change my language, which was extremely difficult, because the word order in Hungarian is not like in western languages. It is also today when I have to write something, I go: 'Hey…reward, that goes there and that there'; so it is a problem." Amused, he continued when asked how long did it take to learn Swedish: "*As far as I see it, 100 years. I'm still making boo-boo's, I presume that even today I feel more familiar with English than with Swedish; English with its mentality, its structure is closer to me than Swedish"*.

Other two great accomplishment of Egon were: the lecture of art held every year in Stockholm and The Egon & Ann Diczfalusy Foundation, he started in Szeged in 2007. About these, he recalled: "*I was thinking back. In the 1960s, Dr Lotte Schenke (who was in charge with the foreign affairs in the pharmaceutical company CIBA in Basel, before they fusion with GEIGY) wanted us to carry out a study that was very difficult to do, as it involved taking daily blood samples from volunteers. It was Britt-Marie Landgren's project, so we did it together. They provided us with a sizeable amount of money. When the research was finished, there still was some money left and we asked them: 'Should we return it to you or would it be agreeable to you if we established a lecture of art?' Dr Schenke said that it was fine with them. Some papers were exchanged between the company and Karolinska Institute, so that this would come to a legal form. Eventually, we had a lecture of art. The very first lecture was organized in 1987. Professor Gyula Telegdy was the first one invited. Since then, every year there have been lectures with the exception of one single year, when there was an international congress. My formal students, my very good friends who happen to be outstanding scientists in the field, all of them, gave a lecture and most of these traditions last even today. Even this year, the lecture will*

take place, though there there will be a special symposium in Stockholm organized by Britt-Marie Landgren and Kristina Gemzell-Danielsson. That is how all started!

Then Professor Bartfai György said they wanted to have something similar in Szeged…'All right, do you want to have a lecture at the University of Szeged?'; 'No, we were thinking of something more ambitious!'; 'Hum…what?' 'How would it be, if we established a foundation? A foundation without funds'. So they did it. I gave them the last pennies I had from the Mahidol Prize to help them register the foundation at the court in Szeged and the foundation started functioning. That was 3 years ago."

When speaking with Egon it is inevitable that you end up talking, in a way or other, about philosophy and you will be mesmerized especially by his philosophy of life and his practical advice.

I wondered what is in his opinion the most important human quality and the answer was: "*To put yourself into the shoes of others, and realize that life, which is not spent for the others, is an incomplete life. And this goes to all significant scientists, starting with Albert Einstein… There is a very deep meaning in it, because 'Whatever you do for yourself, it goes to the grave with you; whatever you do for others, it has a chance to survive'. That was something that interested me very much all my life. I discovered this later in life… In your youth, you are, as I usually say 'living in your salad years'. Don't you agree? And then: 'you are slowly maturing… by recognizing your limitations, the limitations of others, in order to ask the pertinent question: What can I do in life? And the only answer is: try to do something for the others, that is the happy life!' I think it is a very complex process. You do it at the beginning without realizing what you are doing. My wife always said: 'But think of yourself also, not only of the others.' It*

was not a conscious decision for me, but it was kind of an impulsive instinct, an urge to try to do something for others. So maybe, it was not sheer accident that I had these boys and girls here with me."

Asked about his motivation for research Egon confided: "*Your own life conditions are influencing your path to a considerable extent, narrowing your future options. I left Hungary in 1946, I came here, having some 200 dollars in my pocket, big hopes and nothing else… We were struggling… I remember working a week in a factory, where they produced buttons made of leather. And I just couldn't do it. In Sweden, at that time, to practice medicine, you would have to pass examinations, to do this and that. Scientific research could be done only in a few places. Science keeps on motivating science."*

From this, we jumped into defining success and failure. That led to a wonderful discussion: "*They cannot be defined without defining your expectations. What kind of failure are you willing to accept, because it's more or less physiological? And what kind of achievement is really yours and not given by fortunate circumstances. I have a strong belief that: 'SORS BONA NIHIL ALIUM'. Have you ever been given a good faith, things will be rosy, otherwise you may be very talented and there still will be problems. When you are young you don't realize the sensitivity of others, therefore you may say things that later you will regret very much. You forget that once something inappropriate has been said, something that will cause an open wound in the other person that might be impossible to surpass, although sometimes it can be done. One thing I have learned, Cristi, something that came later to me, was that 'Whenever anything bad happened and I wanted to react with anger, it was better not to do anything the same day, to sleep the night over it. Then I tried to reconsider it the following morning. This*

has saved me a lot of problems and embarrassing situations because next day you see all things more lightly. It was not so important anymore. Many things did not go as I expected and only later you understand later, that it was the best that could happen to you, that those things did not go best. But in that moment you do not see it. Later…you do. The realistic approach is that we are never as happy or unhappy as we imagine… It is easy to say it when you are about 90 years old, it is somewhat trickier when you are in your early 20s and for instance get involved in a desperate love affair…The dramatic approach is the privilege of the young. When it remains into high ages, then you get into troubles".

From serious talks, we got, as usual, to funnier ones. The story about the Limoncello Society was most intriguing and made me include here a story from the Society's annals written by Egon. It is the story of the wonderful Zakushka recipe. "T*hen I've complained about headache. Professor Adlercreutz asked: "headache? You should have some 'zakushka'". Zakushka is a kind of a special sandwich in Russia…the Zakushka recipe I learned is that, when you are absolutely depending on your knees to walk further, alcohol being suspected and you need a pick-up then, take a slice of lemon, put on this freshly cut coffee and put all this into your mouth up to the palate and drink some aqua vite through it and the effect is incredible. "*

Aging, although not showing it at all, is always somewhere there in Egon's words. "*When you grow old as Bacon said: 'Prepare for neglecting'. That is very difficult… It is very well known that we live for the others. If you try to cheat and live only for yourself, it will be a miserable life. If you live for others it is a positive feeling that you can be of service … I quoted Tagore saying: 'I slept and dreamt that life is only a pleasure. I got*

awake and I have seen that life is something different and that life is serving others and I found that serving others is the pleasure in life.‘ “

His thoughts and advice for the younger generation are: “*Have faith and trust yourselves. It is still possible to find essential new information. New information is like the perimeter of a circle that is increasing, like your view of the unknown that is also increasing. You can see much more of what was hidden from you before… It doesn’t mean that you can immediately solve them, but now, at least, you can see what the problems are. … Albert Einstein said: ‘All our knowledge is primitive and childlike, but still, it is the best thing we have.’ In a world where the new information doubles every six to seven year, it is impossible to become a new Michelangelo or a Leonardo. Those times passed. You must necessarily specialize in a very small section of the perimeter, if you want to push it further into the unknown. Cirus, The Persian Emperor said that: ‘No one can know what he can do, before he tries it’. That is perhaps the message for the young generation*”.

After the message for the younger generation he continued with the message to take home, from Rönninge: “*The message will be from Euripides, from his drama Heracles, where Heracles says that: ‘He, who believes, that wealth and power are more important than to have devoted friends, makes a major mistake’…I have had a very great privilege in life of having close friends each country I went to… You cannot have friends unless you are one of them. I have many friends that have changed my life… This is to the young generation: ’Try to find many friends because otherwise you will be left alone when you are very old… When you get to the older ages try to see if you can get some friends who are somewhat younger, so that they will not be so handicapped as yourself…’”.*

For those starting Medical School, he said that the most important discovery and achievement humanity made *is that at that age you might not know yourself… You may not know what you really would like to do in this life… This sounds like a hypocritical statement:' You have to live for others in order to have a satisfactory and happy life'… In the medical profession you have all the preconditions to do this, if you have the emotional commitment… Obviously you will have disappointments in life, as they are inherent to the human condition. The Medical School provides a path for a meaningful life. Other professions might get you disillusioned finding out that you have wasted your life*".

I saved, last but not least, one of the most interesting self characterizations I have ever had the opportunity to hear: "*I am a strange mixture between the Hungarian and the Swedish characters. In many aspects I am like a Hungarian trying to surface as much as possible to cover everything and to go further…In other aspects it is the Swedish attitude … very systematic, going into the depth… When I look back I must thank my fate that I have had a very interesting life, that, I have seen more than the average scientist, I have travelled more and through this I have acquired more friends than the average scientist… It is difficult to imagine what the day of tomorrow will bring, but at my age you have to recognize that your future is behind you… I say this with no regrets and no complaints, as Edith Piaf said… Recognize that life is very short, so do what you really would like to do if you can, and of course do not sell your soul…It is very difficult to place yourself into your own age"*

Text by Dr Cristian Furău

Picture 4: Egon and his new friends from Rotaract Cetate Arad

Picture 5: Egon and "Ninni" Mancuso's family

Picture 6: before the ancient Greek drama in Syracuse

Picture 7: a happy reunion with some of the former students, Bijing 1992

Picture 8: "Conferencia de la Fertilidad Humana", Mexico, 1970

Picture 9: Egon and his friends having a glass of beer

Picture 10: Ann and Egon Diczfalusy

Picture 11: Egon and Ann Diczfalusy

Picture 12: Symposium in China

Picture 13: Egon coordinating the activity from his office

Picture 14: Professor Diczfalusy receiving the Prince Mahidol Award

Family tree
Dietz – Diez – Diczfalusy

1.	DIETZ	Wolf	born before 1515, died 1566, Staffelstein, Germany
2.	DIETZ	Alexander	born around 1535, died 1586, Staffelstein, Germany married to Weidner, Barbara
3	DIETZ	Ambrosius	1578 – 1662, married to Forndran, Elisabet
4.	DIETZ	Andreas	1656 – 1730, married to Wiedemann, Rosina
5.	DIETZ	Johann Carl	1694 – 1757, married to Leipold, Catharina Elisabeth
6.	DIETZ	Fransiscus Georg	1737 – 1793, (Dr. Med., Jena 1759) married to Schmidt, Dorothea Barbara
7.	DIETZ	Johann Carl	1777 – 1860, (Dr. Med., Jena) Married to Bencker, Barbara Elisabetha

Changes family name to DIEZ around 1800

8.	DIEZ	Eduard August Hermann	1801 – 1858, married to Decker, Johanna Carolina
9.	DIEZ	Hermann Alfred Gustav	1835 – 1906, married to Edle von Kemenovic, Mila
10.	DIEZ	Ferdinand Julius Alfred	1864 – 1926, married to Sirk, Elvira
11.	DIEZ	Günther Franz Raimond	1895 – 1950, married to Wildmann, Piroska

Changes Family name to DICZFALUSY in 1937

12.	DICZFALUSY (born Diez)	Egon R.	1920 - married to Björk, Ann Eivor Margareta
13.	DICZFALUSY	Ulf Günther	1951 -
	DICZFALUSY	Bo Egon	1952 -

Picture 15: Dietz- Diez- Diczfalusy Family tree

REPRINTS

A LIFETIME DEVOTED TO REPRODUCTIVE HEALTH

INTERVIEW WITH EGON DICZFALUSY

A MAN WHO PUBLISHED HIS FIRST SCIENTIFIC PAPER ALMOST 60 YEARS AGO MIGHT REASONABLY BE EXPECTED TO DWELL ON THE PAST. BUT IT IS THE FUTURE THAT MOST INTERESTS EGON DICZFALUSY, THE EMINENT HUNGARIAN-BORN ENDOCRINOLOGIST WHO INPIRED GENERATIONS OF RESEARCHERS AND HELPED BRING CONTRACEPTION TO HUNDREDS OF MILLIONS OF PEOPLE.

EgonDiczfalusy, 80, professor emeritus at the Karolinska Institute in Stockholm, Sweden, has had a many-faceted career. As a scientist, he is best known for discovering how the fetus uses complex hormonal mechanisms to steer the placenta during gestation. As a respected teacher, he trained some 150 fellows from dozens of countries in endocrinological research, many of whom now run major academic and clinical centres of their own. Perhaps most significantly, he set up, through the World Health Organization (WHO), the structures for research on hormonal contraceptive technologies worldwide - a process that helped make contraception ethically and politically accepted in a wide range of cultures.

But Diczfalusy's latest ideas concern the future. He is focusing his energy on raising awareness of the challenges facing the world's rapidly ageing populations, in which fertility is dropping, creating top-heavy structures where numbers of children, are matched or exceeded by the elderly. "The world is ageing, but its institutions a rest ill those that were designed by our forefathers," he says. He believes that myopic- politicians have paid too little attention to questions about how tomorrow's societies will pay their bills or manage their lives if institutions fail to keep pace with today's demographic and health trends.

For example, many adults now reach age 65 in good health , in economies where physical strength is no longer essential in order to earn. Perhaps people who could stay in paid work for longer should do so. Cities in industrialized countries contain increasingly large numbers of single-person households; the nature of the family is changing radically. "It would not hurt," Diczfalusy suggests with characteristic gentleness, "to have another look at pensions, retirement ages, and health services even simple things like housing."

Diczfalusy is well known as a champion of the health needs of developing countries, where population ageing may have an even greater impact than in the developed world. In Thailand, for example, the scale and pace of change is far greater than in Europe. In 1950, there were 14.2 children to every Thai adult aged 65 and over. Today, here are just 4.3 children to each elderly adult. By 2025, United Nations (UN) projections suggest the figure will be 1.6, falling to 0.9 in 2050. Europe, by comparison, had 3.2 children per elderly adult in 1950. It has 1.2 today. The ratio is projected to fall to 0.6 in 2050. Diczfalusy wants people to think about these trends but he is no doom-monger. Look at the twentieth century, he says: there has been genuine progress. Why should human beings not find new ways to shape future societies?

HISTORICAL VISION

The dramatic changes Diczfalusy has witnessed inform his perspective on the future. "Only those with some knowledge of the past can have a vision of the future," he says, quoting from T S Eliot's poem Four Quartets, the section called Little Gidding': "A people without history / Is not redeemed from time, for history is a pattern / Of timeless moments." It seems appropriate, therefore, to sketch Diczfalusy's life. He was born in Miskolc, Hungary, in 1920. An only child, he grew up in a country devastated by the First World War. He went to the University of Szeged to study medicine, obtaining his MD in 1944, just days before the Russian army occupied the city.

While still a student at Szeged in 1942, he published his first paper - a study in microbiology that was to affect his life more than he could have known. He had been trying to repeat an experiment by the Nobel laureate

Hans von Euler, then at the University of Stockholm. Von Euler's group had found transaminase activity in suspensions of yeast and E. coli bacilli. Diczfalusy could not reproduce the results. His professor urged him to publish. The paper appeared and was instrumental, Diczfalusy believes, in bringing him to Stockholm after the war to work as von Euler's assistant from 1946 to 1947.

But then Diczfalusy's funding ran out. Fortunately, Professor Axel Westman gave him a post in the hormone laboratory, Department of Women's Diseases, and quickly promoted him to laboratory head. Diczfalusy was amazed, he says. He "knew nothing" about endocrinology at the time. Westman told him that, if that was so, it was time he learnt. By 1950, with Westman's support, Diczfalusy had become a Swedish citizen. He obtained his PhD from the Karolinska Institute in 1953 and became associate professor of experimental endocrinology in 1954. By 1967, he was the institute's professor of reproductive endocrinology, and director of the reproductive endocrinology research unit of the Swedish Medical Research Council.

The 1950s and 1960s were highly productive years for Diczfalusy, initially in extraction and purification of gonadotrophins, then in research on the feto-placental unit and the induction of ovulation. "His lab and his publications were the gold standard in these areas of research," says David Baird, another distinguished reproductive biologist, now professor emeritus at Edinburgh University, Scotland.

In the early 1960s, the world's population reached 3 billion. Demographers published dire projections of explosive continuing growth. Those projections have since proved extreme, but few people at the time doubted the need for action. With the aim of encouraging global research and development in contraceptive technologies, the Ford Foundation invested heavily in the field. In 1962, the foundation awarded Diczfalusy's group US$500,000 (a huge amount at the time) to study the endocrinology of early pregnancy. The grant enabled Diczfalusy to set up a world-class laboratory, and to train a succession of scientists from other countries.

"Only those with some knowledge of the past can have a vision of the future”.

One of those trainees, Giuseppe Benagiano, now heads the Istituto Superiore di Sanita in Rome. He remembers Diczfalusy as a tough but caring teacher and a perfectionist. Diczfalusy, who speaks seven languages, expected equally high standards of his proteges. Benagiano recalls a sleepless night after another Italian from the lab had had the misfortune to be first in the group to read a paper at an international meeting. Afterwards, Diczfalusy's only comment had been: "Sergio, you'd better improve your English if you ever want to present a paper again, no kidding."

Demanding as those student years may have been, Diczfalusy's trainees seem to remain devoted to him. "What impresses me is not so much their number and diverse geographic origins but their continued loyalty to him," says his long-time colleague and friend Mahmoud Fathalla. “As a teacher myself, I find this a very enviable record.”

THE CONTRACEPTIVE REVOLUTION

Diczfalusy's own interest in research on hormonal contraception grew during the 1960s. In 1972, he was one of the founders of what was to become the UN human reproduction programme (HRP) at the WHO. The WHO also accepted Diczfalusy's laboratory at the Karolinska Institute as the first WHO collaborating centre for research in human reproduction. From this time on, Diczfalusy's commitment to improving the health of the world's poorer majority drove his work.

For the next 25 years, alongside his Karolinska post, Diczfalusy acted as a consultant to the UN programme and its directors - including Fathalla and, most recently, Giuseppe Benagiano. He has travelled constantly on WHO's behalf to establish a network for research and to help foster the political will to give couples easier access to contraception. The statistics alone provide a measure of success. In 1960, the number of people using contraception worldwide was estimated at fewer than 30 million. By 1993, it was estimated to be nearly 600 million. Baird believes that Diczfalusy was an essential part of the programme's political and scientific success in helping bring about the "contraceptive revolution". "He is an articulate, well educated man," Baird says, "and it was possible for him to get direct access to government ministers in a way that a less civilized person would have found less easy."

It was not just the popularity of contraception that changed markedly during Diczfalusy's time at the WHO. The rationale for promoting contraception also changed. Family planning technologies are no longer used solely to control population growth. Most governments now view contraception as just one part of the larger issue of improved reproductive health - which, in turn, is part of economic and social development and of human rights. This "intellectual revolution", as Diczfalusy calls it, culminated with the 1994 United Nations International Conference on Popularion and Development in Cairo.

TACKLING THE DISEASES OF POVERTY

Six years later, Diczfalusy celebrated his 80th birthday. Aptly enough, he was invited to a conference in Berlin, held in his honour to discuss new pharmacological approaches to reproductive health and healthy ageing. But his preoccupation with global health problems remains as acute as ever. Ask him his top priority for health research and the answer is not, as the uninitiated might expect, a glamorous problem of molecular biology, or a mechanism to halt the ageing process. Instead, he is passionately keen to see a serious effort to tackle the diseases of poverty. In the field of reproductive health, this includes finding ways to prevent the spread of sexually transmitted infections, including HlV; to reduce the continuing and unacceptably high death toll from unsafe pregnancy and childbirth in developing countries; and to provide access to contraception, including a wider choice of technologies, such as male methods, barrier methods for women, and emergency contraception.

Beyond reproductive health, the challenges include the infectious diseases that continue to blight the lives of the poor, and the common noncommunicable diseases such as depression and diabetes - which warns the WHO, will become increasingly significant in the ageing populations of the developed world. If funds cannot be found to develop affordable drugs and vaccines to meet the basic health needs of the world's most disadvantaged populations, Diczfalusy says, then "we are intellectually bankrupt".

Diczfalusy continues to be interested in improving contraceptive technologies, insisting that the revolution is not yet a closed chapter. He is

keen, for example, to see knowledge from the Human Genome project used to tailor drugs to individuals. Recognizing that high costs have deterred much of the pharmaceutical industry from investing further in contraceptive research, Diczfalusy is enthusiastic about a new collaboration between the Rockefeller Foundation and industry to investigate genomic approaches to developing male contraceptives. Under the collaborative programme, for example, researchers in the US, Australia and Europe are trying to identify the genes involved in the maturation of sperm in the epididymal duct, with the aim of interrupting the process.

THE WIDER PICTURE

Diczfalusy also wants to see more research and development in the field of hormone replacement therapies. For example, he would like more studies on the reported differences in menopausal symptoms between women in different populations, believing that Western researchers need to listen to, and learn from, the experiences of researchers in Asia. Physicians, too, need to become more sophisticated in their use of these therapies, he believes. A menopausal woman today is likely to have 30 years of life ahead of her; appropriate therapy can make a major difference to her quality of life.

The prospects for male hormone replacement therapy also interest him, although he maintains a wry skepticism about current research efforts. "When the dust has settled, you will find out whether you are riding a horse or an ass," he says, quoting one of the Arab proverbs of which he is fond. But, once again, his focus is on major global needs. Male hormone replacement therapy is likely to be developed not just because men want to maintain their sexual drive but also because it may help avert an epidemic of osteoporosis and hip fractures in ageing populations.

Diczfalusy comes across as a courteously formal man, disciplined by decades of rigorous work. Tall and fit, he looks much younger than his years. But, despite the formality, he reveals his emotional side. The sadness of leaving his native country and making his home in a society known for its reserve and caution has clearly taken its toll, despite a happy marriage and family life in Sweden. He recalls a terrible visit to Hungary in the early 1970s when he realized he could no longer speak the language fluently.

Subsequent visits have proved happier. One senses that he enjoys the release of being in sunnier and more expressive communities in his regular travel outside Sweden. Then, the lighter side that is often glimpsed in his wry humour is able to emerge more fully. At a conference in Capri, for example, he helped form the Limoncello Society, which invented a country called San Limone, complete with its own gazette.

Diczfalusy looks back on three broad eras: the deeply pessimistic era of war up to 1945; the optimistic postwar era, which ended around 1975; and the "confused era" of the present. If people are not confused in the current epoch, he says, they are probably ill informed. But, despite the confusion, he continues to look ahead with hope, believing that human beings will solve their problems. He quotes another Arabian proverb: "There is a saying that, when a man is young he writes poetry, when he is grown up he speaks in quotations, and when he is old he preaches pessimism. Well, I have not reached that stage yet." •

Phyllida Brown is a science writer based in Exeter, UK.

Reprinted from: Orgyn, no2/2001

CLINICAL EFFECT OF HUMAN PITUITARY FOLLICLE-STIMULATING HORMONE (FSH)

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Reprinted from

ABSTRACT

A partially purified follicle-stimulating hormone preparation (human pituitary FSH) has been obtained from human pituitaries. The ovarian response to this preparation was studied in 7 amenorrheic women. The effect of human chorionic gonadotropin (HCG) was studied in addition.

In 4 patients exhibiting no endometrial activity or only slight proliferation, HCG alone did not induce ovulation and had no effect on the size of the uterus, on the endometrium, or on the urinary excretion of estrogen and pregnanediol. In 2 patients showing endometrial proliferation, the administration of HCG alone was followed by ovulation, a secretory transformation of the endometriun, and a marked increase in urinary pregnanediol excretion.

The administration of human pituitary FSH alone to 2 patients resulted in an increase in the size of the uterine cavity, in polycystic enlargement of the ovaries, and in a pronounced increase in urinary estrogen output.

Treatment with human pituitary FSH followed by HCG produced in all patients polycystic enlargement of the ovaries, ovulation in 4 out of 5, and a secretory transformation of the endometrium in 3 out of these 5 patients. Ovulation was accompanied by a marked increase in the urinary excretion of both estrogen and pregnanediol.

FOLLICLE STIMULATING hormone (FSH) has been purified from sheep (1) and hog (2) pituitaries. Such preparations possess a high degree of purity and have been tried in clinical studies by several investigators, but with inconclusive results. Some investigators report definite hormonal effects, whereas in the opinion of other the results are discouraging. The reason for the inadequate clinical effect of such preparations may be sought in 1) species differences, 2) differences in hormonal requirement, or 3) the formation of antihormone which following repeated administration, might interfere not only with the action of exogenous gonadotropins but also with that of the endogenous ones (3).

It has been demonstrated by Bahn et al. (4) that homogenates of humananterior pituitaries possess FSH activity when administered to hypophysectomized immature female rats. Since the evidence available at present (5) seems to indicate that gonadotropin extracted from human urine has no ability to provoke antihormone formation in clinical experiments, an attempt was made to purify follicle-stimulating hormone from human pituitaries. The data presented in this paper indicate that it is possible to obtain human pituitary follicle-stimulating hormone (hereafter called human pituitary FSH) from autopsy material, and that such preparation exhibit a marked hormonal effect when tested in human beings.

SUBJECTS AND PROCEDURE

The human pituitary FSH preparation was tested in 7 young amenorrheic women. The relevant anamnestic data are listed in Table 1.

Two of the 7 patients had primary amenorrhea and 5 had secondary amenorrhea, of at least two years' duration. Urinary gonadotropin excretion was low or normal. None of the patients has been treated previously with pregnant mare's serum gonadotropin (PMS) or with any other gonadotropic preparation.

*Scheme of treatment.*Since it has been reported by several investigators that a luteinizing factor, such as human chorionic gonadotropin (HCG), by itself may induce ovulation and endometrial changes in amenorrheic women, the effect of HCG ("Gonadex- Leo", manufactured by AB Leo, Hälsingborg, Sweden) was studied in each patient one to two months prior to the start of

treatment with human pituitary FSH. The dosage was 6,000 I. U given intramuscularly daily for a least six days. Human pituitary FSH was injected intramuscularly in daily doses of 10 to 30 mg. It was administered either alone or in combination with 6,000 I.U of HCG daily. No untoward effects, such as fever or local reaction at the site of injection, were observed.

Assessment of clinical effect. In the assessment the clinical effect of human pituitary FSH the following procedures were employed: measurement of the uterine cavity, palpation of the ovaries, inspection of the ovaries by culdoscopy, histologic picture of the endometrium, and determination of urinary estrone, estradiol-17β, estriol, pregnanediol, 17-ketosteroids and 17-hydroxycorticosteroids.

Patient No.Initials Age(yrs)	Type of amenorrhea	Duration of Amenorrhea (yrs)	Size of uterine Cavity(cm.)	Endome-trial activity *	Urinary Gonado-Tropin† (M.U/24 h)	Comments
1)G.K. 22	Primary	-	5	0	<13	Endometrial damage(?)
2)M.H. 25	Primary	-	4	0	>6.5<13	
3)B.ö. 29	Secondary	4	6	0	<6.5	
4)L.F. 21	Secondary	2	6	P(weak)↕	>13<53	
5)E.G. 26	Secondary	6	7	P(medium)	<6.5	
6)I.F 22	Secondary	5	8	P(medium)	>6.5<13	

7)A.S 25	Secondary	3	7	P(strong)	>6.5< 13	Stein-Leventhal syndrome(?). Two menstrual-like bleedings during the previous 3 years.

*Repeated findings.
†Mouse uterus test. A “mouse unit” (M.U) corresponds roughly to the activity of 0.3 to 0.6 mg. of HMG-20 standard.
↕P=proliferative.
METHODS

Preparation of human pituitary FSH
The purification of human pituitary FSH is based on the assumption that (as with sheep FSH) it is the principal pituitary hormone soluble in ammonium sulphate solution above 50 per cent saturation (1). Human pituitaries obtained at autopsy were frozen and lyophilized. The dried glands were cut into small pieces, homogenized, and extracted in cold CaO solution at pH 9.3 under continuous stirring for at least 6 hours. Approximately a 10-ml. volume of CaO solution was used per 100 mg. of dried pituitary. After centrifugation, the clear supernatant liquid was slowly brought to 55 per cent saturation by the addition of a saturated solution of ammonium sulphate. The solution was allowed to stand overnight in a cold room (4° C) and then centrifugated. The precipitate was discarded and the clear supernatant layer was brought to 75 per cent saturation by the addition of solid ammonium sulphate. The material precipitating between 55 and 75 per cent saturation with ammonium sulphate was collected by centrifugation, dissolved in water, dialyzed and lyophilized. This product, provisionally designed as human pituitary FSH, was used in the clinical trials.

Potency of human pituitary FSH when assayed in immature rats

The human pituitary FSH was assayed against the provisional human menopausal gonadotropin (HMG-20) standard preparation. This urinary standard possesses both FSH and LH-activity (6). The uterine weight method was used as described previously (7), the ovarian weight method was carried out according to Albert (8), and the ovarian augmentation test according to Steelman and Pohley (9), with the exception that the amount of HCG used was only 10 I.U. The method based on the enlargement of the ventral prostate in hypophysectomized rats was carried out as described by Greep *et al.* (10), using the modification introduced by Loraine and Brown (11). With the exception of the ventral prostate method, in all assays littermate control was employed. The statistical methods used for calculating the relevant parameters have been described in detail elsewhere (12, 13). All assays satisfied the recongnized criteria of validity. The results are summarized in Table 2.

It may be seen that, on a weight basis, the partially purified human pituitary FSH was 30 to 50 times more potent than the HMG-20 standard preparation, when assayed by various methods measuring total gonadotropic or FSH activities.

Table 2: POTENCY OF PARTIALLY PURIFIED HUMAN PITUITARY FSH WHEN ASSAYED AGAINST HUMAN MENOPAUSAL GONADOTROPIN (HMG-20) STANDARD IN IMMATURE RATS

Type of immature rad	Index of response	No. of rats Per assay	Potency of human pit. FSH(per mg) vs. HMG-20	Fiducial limits Of error (P=0.95)	Index of precision (λ)	Index of discri-mination
Intact female	Uterine weight	28	47.7 □	41.4-54.4	0.07	-
Intact female	Ovarian weight	38	39.4 □	27.3-50.0	0.13	1.21

HCG-treated female	Ovarian weight	36	28.3 ?	22.3-35.7	0.14	1.69
Hypophysectomized male	Ventral prostate weight	14	5.4 ?	2.8-9.5	0.14	8.85

On the other hand, in the ventral prostate test which is considered to be specific for LH-activity, the human pituitary FSH was only approximately 5 times as potent as HMG-20. Gaddum's (14) index of discrimination was also applied to the data, as shown in the last column of Table 2. This index is calculated by dividing the potency ratio obtained in one test by the corresponding figure in another. This index is close to unity when 2 substances possessing identical biologic activity are assayed. When the uterine weight method was employed as a basis for this comparison, only the value obtained by the ventral prostate method showed a greater divergence from unity. This would seem to indicate that the partially purified human pituitary FSH was a richer source of FSH-than of LH-activity, when compared to urinary menopausal gonadotropin. The partially purified human pituitary FSH was also assayed for growth hormone (GH)-activity, using the tibial test of Evans *et al.* (15) in hypophysectomized rats. Only traces of GH-acctivity were found.

The fraction precipitating at 55 per cent ammonium sulphate saturation(see section on preparation of human FSH) was also tested at the 1-mg dose level for FSH- activity in hypophysectomized immature female rats. No follicular growth was observed, but the repair of interstitial wheel cells indicated the presence of luteinizing hormone(LH)- activity. A Steelman-Pohley test (9) was also carried out at the same dose level, with negative results. Thus, it would appear from this data that it is possible to obtain from human pituitaries partially purified fractions exhibiting predominantly FSH- and LH-activities, respectively.

Assessment of endometrial biopsy specimens

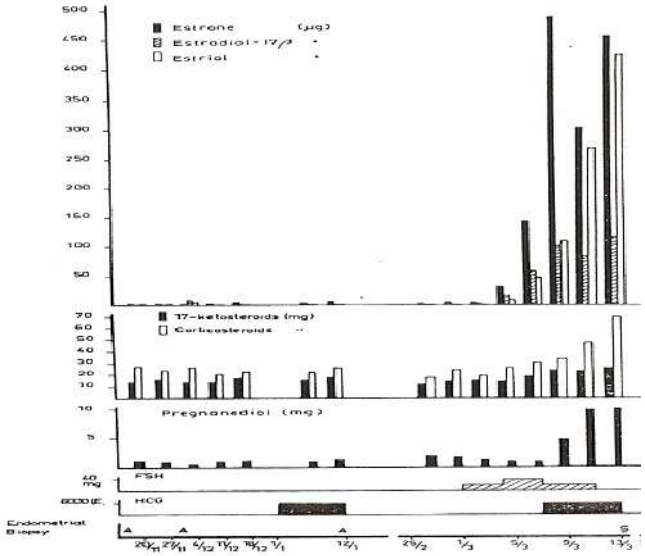
All patients were followed by serial endometrial biopsies. The endometrium was recorded as being:

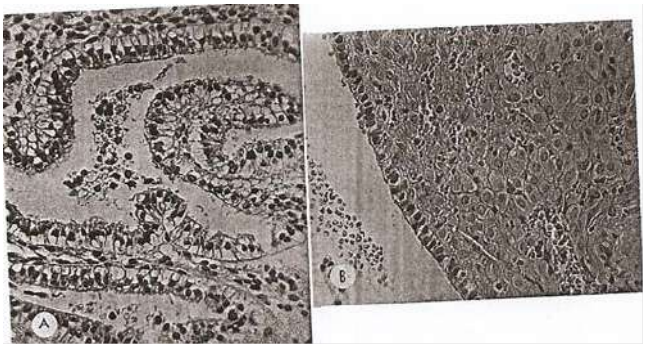
Atrophic (A) i.e., no endometrial material or only a thin endometrium with small inactive glands could be obtained.
Proliferative (P), graded as slight (P), moderate (P) and marked (P)
Secretory(S) sometimes combined with decidual reaction (S)
Each biopsy specimen from the same patient was taken from a different part of the uterine cavity in order to avoid areas of tissue damage from previous biopsies.
Urinary steroid assays
Estrogen assays were restricted to the estimation of 3 "classic" estrogens-estrone, estradiol-17β and estriol. The method of Brown (16) was used, with slight modifications as described by Diczfalusy *et al.* (17) and Brown *et al.* (18). The term "estrogen" will be used in this paper in a loose sense to denote estrone, estradiol-17β and estriol.
Pregnanediol was estimated according to the method of Klopper *et al. (19),* with the exception that the color correction equation of Allen (20) was used. The evidence in favor of this modification has been presented elsewhere (21).
17-ketosteroids (17-KS) were estimated according to the micromethod of Vestergaard (22), with certain modifications, including the use of the Allen (20) formula.
Corticosteroids were assayed as 17-hydroxycorticosteroids (17-OHCS). Using the method described by Appleby *et al.*(23) as modified by Birke *et al.*(24). Thus, the term "urinary corticosteroids" will be used to denote all C21-steroids possessing a 17-hydroxyl group, including compounds with the 17:20-glycol, 17:20-ketol, 17:20:21-triol and dihydroxyacetone side-chain.

RESULTS

Case 1
The results obtained in this patient are shown in Figure 1. It may be seen that HCG alone had no effect on the endometrium or on the urinary excretion of estrogen and pregnanediol.
Human pituitary FSH administered for six days induced a polycystic enlargement of the ovaries and a significant rise in estrogen excretion,

without an Human pituitary FSH administered for six days induced a polycystic enlargement of the ovaries and a significant rise in estrogen excretion, without an effect on pregnanediol excretion. When human pituitary FSH was supplemented with HCG, the urinary estrogen excretion reached levels usually seen only in pregnancy. The rise in estrogen was accompanied by a marked rise in urinary pregnanediol and also in urinary corticosteroid excretion. The endometrium changed from a completely atrophic state to a secretory phase, with an almost decidua-like histologic picture, as shown in Figure 2.





Culdoscopy revealed enlarged polycystic ovaries (the left one with a diameter of at least 10 cm.) and a fresh ovulation in the right ovary. Eight days later the enlargement disappeared and the patient had menstrual bleeding of six days' duration.

Case 2

HCG by itself had no effect on the endometrium or on urinary estrogen and pregnanediol excretion, as shown in Figure 3.

Human pituitary FSH administered alone had no effect on urinary estrogen and pregnanediol excretion, but when it was followed by HCG the excretion of estrogen increased singnificantly. Pregnanediol excretion was not affected in this case.

Culdoscopy revealed moderately enlarged polycystic ovaries without any sign of fresh ovulation. Menstrual bleeding occurred eight days later and lasted for five days.

Case 3

HCG had no major effect on urinary estrogen and pregnanediol excretion, as shown in Figure 4.

Human pituitary FSH caused a minor but significant increase in the estrogen output. Combined treatment with human pituitary FSH+HCG resulted in greatly increased estrogen and pregnanediol excretion accompanied by a rise in urinary corticosteroid excretion.

Culdoscopy showed polycystic enlargement of the ovaries and a fresh ovulation. There were no sings of activation in the endometrium, but the size of the uterine cavity increased by at least 2 cm. Five days later scanty menstrual bleeding occurred, which lasted for five days.

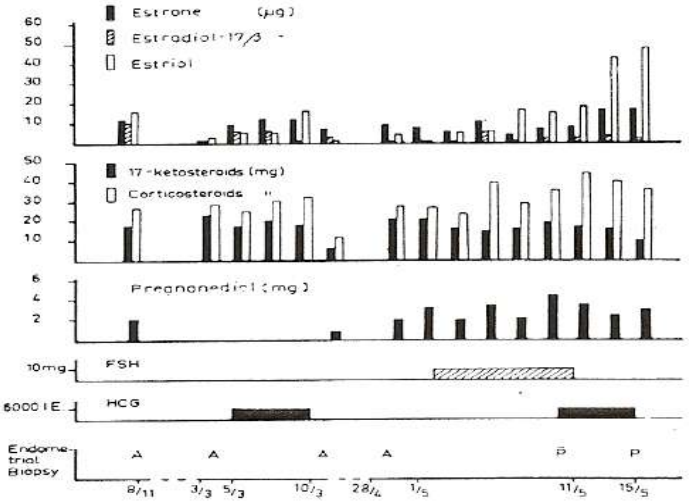


FIG. 3. Steroid excretion (48 hours) and endometrial activity in Case 2, during treatment with HCG and h.pit. FSH.

Case 4

As shown in Figure 5, HCG had no effect on urinary pregnanediol excretion and only a slight effect on urinary estrogen excretion or on the activity of the endometrium.

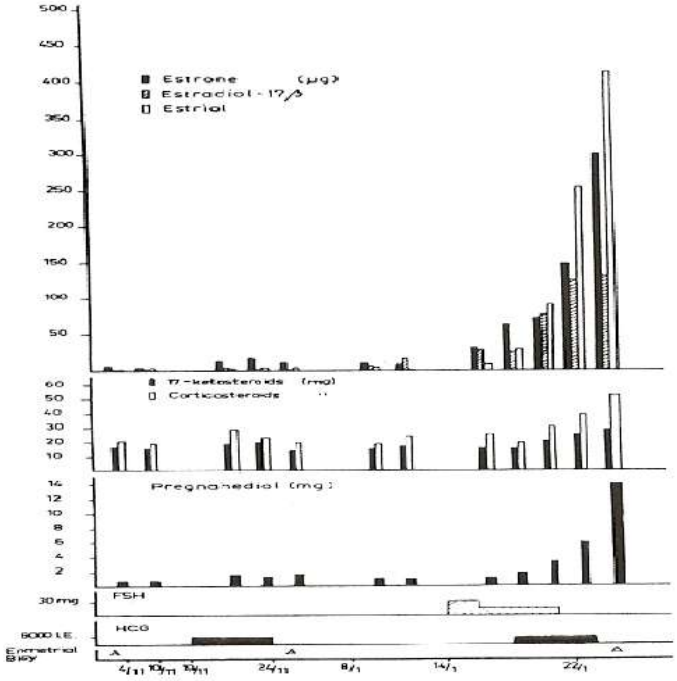
Human FSH by itself had no effect on pregnanediol excretion and but slight if any effect on urinary estrogen output. However, the combination of human pituitary FSH+HCG resulted in a marked increase of both estrogen and pregnanediol excretion.

The endometrium exhibited a secretory reaction with an almost decidua-like histologic picture. The culdoscope revealed enlarged polycystic ovaries and a fresh ovulation in one of them. A week later menstruation occurred, at which time the ovaries had resumed their normal size.

Case 5

As shown in Figure 6, the administration of HCG was followed by a marked rise in urinary pregnanediol excretion without any concomitant increase in estrogen output. The endometrial reaction was secretory and a fresh ovulation was found.

On the another hand, human pituitary FSH by itself induced a marked rise in estrogen excretion without any appreciable increase in pregnanediol output. The proliferative activity of the endometrium was only slightly increased without any signs of a secretory reaction. Culdoscopy revealed polycystic enlarged ovaries but no fresh ovaries.



Case 6

The findings in this patient (Figure 7) resembled those in Case 5. Following administration of HCG there was a definite rise in urinary pregnanediol excretion but only a slight increase in estrogen output. The proliferative endometrium was transformed into a secretory one.

The administration of human pituitary FSH by itself resulted in a pronounced rise in the excretion of urinary estrogen and in a moderate rise in the excretion of pregnanediol. The endometrium did not show any signs of secretory reaction.

Case 7

This adipose, hirsute woman had amenorrhea and a moderate cystic enlargement of the right ovary-Stein-Leventhal syndrome. As shown in Figure 8, she was treated with human pituitary FSH for eight days, and then by combined FSH+HCG therapy for two days.

Following the hormonal treatment, the endometrium showed a secretory reaction. Urinary excretion of pregnanediol, but not of estrogen, increased.

The day following the last injection, the patient was operated upon, and both ovaries were found to be polycystic and enlarged. In the left ovary a fresh ovulation was seen. This is shown in Figure 9.

On histologic examination, the corpus luteum was estimated to be approximately six days old. Wedge resection was performed on both ovaries. Four days after operation menstrual bleeding occurred. Four weeks later there was another episode of menstrual bleeding , and during the next period the patient became pregnant.

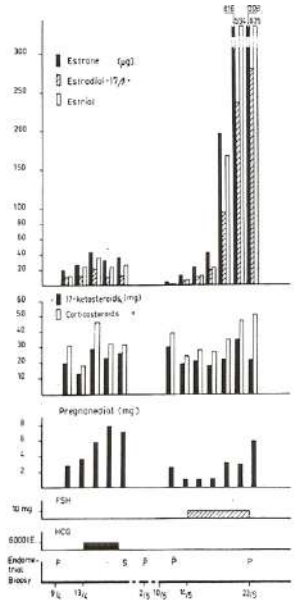


FIG. 7. Steroid excretion (48 hours) and endometrial activity in Case 6 during treatment with HCG and h.pit. FSH.

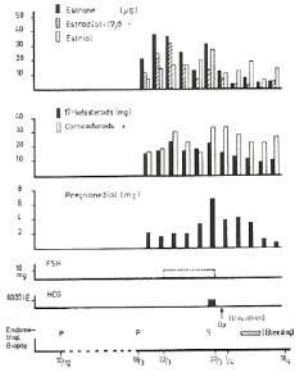


FIG. 8. Steroid excretion (48 hours) and endometrial activity in Case 7 during treatment with h.pit. FSH and HCG.

Summary of response of patients to different hormonal treatments

The responses of the 7 patients to the 3 types of treatment are summarized in Table 3.

In 4 cases HCG by itself did not induce ovulation and had no effect on the endometrium or on the urinary excretion of pregnanediol or of estrogen. In these patients repeated endometrial biopsies carried out before the start of treatment indicated an inactive endometrium or only slight proliferative activity (1 case). On the other hand, in 2 patients showing endometrial proliferation, the administration of HCG was followed by ovulation and a secretory transformation of the endometrium. These changes were accompanied by a marked rise in urinary pregnanediol excretion, whereas estrogen excretion was practically unchanged. The administration of human pituitary FSH produced in 2 patients a pronounced rise in urinary estrogen excretion. This was accompanied by a proliferative reaction of the endometrium. Pregnanediol excretion was virtually unchanged. When human pituitary FSH therapy was combined with or followed by HCG therapy, the result was in all cases a ploycystic enlargement of the ovaries. Ovulation took place in 4 out of 55 patients, and a secretory reaction of the endometrium occurred in 3 of them. These changes were paralleled by a rise in urinary pregnanediol and estrogen excretion, to a level much above that normally found during the menstrual cycle.

FIG. 9. Enlarged, polycystic ovaries with a corpus luteum in the right one, found at laparatomy in Case 7.

There were, however, a few results which require some comments. Thus, in Case 2 there was no ovulation and consequently no rise in pregnanediol excretion. Even the rise in estrogen output was rather limited when

compared to that seen in the other cases. The reason for this is not well understood; it is possible that this patient might have required a higher dosage and/of a repeated course of human pituitary FSH+HCG. It also appears

TABLE 3: RESPONSE TO HUMAN CHORIONIC GONADOTROPIN (HCG), TO HUMAN PITUITARY FSH, AND TO FSH+HCG IN 7 AMMENORRHEIC WOMEN

	HCG			FSH				FSH+HCG				
Case No.	Ovula-tion	Endo-met. Re-Sponse*	Uri-Nary Estro-gen	Uri-Nary Pregnan-ediol	Ovula-tion	Endo-met. Re-Sponse*	Uri-Nary Estro-gen	Uri-Nary Pregnan-ediol	Ovula-tion	Endo-met. Re-Sponse*	Uri-Nary Estro-gen	Uri-Nary Pregnan-ediol
1	0	0	0	0					+	S	+	+
2	0	0	0	0					0	P	+	0
3	0	0	0	0					+	0	+	+
4	0	P	0	0					+	S	+	+
5	+	S	0	+	0	P	+	0				
6	+	S	0	+	0	P	+	0				
7									+	S	0	+

*P = proliferative activity

S= secretory reaction

(Table 3) that in Case 3 there was no endometrial response, although both pregnanediol and estrogen excretion were greatly elevated. It is possible that in this case the endometrium was damaged and could not respond adequately. In Case 7 there was no increase in urinary estrogen excretion following treatment with human pituitary FSH+HCG, although ovulation took place. However, in this instance ovulation most probably occurred early in the course of FSH administration (as shown by the histologic appearance of the corpus luteum). Whether in such a patient a previous ovulation might interfere with the ability of the ovaries to respond to gonadotropic stimulation, remains to be established.

DISCUSSION

Whereas it is well established that at least 2 gonadotropic hormones, FSH and LH, can be separated from animal pituitaries, the existence of these 2 hormones as separate substances in the human pituitary is still under debate. It has been suggested by several workers that in the human subject there may be only 1 gonadotropin molecule exhibiting both FSH and LH-activity (25-27). This suggestion was based on the fact that, so far, attempts to separate these 2 activities from human pituitaries (27) or from human urine (26,27) by electrophoresis or by chemical fractionation (28) have not been successful. Under such circumstances it may be questioned whether it is justifiable to designate the partially purified pituitary gonadotropin used in this study as human pituitary FSH. However, when compared with human menopausal gonadotropin (HMG-20), the pituitary preparation exhibited much less LH-activity than FSH-activity. Furthermore, the fraction precipitating at an ammonium sulphate concentration of 55 per cent was found to possess LH-activity, whereas no FSH-acitivity could be demonstrated in this fraction at the dose levels studied. Although this evidence does not permit a final answer to the problem of duality of these hormones in the human, it suggests at least that the possibility can not be excluded that FSH and LH exist as separate substances in the human pituitary.

When discussing the clinical effects of hormonal therapy is should be recognized that it is extremely difficult, if not impossible, to differentiate between spontaneous occurrences and induced effects when observations are based on a small number of patients. It should also be remembered that our studies were not conducted on hypophysectomized subjects and that the hormone preparation was only partially purified. With these limitations in mind, a few tentative generalizations can be made.

It is apparent from the present study that the human pituitary FSH used is a highly potent hormone when administered to human beings. In amenorrheic women it produced enlarged polycystic ovaries and a great increase in the secretion of estrogen. When FSH was combined with HCG, in most cases ovulation took place and a corpus luteum was formed. This corpus luteum was capable of secreting substantial amounts of progesterone, as shown by

the secretory change of the endometrium and by the greatly elevated output of urinary pregnanediol.

The present study seems also to show that in the human being, stimulation of the follicular apparatus by FSH is essential for the effect of an ovulation-inducing factor. FSH by itself did not produce ovulation in 4 cases. However, when FSH was combined with HCG, in 3 of these patients ovulation occurred.

The ovulation which took place in 2 patients during the course of HCG therapy requires further comments. Since these patients had a previous history of amenorrhea of five to six years' duration, it is tempting to believe that the ovulation was induced by HCG, and that it was not a spontaneous one. These 2 women differed from the 4 HCG-irresponsive patients, since they exhibited a distinct endometrial proliferation and a higher pre-treatment estrogen excretion, indicating a higher degree of ovarian activity. Thus, it may be assumed that the response to HCG might be conditioned by the functional status of the ovaries.

The polycystic condition of the ovaries following the administration of human pituitary FSH indicates that a large number of follicle were stimulated. This suggests that the dose administered was most probably higher than the normal requirement. This view is also supported by the very high urinary estrogen excretion values; such values do not occur during the normal menstrual cycle. In this connection it is of great interest that, although a large number of follicles were stimulated, only 1 follicle was brought to ovulation. The exact hormonal mechanism underlying this phenomenon remains to be elucidated. Also, the marked increase in urinary corticosteroid excretion in 2 patients (Case 1 and 3) which followed combined treatment with human pituitary FSH+HCG, is not well understood. These 2 patients received higher dosages of human pituitary FSH than did the others. It remains to be established whether the increased corticosteroid excretion was due to 1) a direct stimulation of the adrenal cortex by the human pituitary FSH, 2) contamination by ACTH, or 3) an increase in metabolites (e.g pregnanediol) of ovarian precursors, such as 17-α-hydroxyprogesterone. An increase in urinary 17-ketosteroids in 3 out of 16 patients with Stein-Leventhal syndrome, following treatment with hog

or sheep pituitary FSH, has been reported by Keettel *et al.* (29). They also wondered whether the FSH caused stimulation of the adrenal cortex.

Whereas in this study a polycystic enlargement of the ovaries following treatment with human pituitary FSH was found in all patients, Keettel *et al.* (29) using hog or sheep pituitary FSH were unable to show any ovarian response in 35 normal women. In 12 out of 13 patients with Stein-Leventhal syndrome and in 1 normal woman, however, the animal preparations caused a polycystic enlargement of the ovaries. The discrepancy between the results of these 2 studies may be explained by species differences of the preparations used or by differences in dosage.

Follicle- stimulating hormone prepared from sheep pituitaries has been shown to produce ovulation in the rhesus monkey (30); however, it was found difficult to induce repeated ovulation by the use of this preparation. On the other hand, van Wagenen and Simpson (31) were successful in inducing repeated ovulation in this species by the use of a follicle-stimulating hormone preparation obtained from rhesus pituitaries. They interpreted the favorable effect as probably due to lack of antihormone formation following the repeated administration of species-specific protein hormone fraction. The findings of these workers suggest that human pituitary FSH may also be capable of producing repeated ovulation in the human. However, in the present study the effect of human pituitary FSH was investigated only in short-term studies. Except for Patient No.7, who was also treated by wedge resection, none of the patients had a spontaneous second menstrual period. Thus it appears likely that in order to achieve a favorable therapeutic result, further series of injections will be necessary. Whether or not repeated injections will be as effective as the first one is not known. Lack of antihormone formation to this preparation remains to be established. Last, but not least, the question whether ova produced by such means can be fertilized also awaits an answer.

Acknowledgment

We are indebted to Dr. J. A. Loraine (Edinburgh) for the supply of HMG-20 standard preparation.

The expert technical assistance of Mrs. Lisa Gemzell, Miss Berit Martinsen, Miss Leila Nilsson and Miss Ann-Margret Stensgard is gratefully acknowledged.

This work was in part supported by a grant from the KingGustaf V 80th birthday fund.

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Reprinted from: "THE JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM" Vol. XVIII, No. 12, December, 1958, pp.1333-1348 Published for the Endocrine Society by Charles C Thomas, Publisher, Illinois

OESTRIOL METABOLISM IN AN ANENCEPHALIC MONSTER

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A B S T R A C T

Oestriol-16- $_{14}C$ *was injected into the umbilical vein of a newborn anencephalic monster and the circulating and excreted radioactive material was analysed.*

Ten to twelve minutes following injection more than 98 per cent of the circulating radioactive material was in a conjugated form. More than75 per cent of this conjugated material was identified as oestriol-3-sulphate and more than 20 per cent as oestriol-3-sulphate,16(17?)-glucosiduronate. No oestriol-16(17?)-glucosiduronate could be detected in the blood.

Less than 1.0 per cent of the radioactive material was excreted in the urine as unconjugated oestriol. Approximately 50 per cent was present as oestriol-16(17?)-glucosiduronate, more than 16 per cent as oestriol-3-sulphate and more than 25 percent as oestriol-3-sulphate,16(17?)-glucosiduronate. At least 98 per cent of the oestrogen moiety of the various radioactive conjugates was identified as oestriol.

Previous investigations reported from this laboratory established that placental oestriol is extensively sulphurylated by the human foetus (e.g Diczfalusy 1958*; Diczfalusy et al. 1961 a; Mikhail et al. 1963 a,b).* It was also shown that oestriol-3-sulphate dominated the oestrogen pattern of cord blood at term, whereas oestriol-16(17?)-glucosiduronate and a sulpho-glucosiduronate- like double conjugate (most probably oestriol-3-sulphate, 16(17?)-glucosiduronate) were the principal constituents in the urine of newborns (*Troen et al.* 1961). These data suggested that the principal

pathway of oestriol metabolism in the newborn may consist of sulphurylation in position 3, followed by glucosiduronation in position 16(17?) with subsequent partial desulphurylation.

In order to evaluate the quantitative significance of the various steps involved in this pathway, labeled oestriol was injected into the umbilical vein of a newborn anencephalic monster and the radioactive metabolites present in circulating blood and urine were investigated.

E X P E R I M E N T A L

Abbreviations.– The following abbreviations are used throughout this paper: C. C. D. for countercurrent distribution , D. P. M. for disintegrations per minute, K for partition coefficient, OE_3 for oestriol (1,3,5(10)-oestra-triene-3α,16α,17β-triol), OE_3- 3S for oestriol-3-sulphate, OE_3-16(17?)Gl for oestriol-16(17?)-glucosiduronate, OE_3-3S,16(17?)Gl for oestriol-3-sulphate,16(17?)-glucosiduronate, OE_3-3S,16,17-diAC for oestriol-3-sulphate,16,17-diacetate, OE_3-16,17-diAC for oestriol-16,17-diacetate, OE_3-3,16,17-triAC for oestriol-3,16,17-triacetate.

Clinical material.– A newborn female anencephalic monster was used in this study. The monster remained alive for 116 hours.

Injection of radioactive material.– Chromatographically pure oestriol-16-$_{14}C$ with a S.A. of approximately 2 2 μc p er mg was fu rth er p u rified by means of thin-layer chromatography on Silica Gel G, using solvent system "B" of *Lisboa&Diczfalusy* (1962). A polyethylene catheter was inserted into the unbilical vein and 20 ml blood withdrawn. Five μc of oestriol-16-$_{14}C$ dissolved in 2.0 ml of propylene glycol were then mixed with the blood and injected slowly into the umbilical vein.

Collection of material for analysis

a) *Tissues.*– At autopsy on the 6th day, specimens of liver (35 g), lungs (25 g), kidney (10 g) and adrenal tissue (1.3 g) were removed , weighted, immediately disintegrated, extracted with 4 volumes of 95 per cent ethanol and the ethanol extracts stored at - 17° until analysed.

b) *Blood.*-Thirty ml samples were withdrawn at every tenth minute and the amount of blood withdrawn replaced by the same volume of Rh-

negative 0-blood. Eight blood samples were obtained, the first 10 minutes, the last 80 minutes after the administration of the isotope. The first and specimens were analysed separately, whereas the remaining 6 specimens were combined into three samples(20+30 minutes, 40+50 minutes and 60+70 minutes after injection). The blood samples were immediately precipitated by 4 volumes of 95 per cent ethanol and stored at - 17° until analysed.

c) *Urine.* – Urine was collected for 5 days. Unfortunately, complete 24-hour specimens could not be obtained in the study. The urine samples were precipitated with 4 volumes of ethanol and stored in the deep- freeze until analysed.

Extraction procedure. – Extraction by ethanol, methanol-precipitation and ether-water partition for the separation of free (unconjugated) and conjugated material were carried out as described by *Mikhail et al.* (1963 a).

Reference standard preparations, conditions of solvolysis and of *enzymic hydrolysis* were those used in a previous publication (*Troen et al.* 1961) except that the OE_3-16(17?)Gl reference standard was a gift from Professor W.M. Allen, St. Louis, Mo., U.S.A.

Preparation of various derivates and *Kober reaction* according to *Brown* (1955) were carried out as described earlier (*Diczfalusy et al.* 1961 *b*).

Countercurrent distribution.- All distributions were carried out in a manual Craig- Post all glass apparatus , usually at 19±2° C. However, some of the distributions were carried out at a temperature of 25° C. Such a change in temperature alters significantly the K- values. Unless otherwise indicated, 24-transfer distributions were carried out. The following solvent systems were used.

No. 1. : n- Butanol, 0.1 N NaOH (1:1)

2. : n- Butanol, ethyl acetate, 0.2 % NH_4OH

3. : sec. Butanol, 2 N NH4OH(1 :1)

4. : sec. Butanol, water (1:1)

5. : n- Butanol, ethyl acetate, 2 NH_4OH(1:1 :2)

6. : n- Butanol, tert. Butanol, 2 N NH_4OH (3:1:4)

7. : n-Butanol, ethyl acetate, 0.1 N HCl (1:3:4)

8. : sec. Butanol, n-hexane, 0.5% NaCl (4:1:5)
9. : n-Hexane, benzene, methanol, water (7:3:5:5)
10.: Benzene, water (1:1)
11.: Methanol, water, chloroform, carbon tetrachloride(7:3:8:2)
12.: Methanol, water, carbon tetrachloride (2:3:5)
13.: n-Hexane, methanol, water (10:9:1)
14.: n- Butanol, 1 % Na_2CO_3 (1:1)
15.: Ethyl acetate, 0.1 N HCl (1:1)
16.: n- Butanol, n-hexane, 0.1 N NaOH (7:3:10)
17.: Water, chloroform (1:1)

Measurement of radioactivity.– A Tracer lab. Automatic thin- window gas flow counter was used. The efficienty of the instrument was 10.5 per cent. All results are expressed by the addition of an internal standard.

Separation of the radioactive constituents of blood and urine. – The plan of analysis of extracts of blood and urine is shown schematically in Fig. 1.

Following separation of free from conjugated forms, the aqueous phase of each specimen was submitted to C.C.D in system No.1. This distribution invariably resulted in two fractions, one with a K-value of less than 0.2, designated as the "glucosiduronate-fraction" and another distinct peak exhibiting a K-value of 4.5 to 5.5, and designated as the " sulphate-fraction", as shown in Fig.2.

At this stage, the sulphate fractions of all blood specimens were combined, carrier OE_3-3S was added and identification was carried out as described below.

In view of the incompleteness of daily urine collections, all conjugated radioactive material from the urine specimens was combined into a single sample and the OE_3- 3S-like urinary radioactive material obtained following C. C. D in system No.1 was characterized as described below.

The "glucosiduronate-fraction" (contents of tubes 0 to 6) obtained from the pooled urine extract was mixed with authentic OE_3-16(17?)Gl and distributed in system No.2. This distribution resulted in two components, one with a low K-value (0.05) supposed to contain all polar conjugates (such asOE_3-3S,16(17?)Gl or Conjugates B and C of *Troen et al.* 1961) and another one exhibiting the same distribution as carrier OE_3-16(17?)Gl. The latter was submitted to further characterization and identification as

described below, whereas the former was submitted to a 12-transfer C. C. D in system No.3. This distribution resulted in a single component with a K-value of 0.20. In view of previous evidence (e.g. *Troen et al.* 1961), this material was assumed to consist of OE_3-3S,16(17?)Gl. Its further characterisation and identification is described below.

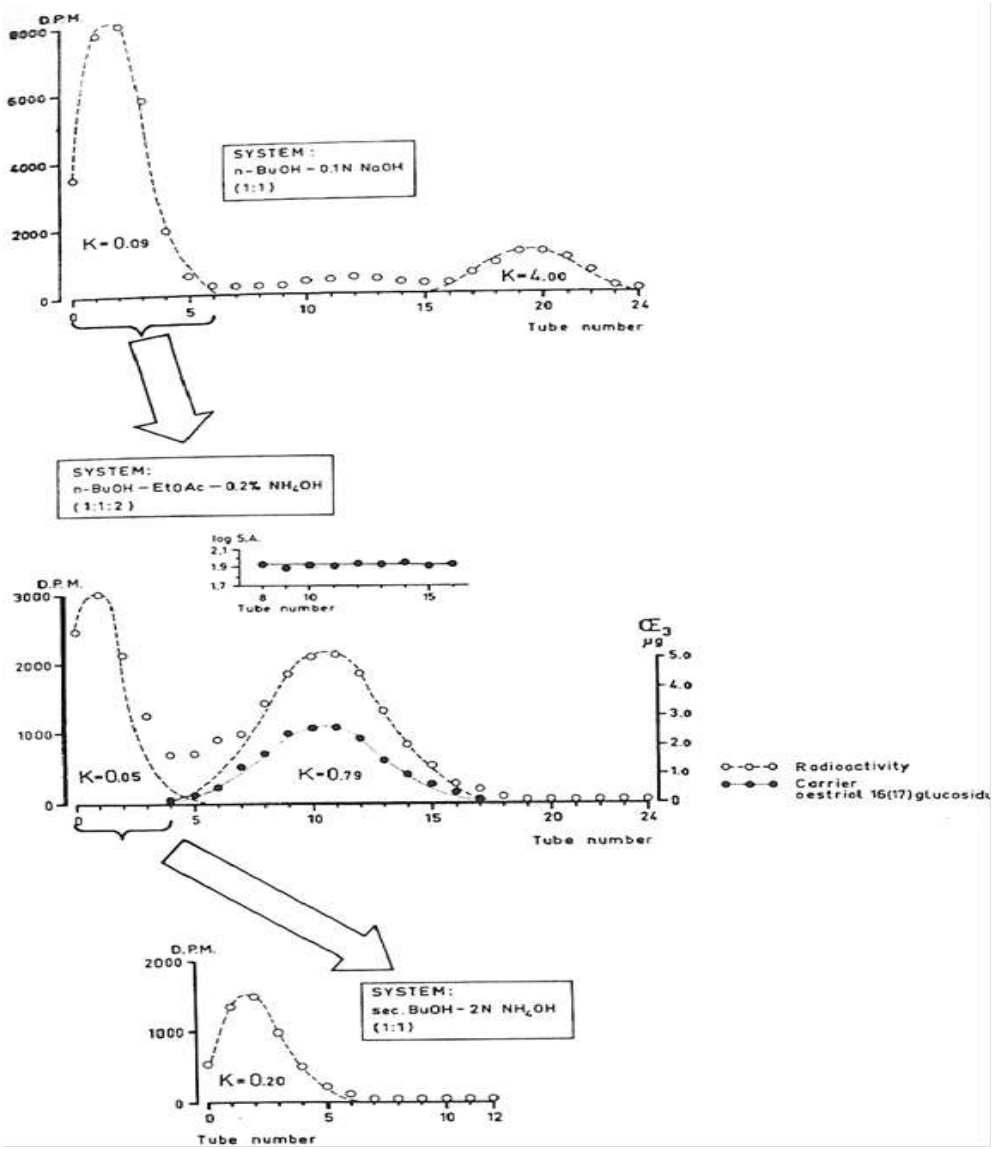
The "glucosiduronate-fraction" of various blood samples was worked up in identical manner. However, C. C. D in system No.2 of the glucosiduronate fractions of blood samples No.1 and 4 (e.g. Table 2) indicated the absence of detectable amounts of OE_3-16(17?)Gl-like radioactive material. This suggested that the glucosiduronate fraction of the various blood samples may consist mainly if not exclusively of polar conjugates. Therefor the glucosiduronate fraction from all blood specimens were combined and submitted to C. C. D in system No.3. A single component with a K-value of 0.25 was .obtained and subsequently identified- as shown below- as OE_3-3S,16(17?)Gl.

R E S U L T S

1. *Free and conjugated radioactive material in various tissues*

Analysis of autopsy material indicated that only a very small amount of radioactive material remained in the various tissues studied, as shown in Table 1.

In contradistinction to the results of short- term perfusion (*Mikhail et al.* 1963 a), among the organs studied in this investigation, the kidneys showed the highest concentration of radioactive material. It can also be seen that some 6 per cent of radioactivity in the liver was present in a free form . Lack of material preluded characterization of the individual components.Fig. 1.General plan of analysis of the radioactive material present in the urine- and blood specimens.


D.P.M.
8000
6000
4000
2000
0
SYSTEM :
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(1:1)
K = 0.09
K = 4.00
5
10
15
20
24
Tube number
SYSTEM:
n-BuOH - EtOAc - 0.2% NH4OH
(1:1:2)
log S.A.
2.1
1.9
1.7
8
10
15
Tube number
D.P.M.
3000
2000
1000
0
K = 0.05
K = 0.79
E3
µg
5.0
4.0
3.0
2.0
1.0
0
5
10
15
20
24
Tube number
Radioactivity
Carrier
oestriol 16(17) glucosid
D.P.M.
2000
1000
0
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SYSTEM:
sec. BuOH - 2N NH4OH
(1:1)
0
5
10
12
Tube number

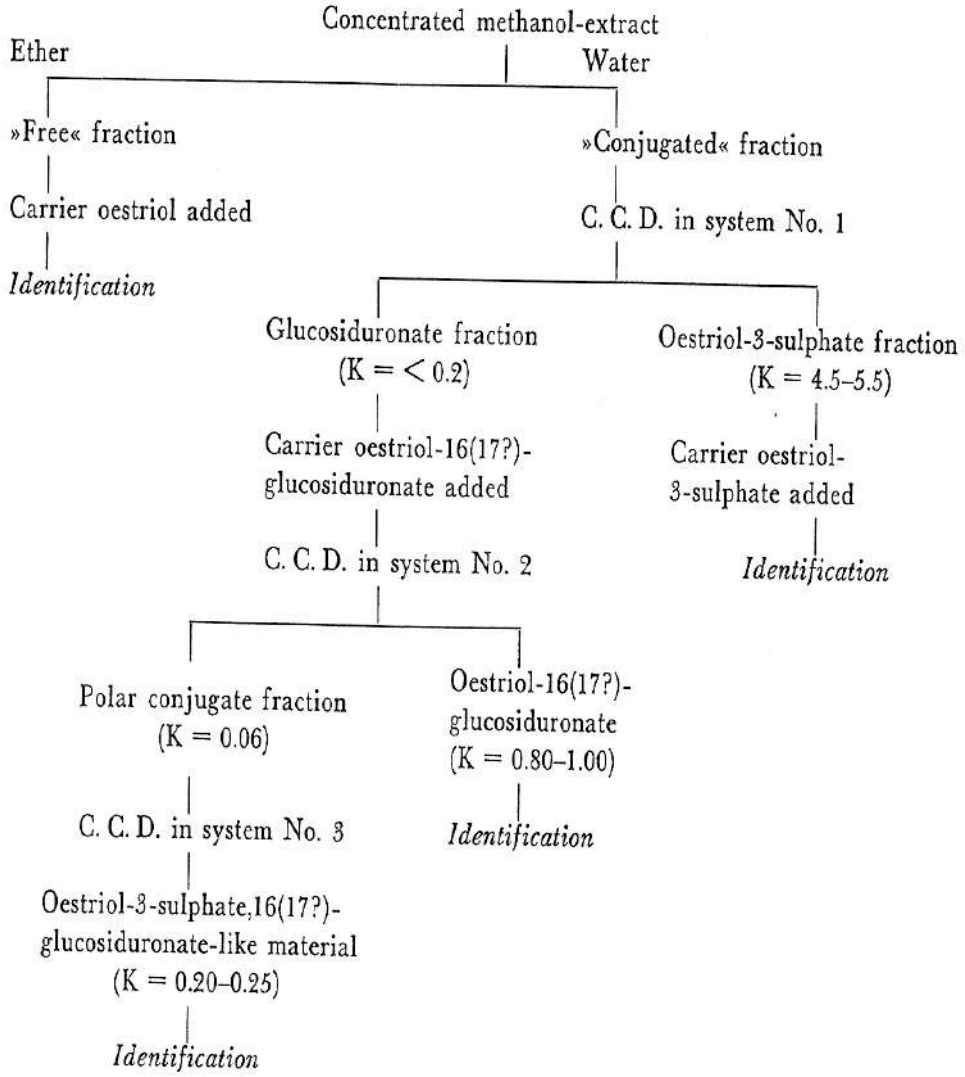

Fig. 2

Sequence of countercurrent distributions adopted for the separation of the oestriol-3-sulphate,16(17?)-glucosiduronate-like radioactive material. Open circles indicate radioactivity measurements, filled circles the results of the Kober reaction on added carrier oestriol 16(17?) glucosiduronide.

Table 1.

Free and conjugated radioactive material (D.P.M per 100 g) in the ethanol extracts of various organs of an anencephalic monster 116 hours following the injection of 5.0 μc of oestriol-16- $_{14}C$ into the umbilical vein.

Organ a)	Free	Conjugated	Free as percentage of total
Lung	0	17.000	0.0
Liver	4000	61.000	6.2
Kidney	2000	168.000	1.2

a) No radioactive material could be demonstrated in the extract of one adrenal.

Table 2.

Distribution of radioactive material (D.P.M per 100 ml blood) in the blood of an anencephalic monster at different intervals following the administration of oestriol-16-$_{14}C$ into the umbilical vein. Figures in parentheses indicate percentages.

Blood sample	Collection period(minutes after injection)	Total radioactivity	Oestriol	Oestriol-3-sulphate	Polar conjugates a)
1.	10- 12	1.154.820	17.820 (1.5)	800.450 (69.3)	336.550 (29.2)
2.	19-31	907.500	5.500 (0.6)	663.870 (73.2)	238.130 (26.2)
3.	39-51	657.330	3.330 (0.5)	494.420 (75.2)	159.580 (24.3)
4.	62-71	722.830	830 (0.1)	641.860 (88.8)	80.140 (11.1)
5.	80-81	351.300	0 (0.0)	268.390 (76.4)	82.910 (23.6)
Mean percentage distribution			0.5	76.6	22.9

a) Subsequent identification of this material revealed that at least 85 per cent of it consisted of oestriol-3 sulphate,16(17?)-glucosiduronate.

2. *Distribution of the radioactive material present in the blood*

The distribution of the material recovered from the blood specimens is shown in Table 2.

It can be seen from the data of Table 2 that the bulk of radioactive material was present in all samples as conjugated material. Ten minutes following the administration of the isotope, as much as 98.5 per cent of the circulating radioactive material was in a conjugated form. It can also be seen that with the exception of blood sample No. 4 (in which case a possible technical error cannot be excluded) the relationship between
OE_3- 3S and OE_3- 3S,16(17?)Gl appears to be remarkably constant during the 80 minutes of experiment.

3. *Distribution of the radioactive material present in the urine*

The results are shown in Table 3.

The data of Table 3 indicate that in all samples studied more than 99 per cent of the radioactive material was in a conjugated form. From the analysis of the combined conjugated radioactive material it can also be seen that some 50 per cent of it consisted of OE_3- 3S,16(17?)Gl, approximately 16 per cent of OE_3- 3S
and more than 30 per cent of polar conjugates. Subsequent identification studies(shown below) indicated that at least 80 per cent of these polar conjugates consisted of OE_3- 3S,16(17?)Gl.

4. *Identification of the individual radioactive components*

a. *Oestriol.* – The small amount of unconjugated OE_3-like radioactive material from all blood and urine specimens was combined, mixed with authentic OE_3 and submitted to C. C. D in system No. 17. There was a close agreement between the distribution of radioactivity and that of the carrier (K=0.79) and very little if any radioactive material could be dissociated from the carrier in this distribution. Lack of material in this fraction precluded further characterisation.

Table 3

Distribution of radioactive material in the urine of an anencephalic monster following the administration of oestriol-16-$_{14}C$ into the umbilical vein. Due to incomplete urine collection, excretion values are expressed as D. P.M per 100 ml urine.

Collection period (hours after injection)	Total radioactivity	Oestriol	Oestriol-3-sulphate	Oestriol-16(17?)-glucosiduronate	Polar Conjugates a)
0-24	11.568.000	68.000	4.270.000	13.100.000	8.600.000
25-48	7.146.000	46.000			
49-72	3.278.600	8.600			
73-96	2.107.300	7.300			
97-116	2.004.170	4.170			

a) Subsequent identification of this material indicated that at least 80 per cent of it consisted of oestril-3-sulphate,16(17?)-glucosiduronate.

b. *Oestriol-3-sulphate.*- For the purposes of identification, approximately equal amounts of OE_3-like radioactive material from pooled blood- and urine specimens were combined into a single sample carrier OE_3-3S was added and the material submitted to a series of C. C. D in a sequential manner, using systems No. 4, 1, 5, 6, 7 and 8. The K-values obtained were 1.9, 4.6, 2.3, 5.3, 1.7 and 0.15, respectively. The material was than acetylated and the OE_3-3S,16(17?)-diAC formed distributed in systems No.8 and 9 (K=2.6 and 0.01) . Following solvolysis, the OE_3-16(17?)-diAC formed was distributed in system No. 9 (K=2.5). In each of this 9 distributions there was an excellent agreement between the behaviour of radioactive material and authentic carrier; at least 95 per cent of the

radioactive material remained associated with the carrier in any of the distributions.

The oestrogen moiety obtained following acid hydrolysis according to *Brown* (1955) was extracted by ether and submitted to C.C. D in systems No. 10, 11 and 12, with K-values of 0.12, 0.89 and 11.5. Following methylation of the phenolic hydroxyl group it was also distributed in systems No.12 and 13(K=0.79 and 0.002)

Another aliquot was hydrolysed with the Helix pomatia enzyme preparation. More than 96 per cent of the radioactive material became ether-soluble. This material was mixed with carrier OE_3 and distributed in system No. 10 (K=0.30). It was then acetylated and the OE_3-3,16,17-triAC formed submitted to the K-values of standard and radioactive material were identical and more than 95 per cent of the radioactivity remained associated with the carrier throughout all distributions.

c. *Oestriol-16(17?)-glucosiduronate.*-When the urinary glucosiduronate fraction was distributed in solvent system No.2 together with authentic OE_3-16(17?)Gl, substantial amounts of radioactive material exhibited the same K-value(0.79) as the reference standard (Fig.2). This material was then submitted to additional C.C.D in a sequential manner in systems No. 14, 3, 15and 1. The K-values obtained were 1.2, 1.1, 1.6 and 0.03, respectively. The distribution of radioactive material and reference standard was identical in all systems, Following methylation and C.C.D in system No.16, two identical peaks of radioactivity and carrier were obtained, with K-values of 0.9 and 3.5 respectively. Enzymatic hydrolysis both with a Helix pomatia preparation and with a bacterial β-glucuronidase preparation converted 100 per cent of the previously ether-insoluble material into an ether-soluble one. However, when hydrolysis with the same amount (12 Sigma units per ml) of β-glucuronidase was carried out in the presence of 10 mg/ml D-saccharic acid lactone, there was a complete inhibition of the enzymic hydrolysis; no radioactive

material could be removed by ether, all of it being recovered from the aqueous phase.

The oestrogen moiety of this conjugate was identified exactly in the same way as that of OE_3-3S; in all 6 solvent systems listed above there was a complete agreement between the distribution of radioactive material and that of authentic OE_3.

d. *Oestriol-3-sulphate,16(17?)-glucosiduronate.* – The first three steps for the separation of this material from OE_3-3S (first distribution), from OE_3-16(17?)Gl (second distribution) and from possible contaminating Conjugates B and C (third distribution) are shown in Fig.2. The fraction obtained after the third distribution of urinary- and blood extracts was then submitted separately to C.C. D. in solvent systems No.1 (K=0.10) and No. 6(K=0.18). This material from the urine and blood was then divided separately into three aliquots and each aliquot submitted to a different type of hydrolysis, as shown in Fig. 3.It appears from the data of Fig.3 that hydrolysis with the Helix pomatia enzyme completely converted this material into OE_3, whereas hydrolysis with β-glucuronidase converted more than 80 per cent of it into OE_3-3S. Finally, when the material was incubated with 0.1 N HCl in an aqueous solution at room temperature evernight, partial solvolysis took place and the end-product of this behaved in C.C.D (in both acid- and alkaline systems) as OE_3-16(17?)Gl. The results of the enzymatic characterisation of the OE_3-3S,16(17?)Gl-like radioactive material from the blood were very similar: more than 90 per cent of this material was converted into β-glucuronidase following hydrolysis with β-glucuronidase.

For further identification the three end-products (OE_3, OE_3-3S and OE_3-16(17?)Gl) obtained following enzymatic hydrolysis of the OE_3-3S,16(17?)Gl-like material from the blood and urine , respectively, were combined and submitted to additional C.C.D. The OE_3-like radioactive material was distributed with carrier OE_3 in systems No. 1. 10 and 11 (K-

values= 2.6, 0.30 and 0.82, respectively) and theOE_3-16(17?)Gl-like materials in systems No.2 and 6 and 15 (K-values=0.79, 0.92 and 1.6, respectively). The OE_3-3S-like material was acetylated and distributed in systems No.8 and 9 (K=2.6 and 0.01). Following solvolysis, the OE_3-16(17?)-diAC submitted to C.C.D. in system No.9 (K=2.5). In all this distributions identical K-value were obtained for the radioactive material and corresponding carrier, and invariably 95 per cent of the radioactive material remained associated with the authentic carrier in question. It is concluded therefore that the polar conjugate formed by the anencephalic monster was identical with OE_3-3S,16(17?)Gl.

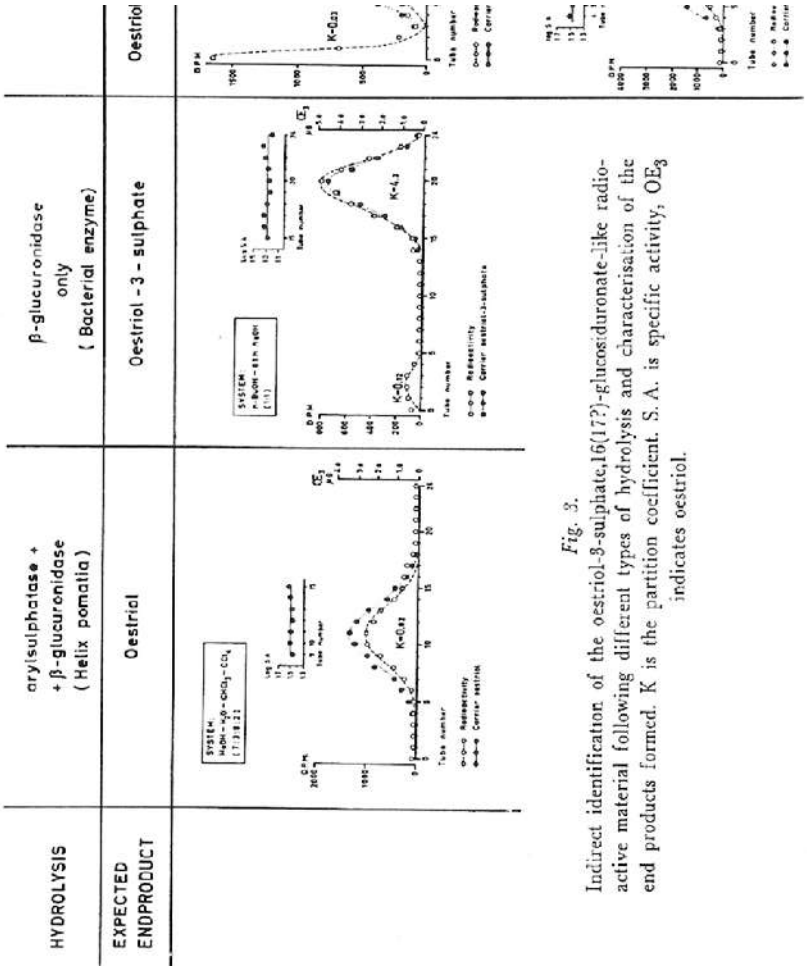

Fig. 3.
Indirect identification of the oestriol-3-sulphate,16(17?)-glucosiduronate-like radioactive material following different types of hydrolysis and characterisation of the end products formed. K is the partition coefficient. S. A. is specific activity, OE_3 indicates oestriol.

D I S C U S S I O N

The most striking feature of the present study in the extremely rapid conjugation of circulating OE_3 by the anencephalic monster studied. Within 10 minutes following intravenous injection, more than 98 per cent of the administered isotope was converted into a conjugated form. This is a much faster rate of conjugation than that found in pregnant women(*Wilson et al.* 1964) and suggests that the extensive oestrogen conjugating ability of the human foetus demonstrated previously in fetuses from the second trimester (e.g. *Diczfalusy* 1958; *Levitz et al.* 1961; *Mikhail et al.* 1963 a,b; *Haynes et al.* 1964) also persists during the third trimester and probably even during a part of infancy.

The rapid conjugation of OE_3 occurred mainly in form of sulphurylation of the phenolic hydroxyl group; more than 75 per cent of the circulating radioactive material was identified as OE_3-3S. An additional 20 to 24 per cent was also shown to be sulphurylated in position , although in this case sulphurylation of the phenolic hydroxyl group was associated with the simultaneous formation of a 16α-(or perhaps also 17β) glucosiduronate. More than 99 per cent of the circulating radioactivity consisted of this two conjugates.

Hydrolysis experiments with different enzymes and subsequent identification of the products of hydrolysis indicated that the sulphoglucosiduronate duble conjugate (resembling in every respect Conjugate A of *Troen et al.* 1961) is identical withOE_3-16(17?)Gl). Since the relationship between circulating OE_3-3S and OE_3-3S,16(17?)Gl.

was the same 10 minutes and 80 minutes following injection, it seems justifiable to conclude that in this case the rapid sulphurylation of circulating al withOE_3 was accompanied by a similarly rapid glucosiduronation.

The two sulphurylated conjugates, OE_3-3S and OE_3-3S,16(17?)Gl were also recovered from the urine in significant quantities, although the predomninant urinary conjugate was OE_3-16(17?)Gl. Since virtually all circulating OE_3 was sulphurylated in position 3, whereas half of the OE_3 excreted in the urine was not, it must be concluded that a considerable part of the circulating OE_3-3-sulphates had to be desulphurylated prior to

excretion in the urine. The site of this sulphatase action remains yet to be determined. It also remains to be established whether all circulating OE_3-3S becomes glucosiduronated prior to desulphurylation, or whether a part of the OE_3-16(17?)Gl excreted is formed via glucosiduronation of unconjugated OE_3.

The objection can be raised, however, that it is unjustifiable to draw conclusions from the metabolism of OE_3 in an anencephalic monster to that taking place in healthy newborns. However, the data presented in Tables 4 and 5 seem to rule out this objection,

In Table 4 the OE_3 pattern of cord blood samples pooled from a large number of normal pregnancies is compared to that found in the present investigation. With the exception of a higher concentration of unconjugated OE_3 in cord blood (reflecting probably the continuous hydrolysis of circulating OE_3-3S by the placenta), the two patterns are very similar indeed. On the another hand, the pattern of circulating radioactive material 10 to 30 minutes following intravenous injection of labeled OE_3 to pregnant women differs markedly from that found in newborns. There is much more unconjugatedOE_3, but considerably less OE_3-3S and OE_3-3S,16(17?)Gl in the blood of pregnant women.

The marked difference between foetal type and adult type of conjugate pattern can also be seen when the urinary excretion patterns are compared, as shown in the Table 5.

Both pregnant women and the anencephalic monster studied excreted huge quantities of OE_3-16(17?)Gl in the urine. On the other hand, only traces of OE_3-3S were excreted by pregnant women, whereas this compound is a quantitatively important constituent among the OE_3 conjugates excreted by the ancephalic monster. Furthermore, in the urine of the pregnant women studied less than 3 per cent of the urinary OE_3-metabolites occurred as OE_3-3S,16(17?)Gl, whereas more than 25 per cent of the urinary radioactive material was excreted by the anencephalic monster in this form. Last but not least it also appears from the data of Table 5 that the urinary OE_3-pattern of healthy newborn boys agrees closely indeed with that found in the anencephalic monster.

The data of Tables 4 and 5 suggest therefore that the OE_3-metabolism of the anencephalic monster studied did not differ appreciably from that normally taking place during the first few days of life.

It is of some interest to note in this connection that in 5 newborns (both boys and girls) with myelomeningocele and/or multiple malformations, the urinary excretion of OE_3-conjugates following intramuscular injection of 1.0 to 1.5 μc amounts of labelled OE_3 was very similar to that found in the present study with more than 12 per cent OE_3-3S, more than 62 per cent OE_3-16(17?)Gl and 19 per cent polar conjugate (*Barr et al.*unpubl. data).

Thus the newborn period as well in the intrauterine life are characterized by a great ability to sulphurylate circulating oestrogens. An inspection of the data of Tables 4 and 5 suggests that this ability is considerably reduced during adult life.

The physiological significance of the extensive oestrogen sulphurylation by the newborn is incompletely understood at present. It is felt that this capacity of the newborn may be just a remnant from the intrauterine life where an extensive ability to sulphurylate 3β-hydroxylated steroids may have an important influence upon placental steroidogenetic reactions by regulating the amount of 3β-hydroxylated C-19 steroids available for placental conversion into oestrone and 17β-oestradiol (*Bolte et al.* 1964).

Table 4

Pattern of oestriol conjugates in the systemic venous blood of pregnant women and of anencephalic monster following in the intravenous administration of oestriol-16-$_{14}C$. For the purposes of comparison the pattern in cord blood from normal pregnancies is also included.Values are expressed as percentages.

	Oestriol	Oestriol -3- sulphate	Oestriol- 16(17?)- glucosid- uronate	Polar conjugates	Oestriol- 3- sulphate, 16(17?)- Glucosid uronate a)	Reference

Pregnant women	45.0	16.5	0.5	38.0	(24)	*Wilson et al.* 1964
Anencepha-lic mons-ter	0.5	76.6	0.0	22.9	(>85)	Present study
Cord blood	12.0	74.8	1.1	12.1	(65)	*Troen et al. 1961*

a) Expressed as percentage of Polar conjugates

Table 5

Pattern of oestriol conjugates in the urine of newborn infants as compared to the urinary patterns found following the intravenous administration of oestriol-16-$_{14}C$ to pregnant women or to an anencephalic monster. Figures indicate percentages.

	Oestriol	Oestriol-3-sulphate	Oestriol-16(17?)-glucosid-uronate	Polar conjugates	Oestriol-3-sulphate,16(17?)-Glucosiduronate a)	Reference
Pregnant Women b)	0.2	0.6	68.1	31.1	(8.0)	*Wilson et al.* 1964
Anencepha-lic mons-ter c)	0.5	16.4	50.2	32.9	(>80)	Present study
Newborn infants(boys only) d)	1.5	10.9	50.8	36.8	(65)	*Troen et al. 1961*

a) Expressed as percentage of Polar conjugates

b) Urine collection: 72 hours

c) Urine collection: 116 hours

d) Urine collection during the first 48 hours of life

number of normal pregnancies is compared to that found in the present investigation. With the exception of a higher concentration of unconjugated OEa in cord blood (reflecting probably the continuous hydrolysis of circulating OEn-3S by the placenta), the two paHerns are very similar indeed. On the other hand, the pattern of circulating radioactive material 10 to 30 minutes following intravenous injection of labeled OEa to pregnant women differs markedly from that found in newborns. There is much more unconjugated OEg but considerably less OEa-3S and OE3-3S,16(17?)GI in the blood of pregnant women.

The marked difference between foetal type and adult type of conjugate pattern can also be seen when the urinary excretion patterns are compared, as shown in Table 5.

Both pregnant women and the anencephalic monster studied excreted huge quantities of OEg-16(17?)Gl in the urine. On the other hand, only traces of OEg-3S were excreted by pregnant women, whereas this compound is a quantitatively important constituent among the OEg conjugates excreted by the anencephalic monster. Fur1thermore, in the .urine of the pregnant women studied less than 3 per cent of the urinary OEg-metabolites occurred as OEg-3S,16(17?)Gl, whereas more than 25 per cent of the urinary radioactive material was excreted by the anencephalic monster in this form. Last but nOI[least, it also appears from the data of Table 5 that the urinary OEa-pattern 01 healthy newborn boys agrees very closely indeed with that found in the anencephalic monster.

The data of Tables 4 and 5 suggest therefore that the OEg metabolism of the anencephalic monster studied did not differ appreciably from that normally taking place during the first few days of life.

It is of some interest to note in this connection that in 5 newborns (both boys and girls) with myelomeningocele and/or multiple malformations, the urinary excretion of OEn-conjugates following intramuscular injection of 1.0 ,to 1.5 fA.c amounts of labeled OEg was very similar to that found in the present study with more than 12 per cent OEg-3S, more than 62 per cent OEg-16(17?)GI and 19 per cent polar conjugates (Barr et aI., unpubl. data).

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ACKNOWLEDGENTS

The authors are indebted to Miss Monica Westerlund and Miss Marianne Edev1g for their expert technical assistance, and to Professor Willard M. Allen (Saint Louis, Mo., U.S. A.) for a generous gift of oestriol-IG(17?)-glueosiduronate.

The expenses of this investigation were defrayed by a Research Grant from the Ford Fowldation and by Grant HD-0041S (formerly AM-0277I) of the National; Institutes of Health, United States Public Health Service.

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Reprinted from: Acta Endocrinologica, vol 46, no 4/1964, pages 511-524

Periodica Copenhagen

AN IMPROVED IN VITRO BIOASSAY METHOD FOR MEASURING LUTEINIZING HORMONE (LH) ACTIVITY USING MOUSE LEYDIG CELL PREPARATIONS

M.-P. VAN DAMME, D. M. ROBERTSON and E. DICZFALUSY

ABSTRACT

An improved in *vitro* bioassay method for the measurement of LH-activity is presented. The method is based on the assay of testosterone produced by "Leydig cell" preparations from mouse testes in the presence of added gonadotropin. The method is significantly improved in terms of sensitivity, precision and practicability when compared to the previously described bioassay method employing decapsulated testes from adult mice. The sensitivity of the improved method is 15 μIU for HCG and 50 μIU for HMG. The useful range of the method is 15-260 μIU for HCG and 50-900 μIU for HMG. Using a 3+ 3 point assay design with each dose in quadruplicate, a mean index of precision (λ) of 0.044 was obtained in 19 assays.

Human FSH, TSH, ACTH, LTH, STH, oxytocin, vasopressin and LHRH preparations did not influence the bioassay method at levels likely to be found in biological samples. A good correlation was found between estimates obtained by the "Leydig cell" * method and by the method using decapsulated testes when various HCG and HMG preparations were used. With the proposed method at least 30 samples can be assayed each week by 2 persons, with a marked reduction in cost.

In a previous paper (*Van Damme et al.*1973) an *in vitro* bioassay method for measuring LH activity was described. Evidence was presented indicating that the method, which is based on the assay of testosterone produced by decapsulated mouse testes in the presence of LH preparations, fulfils the recognized criteria of reliability. There were, however, 2 limitation of the method; the relatively poor precision (λ=0.22) and a limited practicability.

The present paper describes a significantly improved modification of the previous method. “Leydig cell” preparations from disrupted mouse testes are used in the bioassay. This modification results in a considerable increase in precision, sensitivity and practicability.

M A T E R I A L S

Abbreviations and trivial names

CV= coefficient of variation; HCG= human hypophyseal gonadotropin; HHLH= human hypophyseal luteinizing hormone; HTSH= human thyroid stimulating hormone; λ= index of precision; LHRH= luteinizing hormone releasing hormone; OAAD= ovarian ascorbic acid depletion assay; RIA= radioimmunoassay; WITARO= weight increase of the total accessory reproductive organs in intact immature rats.

Materials

Sprague-Dawley rats and mice of the Naval Medical Reseach Institute strain (Bethesda, USA) were purchased from AB Anticimex, Stockholm, and caged for a minimum of 24 h prior to sacrifice.

The majority of the hormones used have been described previously (*Van Damme et al. 1973*) . Additional preparations used are indicated in the text.

All glassware used was siliconized (Silikonvätska MS 1107, Kebo, Sweden; 2% in acetone) and dried at 50˚C overnight.

Eagle’s Minimum Essential Medium and calf serum were obtained from Statens Bakteriologiska LAboratorium, Stockholm, Sweden. The medium containing the calf serum was bubbled with O_2CO_2 (93.5:6.5) for several minutes before use. The resulting pH was 7.2. The serum was obtained from calves aged 1-3 months. As assessed by the present “ Leydig cell” bioassay method LH activity in 45 µl or serum was non-detectable.

The collagenase (137 U/mg) and the Lima bean inhibitor were obtained from Worthington Biochemical Co., New Jersey, USA.

M E T H O D S

Proposed method

Assay design.-A 3+3 point assay is used in quadruplicate for each dose level with a log dose interval of 1.5. Usually 10 unknown preparations are

assayed against 1 standard. Validity tests are performed as described in a previous publication (*Van Damme* et al. 1973).

Preparation of cells. – Adult mice (3 ½ months) are sacrified by cervical dislocation, the testes decapsulated and placed in a Petri-dish containing Eagle's medium + 2& calf serum. Usually 3 animals are used in each experiment. The testes are cut with scissors into small pieces and Eagle's medium +2% calf serum are added to a final concentration of 6 testes/50 ml. The medium containing the testes is gently stirred by a magnetic stirrer for 10 min at room temperature. The medium is then filtered through a fine nylon mesh.

The filtrate is pre -incubated for 1 h at 34℃ in an O_2CO_2 (93.5:6.5) atmosphere in a Metabolyte shaker (New Brunswick Scientific Co., Inc., New Jersey, USA) at 80 r. p.m. After pre-incubation the cell suspension is placed in ice and centrifuged at 4℃ at 130 *g for 15 min. The supernatant is discarded and the cells are resuspended in the same volume of fresh Eagle's medium +2% calf serum.

The number of cells in the cell preparation and the percentage of broken cells are assessed by counting in a haemocytometer in the presence of Trypan Blue (0.1 %, Fluka AG, Switzerland). No attempts were made to classify the various cell types present. Seminiferous tubules were not found in any preparation. When the testosterone produced by the Leydig cells was expressed *per cell*, the total number of cells present was used in the calculation and not the number of cells considered viable.

Incubation conditions.-A portion (0.1 ml containing 6* 10000 cells) of cell suspension is added to tubes in ice containing 0.1 ml of the appropriate amount of the gonadotrophic hormone. In the standard assay the doses are 22-114 μIU HCG and 79-400 μIU HMG and HHG in Eagle's medium containing 2% calf serum. The samples are incubated at 34℃ for 3 h under an O_2CO_2 atmosphere at 80 r.p.m.

Radioimmnunoassay method (RIA).- At the completion of the incubation, the tubes are placed in ice and a portion (0.2 ml) of a mixture containing tritium labeled testosterone and the appropriate dilution of the antiserum is added. The mixture is incubated at 60℃ for 10 min followed by incubation at 30℃ for 30 min or at 4℃ overnight. A testosterone calibration curve (0-1600 pg) which is processed in parallel, contains the same concentration of

calf serum present in the unknown samples. This corrects for the influence of the testosterone binding components in the calf serum on the RIA. Further detail of the RIA method (e.g. specificity) are presentment in a previous paper (*Van Damme et al. 1973).*

Linearization of the dose response relationship.-When the testosterone produced (pg) was plotted against the logarithm of the dose, the dose response line deviated from linearity at the lower dose levels (Fig. 1a). In an attempt to extend the working range to include lower doses, various metametric transformations (e.g. *Finney* 1964) were tried. It was found that by plotting the square root of the amount of testosterone produced (y 1/2) as the response metameter against the logarithm of the dose, a straight line is obtained over a wide range of doses (Fig.1a). This improvement in linearity was consistent when 10 subsequent assays were analysed.

The homogeneity of variances was assessed by the use of *Bartlett's* (1937) test. A stable variance was obtained over the useful range employing the X2 tables applicable to low numbers of replicates at each dose at the 5 % level (*Pearson&Hartley 1954*). However, in 17 out of 684 quadruplicates, "outliers" were found. The presence of such "outliers" resulted in heterogeneity of variances when tested as described above. If the "outliers" were removed, Bartlett's test indicated homogeneity of variances.

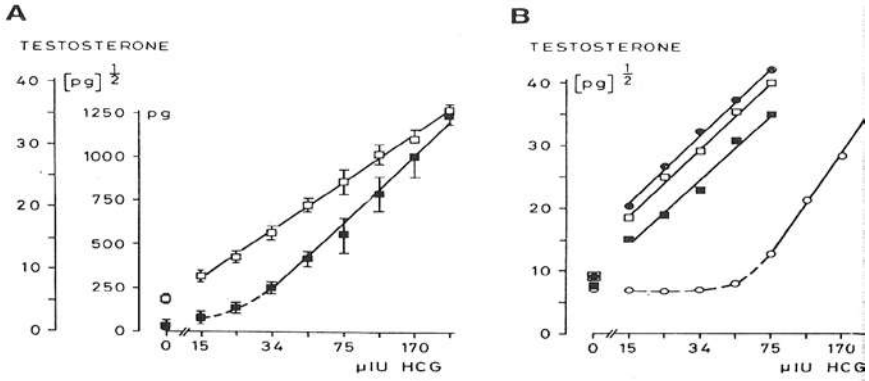

Fig. 1 a.
Linearization of the dose response curve, using the amount of testosterone produced (■) and the square root of the testosterone produced (□) as response metameters. Lambda values of 0.084 and 0.060 respectively, were obtained for the 2 transformations from the regions of the 2 curves yielding a straight line. The log dose ratio was 1.5. Standard deviations are indicated by vertical bars.

Fig. 1 b.
The influence of calf serum at several concentrations on the dose response curve. (○) No calf serum added, (●) 0.5 %, (□) 2 % and (■) 5 % calf serum.

For this reason 5 observations per dose were recently introduced to reduce the influence of these "outliers" on the assay. In view of these findings, a statistical analysis of each assay invariably includes a test for homogeneity. The reason for these "outliers" is incompletely understood.

The use of some other metametric transformations such as 1/y, log y, 1/y, y la 2/3, logit y and logit 1/y were also studied. Using the log y transformation a lower variance was obtained although the useful range was smaller and variable. When y la 2/3 was examined similar results were obtained to y la ½ in terms of useful range, precision and sensitivity with, however, a higher but still acceptable x 2 value. All other mtametric transformations studied did not result in statistically useful dose response relationships.

Variables affecting the assay

Choice of animals.-Cell preparation from adult and immature rats and mature mice were examined for their responsiveness to HCG and HMG. Cells from rat testes were unresponsive to gonadotrophic stimulation over a wide range of cell concentrations (4-98testes/100 ml). Similar observations have been reported by *Dufau et al.* (1971)*. In contrast, cell preparations from adult mice (3 ½ months) gave a good response with high sensitivity, slope and working range.

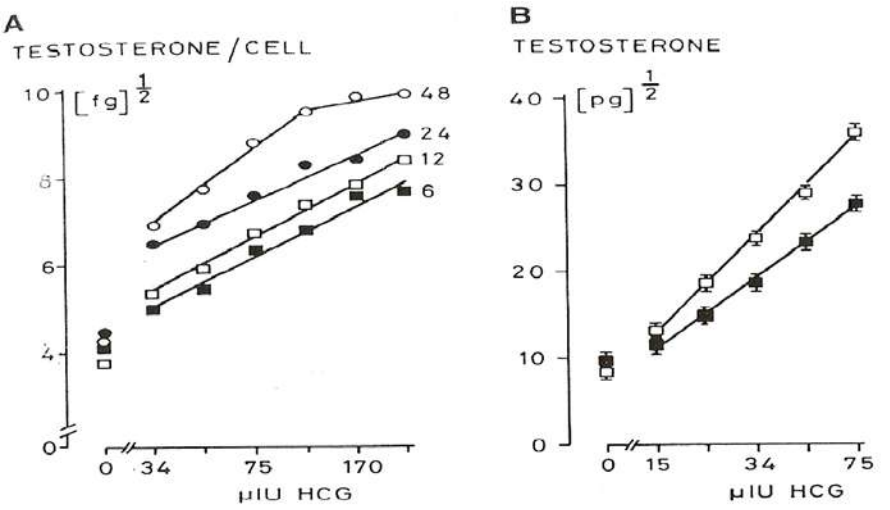

Fig. 2 a.

Dose response curves at different cell concentrations in Eagle's medium with 2 % calf serum. (■) 0.15×10^6 cells/ml, (□) 0.3×10^6 cells/ml, (●) 0.6×10^6 cells/ml, (○) 1.2×10^6 cells/ml.

*However, “Leydig cell” suspensions prepared by collagenase digestion from rat testes are responsive to gonadotrophin stimulation.(*Moyle& Ramachandran 1973*)

Choice of incubation medium.-Different incubation media were investigated in the proposed method. Krebs-Ringer bicarbonate buffer and Eagle’s medium gave comparable responses while the addition of calf serum lead to a 3 fold increase in sensitivity with Eagle’s medium (Fig.1b) and 1.5 fold with Krebs-Ringer bicarbonate buffer. The same effect was obtained with extensively dialysed calf serum. Several concentrations of calf serum (0.5, 2 and 5%) were examined with 0.5- 2% giving an optimal effect. Although 2% calf serum was used in the present studies, this concentration can be reduced without any change in sensitivity.

Choice of cell concentration. – Dose response curves to HCG were prepared at cell concentrations equivalent to 6, 12, 24 and 48 testes/100 ml (total cell concentration ranging from 0.15-1.2 *1000000 cells/ml) (Fig.2a), and the sensitivity, slope and working range were investigated using the square root of the testosterone produced *per cell* as the response metameter. At higher cell concentrations there was an increase in sensitivity; a finding which can be utilized for assays requiring a specially high sensitivity. However, the high levels of testosterone produced under these conditions would require a dilution step before RIA and such a lengthening of the procedure was not considered necessary in the present study. Suitable sensitivity and precision were obtained with 12 testes/100 ml (3*100000 cells/ml, i.e 6* 10000 cells/assay) without the need to dilute the sample before RIA.

Conditions of assay.-Pre-incubation of the cells in the absence of added gonadotrophin for 60 min followed by centrifugation and resuspension in fresh medium gave an increase in sensitivity and a reduction of the testosterone produced by the cells containing no added gonadotrophin (Fig.2b). The toal amount of testosterone produced by the cells in the presence of added gonadotrophin was still increasing after 4 h of incubation. A 3 h incubation period was used routinely in the assays.

Preparation of the cells.-To assess the reliability of the method for the preparation of cells, several variables affecting the responsiveness of the cells to gonadotrophins were examined. From all the multiple assays in this study (n=19) the cell breakage observed was 32 ± 5% (mean ± SD) , with a

cell yield per testis of 3.0 ± 1.0*10000. The mean slope of the dose response curves when expressed in terms of the square root of testosterone produced *per cell* was 1.11 * 1/1000 ± 0.37. The testosterone levels per cell in the absence of gonadotrophin were 1.4 ± 0.4 fg and at one dose level (76 μIU HCG corresponding to 266 μIU HMG) was 38.5 ±9.8 fg. The above variations did not influence the validity of any assay.

Separation of the cells using collagenase treatment

As indicated above, when the cells were prepared by the mechanical separation method, a high cell breakage was observed. It is conceivable that by reducing the extent of cell damage, the responsiveness of the cell preparations and thus the sensitivity of the assay could be increased. To examine this possibility, "Leydig cell" suspensions were prepared from adult mice by collagenase digestion using the method of *Moyle& Ramachandran (1973).* The medium used was Eagle's medium (MEM) containing 2% calf serum and collagenase. After enzymatic digestion of the testes, Lima bean trypsin inhibitor was added to all incubation medial thereafter the cell breakage was observed 18 ± 1 %, n=5). However, dose response curves to HCG, HMG and HHG were less sensitive (35-50 μIU for HCG (2^{nd} IS) and 117-167 μIU for HMG (2^{nd} IRP)) than those obtained by the mechanical separation method, although similar potency assayed against their appropriate standards (Table 4). In these series of assays, a relatively high frequency of invalid assays was observed due to either non-parallelism or non-linearity. Whether this is a property of the collagenase treated cells remains to be investigated.

R E S U L T S

Reliability of the in vitro bioassay method

The useful range of doses giving a linear-log dose response line when plotted against the square root of testosterone produced was 15-269 μIU for HCG and 50-900 μIU for HMG. Usually, the doses chosen for assay were 22-114 μIU for HCG and 79-400 μIU for HMG and HHG. The sensitivity of the method, assessed as the smallest dose giving a response significantly different from that given in the absence of added gonadotropin, was 15 μIU for HCG and 50 μIU for HMG as assessed by t-test analyses ($P<0.01$, n=5). The precision of the method was assessed from the mean λ value of the

potency estimates of all preparations and from repeated assays of an HCG and an HHG preparation. From 19 assays a $\bar{\lambda}$ value of 0.044 was calculated. In each case a 3+3 point bioassay was used. In 5 repeated assays the weighted mean relative potency of HCG (2^{nd} IS) in terms of HMG (2^{nd} IRP) was 3.21 with 95 % fiducial limits at 3.09 and 3.34, and the weighted mean relative potency of HHG (68/40) in terms of HMG (2^{nd} IRP) was 1.11 with 95 % fiducial limits at 1.07 and 1.14. However, homogeneity was observed in the combination of estimates to establish the weighted mean relative potency (*Finney* 1964) with an HCG and 2 HHG estimates which were not included in the assessment of the weighted mean relative potency.

All hormone preparations possessing LH activity (HCG,HMG, HHG, FSH, and TSH) gave linear dose response lines. Parallelism was observed with gonadotrophins from the same source between HCG (2^{nd} IS) and HHG (69/104) (n=5); however, deviations from parallelism were observed in 2 out of 8 assays when HCG (2^{nd} IS) was assayed against HMG (2^{nd} IRP).

In terms of practicability, the assay of 10 unknown hormone preparations against 1 standard can be performed by 2 persons in 1 ½ days.

The specificity of the method was investigated in a similar way to the previous study (*Van Damme et al. 1973*). Firstly, the LH activity of a number of hormone preparations was assessed, and with the exception of FSH and TSH preparations, no activity could be detected. (Table 1). Secondly, the synergistic or antagonistic effect of the same hormones on HCG, HMG, or HHG preparations was studied. The lowest level of hormone giving an antagonistic effect was 1 mIU for STH, 0.05 mIU for oxytocin, 5mIU for vasopressin and 0.15 μg for LHRH. Derivations from parallelism were observed at higher concentrations of oxytocin (5 mIU). Although a multiple assay design was used, individual statistical treatment against the standard was employed in order to assess the synergistic or antagonistic effect of each hormone against the standard. Thirdly, the LH activity of several HCG (Table 2), HMG and HHG preparations (Table 3) was determined and compared with potencies obtained by the decapsulated testes method. The correlation coefficient (r) for the 10 HCG preparations was 0.99 and for the 8 HHG preparation 0.99. The potency estimates obtained with the decapsulated testes method were shown previously (*Van Damme et al. 1973*) to agree very closely with those obtained by the

WITARO (*Diczfalusy 1954*) or the OAAD (*Parlow 1961*) in vitro bioassay methods.

A pituitary reference preparation (HHG 69/104) was used as standard for all HHG preparations in contrast to the previous study where HMG was used.

For the purposes of comparison HHG values obtained with the method based on the decapsulated testes method, have been recalculatd in terms of the new standard.

The presence of calf serum in the incubation medium did not alter the potency estimates of HCG, HMG and HHG preparations (Table 2 and 3).

Table 1.

Specificity of the in vitro"Leydig cell" bioassay method: The effect of adding various hormones to HCG, HMG and HHG preparations

		HCG (2nd IS)		HMG (2nd IRP)		HHG (HHLH 66/40)	
Hormone added	Levels Tested a)	Relative potency	Index of precision(λ)	Relative potency	Index of precision(λ)	Relative potency	Index of Precision(λ)
ACTH	40mIU b)	0.97(0.88-1.07)	0.046	0.91(0.80-1.04)	0.063	0.95(0.81-1.12)	0.075
LTH	0.1µg b,c)	0.95(0.87-1.06)	0.043	1.00(0.92-1.08)	0.024	1.04(0.90-1.22)	0.077
STH	1mIU b)	0.85(0.76-0.94)	0.049	0.89(0.81-0.98)	0.047	0.82(0.77-0.88)	0.034
	34µIU b)	1.08(0.98-1.19)	0.042	1.04(0.98-1.09)	0.030	1.00(0.92-1.08)	0.042
Oxytocin	5 mIU b)	d)	-	0.63(0.58-0.70)	0.044	d)	-
	0.5mIU b)	0.74(0.66-0.82)	0.056	0.75(0.72-0.79)	0.020	0.93(0.80-1.07)	0.072
	0.05mIU b)	0.94(0.88-1.02)	0.038	0.92(0.88-0.98)	0.022	0.87(0.79-0.95)	0.051
Vasopresin	5mIU b)	0.71(0.66-0.78)	0.039	0.87(0.79-0.97)	0.049	0.92(0.80-1.06)	0.069
	0.05 mIU b)	1.08(0.95-1.23)	0.057	1.10(0.98-1.25)	0.061	0.93(0.84-1.02)	0.048
LH-RH	150ng b)	0.61(0.57-0.64)	0.030	0.49(0.43-0.55)	0.050	0.89(0.81-0.95)	0.036
	10 ng b)	0.90(0.87-1.06)	0.043	1.01(0.95-1.07)	0.027	1.03(0.84-1.28)	0.103
FSH	28-92mIU e)	0.94(0.88-1.02)	0.037	1.03(0.98-1.09)	0.021	1.00(0.92-1.08)	0.039
HTSH	1.05-3.51µUSP e,f)	1.07(0.98-1.18)	0.035	1.03(0.95-1.11)	0.043	1.04(0.98-1.10)	0.035
	0.30-1.01 µUSP e,g)	0.97(0.89-1.04)	0.037	0.92(0.87-1.01)	0.039	0.98(0.89-1.05)	0.039

HMG (2^{nd} IRP)	60-200 µIU	1.06(0.99-1.14)	0.036	-	-	-	-
HHLH 68/40	60-200 µIU e)	1.07(1.00-1.15)	0.037	1.05(0.98-1.13)	0.039	-	-

Figures in parantheses indicate 95 % fiducial limits.

a) The amount of hormone present per tube (0.2 ml).

b) No LH acitivity was observed when this hormones were assayed at the following levels: ACTH 0.1 IU, LTH 0.2 µg, STH 1 mIU, oxytocin 0.1 IU, vasopressin 0.1 IU and LH-RH 0.25 µg.

c) Highly purified human LTh preparation provided by Dr. Friesen.

d) No parallelism could be obtained of this level of oxytocin.

e) The LH acitivity of this hormones is given in Table 3. Graded doses of hormone where added to graded doses of HCG, HMG, or HHG (cf. Van Damme et al. 1973).

f) LER 1928- 2b provided by Dr. Reichert (LH/TSH=57).

g) Rechrom C.M cellulose Column 1 FR. 2 preparation provided by Dr. Pierce (LH/TSH=198).

T*able 2.*

The Lh activity of various HCG preparations was estimated by the vitro bioassay method in the presence and absence of calf serum, using " Leydig cell" suspensions prepared from mouse testes by mechanical separation. Comparisons are made with the previously reported values obtained by the in vitro bioassay method using decapsulated testes and by the in vivo WITARO method (Van Damme et al.1973)

Figures in parentheses represents 95% fiducial limits *.

	"Leydig cell"method (Eagle's medium with 2% calf serum)		*"Leydig cell"method (Eagle's medium without calf serum)*			
Batch No.	IU/mg	Index Of precision (λ)	*IU/mg*	*Index Of precision (λ)*	*Decapsulated Testes method IU/mg*	*In vivo method (WITARO) IU/mg*
MSKG 03	*16780 (15 580-17 960)*	*0.041*	*17 150 (16 440-17 900)*	*0.023*	*17 140 (10 270-30 340)*	*17 150 (14 010-21 130)*
MSKG 02	*11 320 (9900-13 180)*	*0.050*	*12 920 (12 390-13 480)*	*0.023*	*9310 (5700-13 690)*	*12 250 (8860-16 580)*
E 231-2	*8340 (7230-9500)*	*0.051*	*8040 (7710-8390)*	*0.023*	*8320 (5330-12 090)*	*9820 (7480-12 160)*
71:75:16	*8270 (7280-9390)*	*0.060*	*9730 (9330-10 150)*	*0.023*	*7650 (5700-10 010)*	*9240 (7360-11 350)*
J 26:30	*8070 (7110-9290)*	*0.064*	*7460 (6670-7260)*	*0.023*	*8580 (5880-13200)*	*7130 (5570-9600)*
428 ZEP	*2570 (2380-2790)*	*0.041*	*2700 (2580-2810)*	*0.023*	*2460 (1660-3570)*	*2720 (1640-3890)*
760-531	*2970 (2730-3300)*	*0.064*	*2990 (2860-3120)*	*0.023*	*2560 (1810-3560)*	*2660 (2070-3460)*
MPPD 02	*1680 (1500-1910)*	*0.055*	*1690 (1610-1770)*	*0.023*	*1660 (1140-2510)*	*1680 (1340-2200)*
MPPD 03	*1420 (1320-1530)*	*0.041*	*1350 (1290-1400)*	*0.023*	*1510 (1170-1950)*	*1250 (940-1680)*
MPPD 04	*1220 (1090-1340)*	*0.055*	*1310 (1240-1370)*	*0.023*	*1040 (712-1560)*	*1200 (993-1530)*

* Standard used: HCG (2nd IS)

Table 3.

The LH activity of various HMG and HHG preparations as estimated by the in vitro bioassay method using"Leydig cell" suspensions prepared by mechanical separation from mouse testes. Comparisons are made with the in vitro bioassay method using decapsulated testes and with the in vivo OAAD

method(Van Damme et al.1973). Figures in parentheses represent 95% fiducial limits.

	"Leydig cell"method (Eagle's medium with Calf serum)		"Leydig cell"method (Eagle's medium without Calf serum)		Decapsulated testes method		In vivo method (OAAD)	
Hormone Preparat.	IU/amp	Index of Prec. (λ)	IU/amp	Index of Prec. (λ)	IU/amp	Index of Prec. (λ)	IU/amp	Index of Prec. (λ)
Urinary gonadotrophins a)								
HMG								
Homogonal	65(61-69)	0.031	58(54-64)	0.044	50(38-70)	0.18	49(30-95)	0.22
Humegon	69(65-73)	0.031	69(64-76)	0.044	75(64-87)	0.19	52(33-92)	0.19
70/45	58(55-61)	0.031	63(58-69)	0.044	59(41-88)	0.19	45(37-54)	0.12
FSH								
E 161 Ter-4	2.1(1.9-2.3)	0.060	-	-	3.4(2.6-4.5)	0.17	-	-
Pituitary gonadotrophins d)								
HHG								
HHLH	140(127-150)	0.031	135(122-150)	0.034	74(55-100)	0.19	92(67-131)a)	0.20
XL-E3	190(172-202)	0.031	1380(1300-1490)	0.034	240(180-330)	0.18	440(270-770)	0.22
B/62	1380(1260-1490)	0.031	1380(1300-1490)	0.034	865(640-1230)	0.19	770(550-1050)	0.16
HTSH								

Table 4.

The LH activity of several HCG,HMG and HHG preparations as estimated by the in vitro bioassay method using"Leydig cell" prepared by collagenase digestion from mouse testes.Comparisons are made with the method using cells prepared by mechanical separation. Eagle's medium containing 2 % calf serum was used in both methods. Figures in parentheses represent 95%fiducial limits.

	"Leydig cell"prepared by collagenase	"Leydig cell"prepared by mechanical

	digestion		separation
Hormone preparation	IU/mg	Index of Precision(λ)	IU/mg a)
MSKG	16 700(15 720-17 770)	0.030	16 780
J 26-30	7380(6840-7980)	0.038	8070
428 Zep	2880(2660-3110)	0.038	2570
MPPD 02	1780(1660-1920)	0.036	1680
MPPD 04	1390(1300-1500)	0.036	1220
Homogonal b)	68(63-74)	0.039	65
HHLH 68/40 b)	120(115/123)	0.016	140
B/62	1460(1380-1540)	0.030	1380
HTSH 1728-3	208(195-221)	0.033	210
HTSH 1728-2B	243(225-260)	0.033	196
HTSH 1728-1	4530(4240-4830)	0.032	4900

A) Cf. Table 2 and 3

B) IU/amp

D I S C U S S I O N

The use of mouse “Leydig cell” in the bioassay method leads to a considerable increase in sensitivity, precision and practicability over the decapsulated testes method. The sensitivity is increased at least 25 times. This increase is attributed to a reduction in incubation volume (5 times), calf serum (3 times) and the remainder to the increase in sensitivity attained in its presence was considered necessary when the method was to be applied to the measurement of very low activity in biological samples. For preparations with high activity there was no added advantage in including the calf serum as equally valid assays can be conducted in its absence. The

precision of the method was greatly improved as indicated by the change in mean λ value from 0.22 to 0.044. This has a significant effect on the fiducial limits, which are reduced from 68% (decapsulated testes method) to 15% for the present method.

In terms or practicability 10 unknown preparations can be assayed in 1 1/2 day by 2 persons. The costs of the assay are also considerably reduced; to assay 10 preparations 100 mice were necessary using the decapsulated testes method while 3 mice sufficient with the "Leydig cell" technique. As many as 300 rats were needed for the same purpose, with the WITARO method.

The specifity studies have been extended with the inclusion of human prolactin and 2 highly purified human TSH preparations. Luteinizing hormone activity was only found in FSH and TSH preparations. This was attributed to contamination by LH as indicated by the results obtained by radioimmunoassay or in vitro bioassay methods.

Antagonistic effects were observed when HCG, HMG or HHG preparations were assayed in the presence of high levels of a number of hormones(STH, oxytocin, vasopressin and LHRH). These high levels are unlikely to be found in biological samples such as peripheral plasma (Della Casa 1968; Chard et al. 1970; Walker 1961). Thus it can be concluded that human FSH, TSH, ACTH, LTH, STH, oxytocin, vasopressin and LHRH have no effect on the assay at levels expected to be found in biological samples.

All HCG, HMG and HHG preparations gave parallel dose response relationships when assayed against their respective standard.

A good correlation was found between potency estimates obtained by the use of decapsulated testes and the "Leydig cell" method for both HCG and HHG preparations ($r=0.99$, $n=10$; $r=0.99$, $n=8$). However, closer inspection of the data (Table 3) shows considerable discrepancies between potency estimates of several HHG preparations obtained by the various methods. Marked differences in potency estimates for a number of HHG preparations were also observed when the radioligand method was compared with the OAAD method (Leidenberger & Reichert 1972). A more extensive study of HHG preparations using a variety of in vitro and in vivo techniques and radioligand methods will be necessary to classify the nature of these discrepancies.

The present method is similar to the radioligand method of Leidenberger & Reichert 1972, in terms or precision and practicability in the estimation of some gonadotrophins, In the assessment of the relative potency of HCG (2nd IS) in terms of HMG(2nd IRP) these authors reported a mean relative potency of 3.90(3.68-4.85) while in the present study a lower value of 3.21 (3.09-3.34) was obtained. With the decapsulated testes method a value of 3.37 (2.72-4.02) was previously reported. Further comparisons with other preparations, particularly HHG preparations were not possible since different standards were used in the 2 studies.

In preliminary studies, "Leydig cell" suspension were prepared by the method of Moyle& Ramanchandran 1973 using collagenase digestion, and introduced into the proposed bioassay system. A relatively high dose response curve to HCG, HMG and HHG. Potency estimates comparable to those obtained by other methods were found when various gonadotrophin preparations were assayed against the appropriate standards. It was concluded that under the experimental conditions employed, collagenase treatment offers no major advantage over mechanical disruption of the testes. A comparison of the data in the present study with the results reported by Moyle& Ramanchandran 1973 was not possible as different gonadotrophin preparations had been used. The synergistic effect of calf serum was also observed with cells prepared by the enzymatic digestion method.

The high sensitivity, precision and practicability of the present method open up the way to measure circulating LH activity in various clinical conditions and to compare the results in such bioassay with those obtained by various immunological techniques. Such studies are now in progress in this laboratory.

A C K N O W L E D G M E N T S

We are much indebted to Dr. L. Reichert Jr. (Atlanta) and to Dr. J. Pierce (Los Angeles) for the highly purified human TSH preparations, to Dr. H. Friesen (Montreal) for the highly purified human LTH preparations, to Dr. E. Nieschlag (Düsseldorf) for the gift of the testosterone antibody, to the MRC National Institut for Biological Standards and Control (London) for

the HHLH preparations and to Dr. Bettendorf (Hamburg) for the HHG preparations. Synthetic LH-RH was a gift from Hoechst AB. Frankfurt.
The expert technical assistance of Mrs. Ulla-Britta Edqvist and Miss Gun Thorell is gratefully acknowledged.
The expenses of this investigation were defrayed by reaseach grants from the Swedish Medical Research Council, the World Health Organization Expanded Programme of Research, Development and Research Training in Human Reproduction (Geneva), the Ford Foundation (New York) and the AB Leo Research Foundation (Helsingborg).

R E F E R E N C E S

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Received on February 21st, 1974
VOL.77 No.4.
PERIODICA COPENHAGEN
ACTA ENDOCRINOLOGICA
77 (1974) 655- 671

Swedish Medical Research Council, Reproductive Endocrinology Research Unit, Karolinska sjukhuset, Stockholm 60

Reprinted from: Periodica Copenhagen, Acta Endocrinologica, 77 (1974) 655-671

BIOLOGICAL AND IMMUNOLOGICAL CHARACTERIZATION OF HUMAN LUTEINIZING HORMONE: I.BIOLOGICAL PROFILE IN PITUITARY AND PLASMA SAMPLES AFTER ELECTROFOCUSING.

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Received 14 April 1977; accepted 1 July 1977

Pituitary and plasma pools from postmenopausal women and plasma pools from women at midcycle were fractionated by electrofocusing in sucrose density gradients. The biological LH activity was determined in each of the electrofocusing fractions by the use of an in vitro bioassay method.

A heterogeneous profile of LH activity was found in both pituitary and plasma samples with a large proportion present within the pH range 6.5-10. In a total of 11 electrofocusing runs 7 main regions of high LH activity were found within this range with mean pl values (±SD) of 6.75±0.08 (n=6), 7.33±0.08 (11), 8.81±0.04 (7), 9.17 ± 0.05 (6) and 9.55(2).

A significantly higher proportion of LH activity was found in the midcycle plasma samples (36%) in the pH regions with mean pI values of 8.81, 9.17 and 9.55 than in postmenopausal plasma (7%) and pituitary extracts (5%). This indicates that the profile of biologically active LH in women in the fertile age is different from that present in postmenopausal women.

By detailed fractionation based on narrow pH range studies and the refocusing of specific peak fractions it was shown that each of the regions

studied consisted of several peaks of LH activity indicating the presence of a large number of molecular species exhibiting varying degrees of LH activity. The relative proportions of these species showed considerable differences between sources but also between samples from same source.

Keywords: human luteinizing hormone from pituitary and blood; biological and immunological characterization; electrofocusing.

Previous studies (Qazi et al., 1974; Romani et al., 1977) have shown that the levels of biologically active LH in plasma from women as measured by an in vitro bioassay method were considerably higher than those measured by radioimmunoassay methods. In addition, after fractionation of plasma pools from women by gel filtration, a heterogeneous LH profile was obtained with marked disparities between estimates obtained by two methods (Qazi et al., 1974).It was concluded from these studies that various LH species with different biological and immunological properties may be present in plasma.

The purpose of the present study was to further analysesthe heterogeneity of LH in plasma by the use of electrogocusing methods. Plasma from postmenopausal women and from normally menstruating women at midcycle was examined. In order to differentiate the LH species which were structurally modified as a result of entering the circulation, electrofocusing studies were also performed on aqueous extracts of human pituitaries. Electrofocusing fractionation was chosen because of its high resolving capacity for proteins and because it gave almost quantitative recoveries after fractionation of pituitary LH preparations (Rathnam and Saxena, 1970). In this publication the profiles of biological LH activity in plasma and pituitary samples after electrofocusing are presented. An investigation of the immunological characteristics of the pituitary samples is reported in a subsequent paper (Robertson and Diczfalusy, 1977).

MATERIALS AND METHODS

Abbreviations MCP, midcycle plasma; PMP, postmenopausal plasma; Pit, pituitary extract; BSA, bovine serum albumin.

Reagensts Human menopausal gonadotrophin (2nd IRP of Human Menopausal Gonadotrophins (FSH and LH (ICSH)), urinary, for bioassay), human pituitary gonadotrophin (1st IRP of Human Pituitary Gonadotrophin (FSH and LH(ICSH)) for bioassay (hereafter designated as 69/104) and Human Pituitary Luteinizing Hormone (LH (ICSH)) for immunoassay (hereafter designated as 68/40) were obtained from the MRC National Institute for Biological Standards and Control (London). The composition of the phosphate buffer was 6.5 mM Na2HPO4, 1.4 mM KH2PO4, 0.13 M NaCl, pH 7.4.

Beef haemoglobin (Type 1) was obtained from Sigma Chemical Co. When assayed for biological LH activity by the in vitro bioassay method, no detectable activity was observed at a concentration of 3 mg/ml corresponding to an LH potency of less than 0.03 mlU/mg protein.

Clinical material Plasma was obtained from postmenopausal women and from normally menstruating women on the day of the midcycle LH surge. The criteria used to characterize normal menstrual cycle have been previously reported (Guerro et al., 1976). Three plasma pools, each from at least five women, were collected and designated as PMP-1, PMP-2 and PMP-3 in the case of postmenopausal plasma and MCP-1, MCP-2 and MCP-3 in the case of midcycle plasma. In addition, plasma was collected from women using oestrogen-progestagen combination pills. The level of biologically active LH in these latter samples (<3mlU/ml) were less than the sensitivity of the bioassay method when applied to plasma (Romani et al., 1976).

Human pituitaries from postmenopausal women were obtained at autopsy and frozen until required. The first pituitary extract (Pit-1) was obtained from a single pituitary. The second (Pit-2) and third (Pit-3) extracts were obtained from pools of 4 and 7 pituitaries, respectively.

Preparation of samples for electrofocusing

Plasma pools (7-20 ml) were dialysed against 0.15 M NaCl or distilled water for 24 h and then applied to a G-100 Sephadex column (89x5 or 90 x 2.5 cm) and eluted with 0.15 M NaCl or phosphate buffer. The fractions containing the biologically active LH (corresponding to a molecular weight range of 25.000-65.000) (e.g. Qazi el al., 1974) were pooled and

concentrated by dialysis against 50% sucrose for 24-48 h prior to electrofocusing.

Plasma samples devoid of biological LH activity, which were obtained from women using oestrogen-progestagen combination pills, were also processed by the above procedure. The chromatographic fractions corresponding to the biologically active region (hereafter plasma carrier) were pooled and frozen.

The pituitaries were homogenized at 4o C in 2- 5 volumes of 0.25 M sucrose containing 0.14 M NaCl followed by centrifugation at 100.000 g for 1 h. The supernatant fractions were dialysed and applied directly to the electrofocusing column.

Electrofocusing procedure

Electrofocusing experiments in sucrose density gradients were performed on 110 ml of 440 ml columns (LKB-Produkter AB, Bromma, Sweden) using carrier ampholytes (Ampholine, LKB-Produkter AB) at 1% or 2% concentrations in the pH ranges 3-10,3.5-10,7-9 and 6-9. The temperature of the cooling water was 3-13oC.

Three electrofocusing procedures were employed (table 1). The first procedure (I) was similar to that described in the LKB manual (1972 edition). The first procedure requires long time periods for focusing which may lead to losses of biological activity (table 3). Therefore attempts were made to shorten the focusing time in order to obtain higher recoveries. In the second procedure (II) the starting power was increased to 10 W with the 440 ml column or to 5 W with the 110 ml column, which resulted in shorter focusing times. When the high-speed electrofocusing produce with constant power (LKB Application Note 194) became available it enable even shorter focusing time periods. This electrofocusing procedure (III) employed starting voltage of 2000 V or 30W on the 440 ml column or 1600 V or 10W on the 110 ml column. Further details are presented in table 1.

At the completion of the run, the column contents were withdrawn by means of a peristaltic pump and 3 ml fractions (440ml column) or 1 ml fractions (110 ml columns) were collected. The pH was read directly at 4oC with a digital pH meter (PHM 63,Radiometer, Copenhagen) with an accompanying strip chart recorder (Servograph Type REC51b, Radiometer, Copenhagen).

The samples were then either diaysed against 0.15 M NaCl for 4- 6 days (procedures I and II) or against a phosphate buffer for 36 h(procedure III, 440 ml column).

Table 1

Samples and condition used in the electrofocusing experiments

Sample	Protein mg	pH range	IEF Column (ml)	Ampholine Conc. (%)	Separation Time (h)	Precipitate formation
Pstmenopausal plasma :						
PMP-1[a)]	409	3-10	440	2	163	++
PMP-2[a)]	285	3-10	440	2	187	++
PMP-3[c)]	1100	3.5-10	440	1	16.4	-d)
PMP-3[c,g)]		6-9	440	1	16.5	-d)
Midcycle plasma:						
MCP-1	127	3.5-10	440	2	135	+
MCP-2	41	3.5-10	110	2	67	-d)
MCP-3	1200	3.5-10	440	1	15	-d)
MCP-3		6-9	440	1	15.5	-d)
Pituitary extracts:						
Pit-1	e)	3.5-10	440	1	86	-d)
Pit-2	55	3.5-10	440	1	61	++
Pit-2	18	7-9	440	1	114	-d)
Pit-2	40	6-9	110	1	15-19	-d)
Pit-3	7.5	3.5-10	110	1	20	-d)
Pit-3	22.5	6-9	110	1	21	-d)

a), b) and c) indicate IEF procedures I, II and III, respectively (see Methods section)

d) Samples predialysed against distilled water.

e) Estimated 5-10 mg.

f) Refocusing experiments (see text)

g) The pH 6-9 region of the preceding broad pH range (3.5-10) electrofocusing was refocused over the pH 6-9 range.

Fractions from the 110 ml column using procedure III were separated by gel filtration (Sephadex G-25 medium; column dimensions 11.5X1 cm or 13X1 cm) using phosphate buffer containing 0.1% BSA as eluent buffer. Unless otherwise stated, bovine serum albumin in a final concentration of 0.1% was added before dialysis or gel filtration. At the completion of the dialysis the fractions were measured for volume, the samples were aliquoted and frozen until further required.

Precipitation was observed after electrofocusing in those runs (table 1) in which the sample had been previously dialysed against 0.15 M NaCl and not distilled water. In most cases the precipitate was observed in the acidic region of the pH gradient separate from most of the LH fractions. There was no marked difference in resolution or recovery of LH activity when electrofocusing runs in which precipitation did occur were comared with those runs in which precipitation did not occurred (cf. table 1). The use of a higher ampholyte carrier concentration (2%) did not result in a appreciable reduction in precipitation.

In all studies in the pH ranges 6-9 and 7-9 in which a plasma carrier was used refraction lines were observed. These originated at the beginning of the electrofocusing run at the boundary of the buffer surrounding the anode and the sucrose gradient and migrated into the gradient during the course of the experiment which resulted in the degereration of the pH in this region, This phenomenon was not overcome by reducing the power or voltage (e.g. to 3 W or 700 V) or by increasing the concentration of the carrier ampholytes from 1 % to 2 %. However, the resolution of the remaining pH gradient and the recoveries of added LH were not adversely influenced by this artifact.

In order to obtain maximum resolution, plasma pools PMP-3 and MCP-3 wwere first electrofocused in a broad pH range (3.5-10.0), which was then followed by a refocusing run over a narrow pH range (6-9).

The reliability of the electrofocusing procedure.

This was investigated as follows: Firstly, by comparing the electrofocusing profiles of two pituitary extracts (Pit-2 and Pit-3) obtained over a broad (pH 3.5-10) and narrow (pH 6(7)-9) pH range in terms of the pI values of the major biologically active fractions and the recovery of

bioactivity in the corresponding pH regions (table 3). The profiles, considering the different degrees of resolution, were similar, with no systematic bias observed between the two pH ranges with the two preparations.

Secondly, by the reproducibility of the pI value of four selected fractions (fractions1-4mcf. Fig.1) obtained from a previous narrow range electrofocusing run of a pituitary extract (Pit-2) when refocused in four separate narrow range (pH 6-9) electrofocusing runs. A plasma fraction devoid of biological activity (plasma carrier 40mg) was also added to reduce losses and to simulate conditions which exist in plasma. The pI value of the first fraction (7.35) gave two closely adjacent peaks with pI values of 7.25 and 7.35, the second fraction (pI7.72) gave two peaks (pI 7.67 and 7.75), the third peak (pI 7.89) two peaks (pI 7.75 and 7.84) and the fourth (pI 8.18) a single peak at pI 8.14. The recovery of biological activity ranged from 71% to 93% (mean: 84%).

Thirdly, by the reproducibility of the pI value of beef haemoglobin added as marker protein in 9 narrow pH range (6-9) electrofocusing runs (procedure III). A mean (±SD) pI value of 7.90±0.03 (n=9) was obtained. The pI values of beef haemoglobin in the presence (7.87±0.02; n=5) or absence (7.0±0.04; n=4) of plasma carrier were not significantly different.

Bioassay method

An in vitro bioassay method based on the reproduction of testosterone by mouse interstitial cell preparations incubated in the presence of graded doses of LH was employed (Van Damme et al., 1974). Pituitary hormones other than LH do not cross-react in this system (Van Damme el all., 1974) and the constituents of normal female plasma do not interfere with the validity of the assays (Romani et al.,).

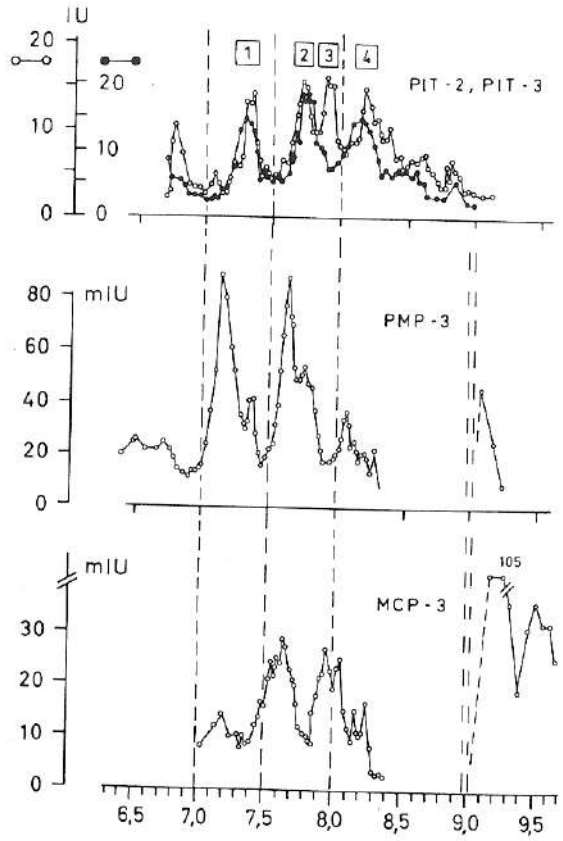

Fig.1. Narrow pH range electrofocusing profile of bioactive LH in two pituitary (Pit-2 (0) and Pit-3 (0)) and two plasma pools (postmenopausal PMP-3 and midcycle MCP-3) within the pH range 6.5-9.5. In the plasma samples an initial electrofocusing in the pH range 3.5 – 10 was carried out which was followed by the refocusing of the pH 6-9 region. In these samples the activity observed in the pH range 9-10 originated from the first electrofocusing, the remainder from the second (partitioned by a vertical double dotted line). The vertical single dotted lines were introduced to aid comparison between figures. The peak tubes in the biologically active regions with mean p I values of 7.35 (fraction 1), 7.72 (2), 7.89(3) and 8.18(4) were pooled before being used in the refocusing experiments.

A mean index of precision (λ) of 0.040 was obtained in 230 2+2 multiple parallel line assays in the latter study.

All bioassays consisted of a minimum 2+2 point parallel line assay design with 5 replicates/dose level. Samples from the major peaks were also assessed for linearity and parallelism with the standard using a 3+3 point parallel line assay design (e.g. Van Damme et al., 1974; Romani et al., 1976). A mean index of precision (λ) of 0.044 from 127 2+2 point assays was obtained in the present study.

Table 2

Mean potency and 95% confidence limits (in parentheses) of various International Reference Preparations for human luteinizing hormone (hLH) estimated by an in vitro bioassay method (cf. Van Damme et al., 1974).

Reference preparation assayed	Stated potency of preparation (IU/ampoule)	Reference preparation as standard	Estimated mean potency	No. of assays
69/104	25	hMG (2nd IRP)	51.5 (49.1-53.9)	24
68/40	77	69/104	136.9(133.8-141.5)	17
68/40	77	hMG (2nd IRP)	249.6(238.7-260.0)	10

In the present investigation the buffer used for diluting the samples and standards was changed from Eagle's medium to either 0.15 M NaCl containing 0.1% BSA or phosphate buffer containing 0.1% BSA. No significant difference was observed in the dose-response curves of the standard or unknown LH preparations in terms of sensitivity or slope with any of the three buffers used.

To assess whether or not the carrier ampholytes interfere in the bioassay, 1% ampholyte solutions in the pH ranges 3.5-10.0 and 7-9 were neutralized with hydrochloric acid or phosphate buffer and bioassayed in the presence of increasing dose of the standard. No significant effect in terms of changes in the sensitivity or the slope of the dose-response curves was observed. Suncrose in a final concentration of 0.2% showed no significant effect on the results of bioassays; however, with higher concentrations (0.3-3.3%) an inhibitory effect was observed.

Protein was assayed by the method of Lowry el al. (1951), using bovine serum albumin as standard.

Standard preparation hMG (2nd IRP) and 69/104 were used as laboratory standards throughout the study. Since these standard preparations as well as 68/40 give parallel dose-response lines in the bioassay, data obtained with the use of one standard can be restarted in terms of the other or of the 68/40 standard. The conversion factors are presented in table 2. Throughout this study all results are expressed in terms of the 69/104 standard preparation unless otherwise stated.

RESULTS

15 electrofocusing experiments consisting of 11 broad and narrow pH range studies and 4 refocusing experiments of selected fractions were conducted on 3 pituitary extracts, 3 postmenopausal plasma pools and 3 midcycle plasma pools. The plasma pools had been previously fractionated by gel filtration and the biologically active fractions were pooled and used for electrofocusing. The conditions used in these electrofocusing experiments are presented in table 1.

The biological LH activity was determined in 180 3 ml fractions from 440 ml column and 120 1 ml fractions from the 110 ml column after fraction by electrofocusing. The profiles of biological activity have been arranged according to the pI values of the bioactive peak fractions as best seen in the broad pH range electrofocusing studies, and the relative proportion of the biological activity in the various pH regions for the various electrofocusing runs has been tabulated (table 3). This graphical presentation enables a more direct reading of the pI values of each fraction, although it is limited in that the distribution of the bioactivity is not directly related to the corresponding fraction number since the pH gradient is not linear over the whole pH range.

Broad pH range (3-10) electrofocusing studies of pituitary and plasma samples gave profiles of biological activity in which the bulk of activity was found in the pH region 6.5-10.0 (cf. IEF procedures II and III, table 3).

Bioactivity was also present in some electrofocusing runs over the acid pH range, although no distinct peak fractions could be noted (cf. table 1).

The mean pI values (±SD) for all electrofocusing studies (n=11) for the main bioactive fractions were 6.75±0.08 (n=6), 7.33±0.08 (11), 7.80±0.09 (11), 8.23±0.10 (11), 8.81 ±0.04 (7) 9.17±0.05 (6) and 9.55(2). In addition, a peak with pI 4.5 was observed in each of one PMP and one MCP electrofocusing run.

A significantly higher proportion of the total biological activity recovered was found in the pH region 8.5-10.0 following electrofocusing of MCP samples (36%) than after electrofocusing of Pit (17%P<0.01) and PMP (7%;P<0.001) samples. Conversely, PMP samples contained higher proportions of biological activity n the pH region 6.5-7.0 (10%) than either MCP (<2%; P<0.001) or Pit (5% <0.05) samples. The above comparisons were only made with results obtained from electrofocusing studies over a broad pH range (pH 3.5-10) (table 3).

When pituitary and plasma samples were subjected to narrow range electrofocusing the main bioactive fractions proved to be heterogeneous (fig.1), although the average pI value for each peak region was comparable to that observed in the broad range studies. The microheterogeneity observed with these peak fractions between sources could also be observed in varying degrees between samples from the same source (cf. Pit-2 and Pit-3, fig. 1).

The overall recovery of biological activity after electrofocusing ranged from 41% to 102% (average78%, cf. table 3). An average of 61% of the bioactivity was recovered using electrofocusing procedure I. Higher recoveries (90%) were obtained with procedure III (P<0.05). These differences can be accounted for by the duration of the electrofocusing. Procedure, the dialysis step and the difficulty of determining low levels of LH activity spread over the acid pH region in some of the samples obtained by procedure I.

The protein concentration was determined in a pituitary (Pit-2) preparation after electrofocusing in the pH range 3.5-10.0. Peak fractions with pI values of 8.84, 8.44, 7.99 and 7.50 were obtained with specific activities of 1640, 1231, 616 and 455 IU/mg protein, respectively.

455 IU/mg protein, respectively. The bioactivity of the original pituitary extracts (Pit-2 and Pit-3) was 55 and 36 IU/mg protein, respectively. It is realized that protein determination alone provides only an approximate

estimate of the weight of LH, since it does not take into account differences in the carbohydrate content.

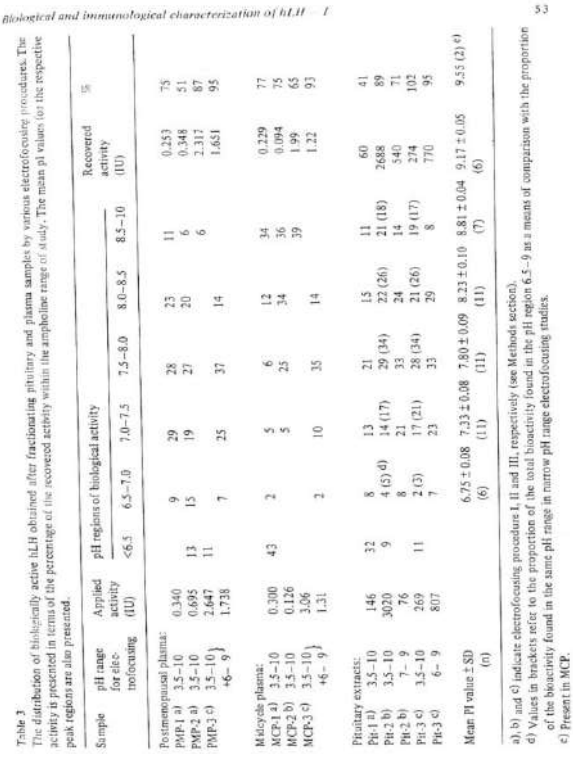

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Table 3
The distribution of biologically active hLH obtained after fractionating pituitary and plasma samples by various electrofocusing procedures. The activity is presented in terms of the percentage of the recovered activity within the ampholine range of study. The mean pI values for the respective peak regions are also presented.

Sample	pH range for electrofocusing	Applied activity (IU)	pH regions of biological activity							Recovered activity (IU)	%
			<6.5	6.5–7.0	7.0–7.5	7.5–8.0	8.0–8.5	8.5–10			
Postmenopausal plasma:											
PMP-1 a)	3.5–10	0.340		9	29	28	23	11		0.253	75
PMP-2 a)	3.5–10	0.695	13	15	19	27	20	6		0.348	51
PMP-3 c)	3.5–10	2.647	11	7	25	37	14	6		2.317	87
	6– 9	1.738								1.651	95
Malcycle plasma:											
MCP-1 a)	3.5–10	0.300	43	2	5	6	12	34		0.129	77
MCP-2 b)	3.5–10	0.126			5	25	34	36		0.094	75
MCP-3 c)	3.5–10	3.06						39		1.99	65
	6– 9	1.31		2	10	35	14			1.22	93
Pituitary extracts:											
Pit-1 a)	3.5–10	146	32	8	13	21	13	11		60	41
Pit-2 b)	3.5–10	3020	9	4 (5) d)	14 (17)	29 (34)	22 (26)	21 (18)		2688	89
Pit-2 b)	7– 9	76		8	21	33	24	14		540	71
Pit-3 c)	3.5–10	269	11	2 (3)	17 (21)	28 (34)	21 (26)	19 (17)		274	102
Pit-3 c)	6– 9	807		7	23	35	29	8		770	95
Mean PI value ± SD (n)				6.75 ± 0.08 (6)	7.33 ± 0.08 (11)	7.80 ± 0.09 (11)	8.23 ± 0.10 (11)	8.81 ± 0.04 (7)	9.17 ± 0.05 (6)		9.55 (2) e)

a), b) and c) indicate electrofocusing procedure I, II and III, respectively (see Methods section).
d) Values in brackets refer to the proportion of the total bioactivity found in the pH region 6.5–9 as a means of comparison with the proportion of the bioactivity found in the same pH range in narrow pH range electrofocusing studies.
e) Present in MCP.

DISCUSSION

A number of studies have been carried out on the heterogeneity of purified human pituitary LH preparations (Peckham and Parlow, 1969; Rathnam and Saxena, 1971; Reichert, 1971; Graesslin et aI., 1973; Rooset aI., 1975).

Three fractions with pI values ranging from 6.1 to 7.1, 7.3 to 7 .6 and 7.7 to 8.1 are common to all these studies. A fourth fraction with a pI value of 9.2 was also reported in one study (Reichert, 1971). It has been shown that these fractions have otherwise similar physico-chemical properties (molecular weight, amino acid content), although in some reports (Roos et aI., 1975; Rathnam and Saxena, 1971) differences in sialic acid content were noted.

In the present study an attempt was made to assess in detail the heterogeneity of biologically active LH in unprocessed aqueous pituitary extracts in order to compare it with LH from plasma. A comparison of the pI values for the various fractions in purified preparations (above) and in unprocessed pituitary extracts shows apparent similarities between 6.1 and 8.1 with apparent dissimilarities between 8.1 and 9.2. Studies are currently in progress in order to clarify this aspect.

It is apparent that the profile of biological activity obtained after electro-focusing is complex, with at least 20 discernible entities observed in the pH range 6.5-9.5. However, as best seen after broad range electro focusing, these activities appear to fall into 7 principal regions; to each of these an average pI value can be assigned.

Using this classification as a first appraisal of the data, it appears that there are statistically significant differences in the distribution of bioactivity with the most pronounced differences observed in the relatively more alkaline pH regions of the midcycle plasma pools. These data suggest that there is an endocrinological basic for these differences.

Similar conclusions based on the change in apparent molecular weight and half-life of the circulating hormone under various physiological conditions have been reported for LH (and FSH) in the rhesus monkey (Peckham and Knobil, 1976a,b). Differences in half-lives under different physiological conditions have also been observed in the rat (Bogdanove et aI., 1975).

The causes for the microheterogeneity of these LH regions are uncertain since differences were also observed not only between samples obtained from different sources, but also between samples from the same source. These differences can be large, as especially evidenced in the pH region 7.5-8.0 where pI values of 7.67, 7.75, 7.84 and 7.90 were observed in different proportions. From the evidence pre sented it seems unlikely that these differences are due to methodological problems related to the electrofocusing procedure; whether they can be attributed to other changes (e.g. post-mortem changes in pituitary LH) remains to be established.

It has been suggested that the observed heterogeneity of pituitary LH is due to differences in the content of sialic acid in its various components (Reichert, 1971). This would imply different clearance rates and therefore different in vivo biological activities. Reichert (1971) reported that after

desialylation by neuraminidase treatment of a purified human LH preparation consisting of four major fractions, a single peak with a pI value of 9.35 was obtained. However, the literature reports are at variance as to the sialic acid content of the various LH components in different LH preparations. Rathnam and Saxena (1971) reported 0.39, 1.09 and 1.57 residues/LH molecule, while Rooset al. (1975) obtained in four components an average of 5, 7, 7 and 5 residues/molecule. This aspect requires further examination.

As indicated in table 3, biological activity was found throughout the acid pH range in several, but not all, samples after electrofocusing. Similar results were obtained by Kercret and Duval (1975) who observed the presence of LH fractions in the acid pH range in purified but not in crude rat LH preparations. It was suggested by these authors that this component was a result of modifications during purification or storage. In the present study, relatively large amounts of this component were obtained when the more lengthy electro focusing procedure was employed. Hence it is possible that the LH found in the acid region is a modified form which is not secreted as such.

Discrepancies in pI values of human pituitary LH have been reported after electrofocusing the same preparation in different ampholine pH ranges which were attributed to interactions between the carrier ampholytes and the hormone (Reichert, 1971). This possibility was assessed in the present study by comparing electrofocusing profiles over more than one pH range and by refocusing specific fractions. No evidence was obtained indicating that artifacts of this type are produced by the electrofocusing procedure. In support of this there have been several recent reports (Dean and Messer, 1975; Baumann and Chrambach, 1975) which have shown that ampholyte-protein complexes do not in fact occur with a large number of protein preparations.

The use of isoelectric focusing in fractionating LH underwent several modifications before optimal conditions were established. The final procedure, employing high-speed electrofocusing followed by gel filtration, as seen in the refocusing studies, requires a relatively short time period for completion (24-30 h), yields high resolution (±0.03 pH units) and reproducibility (±0.03 pH units) and results in high (90 ± 13%) recoveries;

ACKNOWLEDGEMENTS

The authors are indebted to Dr. B.-M. Landgren for collection of the clinical material, to Mrs. B. Fraysa, Mrs. *V.-B.*Edqvist and Mrs. M. Lindberg for their expert

technical assistance and to Mr. H. Perlmutter (LKB-Produkter, Bromma, Swden) for his cooperation with the high-speed clectrofocusing experiments.

The **LH** standards (hMG 2nd **IRP,** 69/1 04 and 68/40) were a gift from the National Institute for Biological Standards and Control (London).

The expenses of this investigation were defrayed by grants from the Swedish Medical Research Council, the Ford Foundation, the World Health Organization Expanded Programme on Research, Development and Research Training in Human Reproduction, and the Leo Research Foundation, Helsingborg, Sweden.

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Reprinted from: Molecular and Cellular Endocrinology, 9 (1977) 45-56

PHARMACOKINETIC AND PHARMACODYNAMIC EFFECTS OF SMALL DOSES OF NORETHISTERONE RELEASED FROM VAGINAL RINGS CONTINUOUSLY DURING 90 DAYS.

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ABSTRACT

The pharmacokinetic and pharmacodynamics effects of norethisterone (NET) released continuously from vaginal devices at rate of 50 μg/24 h and 20 μg/24 h, respectively, were investigated during a 90-day period in two groups of 7 women each with regular menstrual periods. Blood samples were taken three times weekly (Mondays, Wednesdays and Fridays) during a control cycle and during the entire levels, and hourly blood samples were collected throughout a 24-hour period after 6 weeks of exposure to assess the short-term variation in NET levels. In addition, an endometrial biopsy was taken on days 21-23 of the control cycle and after 6 and 10 weeks with the device in situ.

There was little, if any, initial "burst" effect on the plasma levels of NET following the insertion of the devices, and a statistically significant linear relationship was found in each case when the NET levels were plotted against the 90 days of exposure. However, the average slope of the regression lines with the 50qg-releasing devices (-0.44) was significantly flatter than with the 20 μg-releasing devices (-1.63). Also the mean plasma level in the former group (238 μg/ ml) was significantly lower than that (666 μg/ml) observed in the latter group. The average daily decline in plasma NET levels was 0.16% and 0.24%, respectively, and the 95% confidence limits of the hour-to-hour variation in NET levels were at 92 and 106%.

All control cycles were of normal length (26-35 days) and exhibited a normal luteal activity. There were a total of 16 cycles with normal luteal function among the 7 subjects with 50 μg-releasing devices; each of them

had a minimum of 2 such cycles. However, 11 of these 16 cycles were of abnormal length, indicating a significant effect of norethisterone on the follicular phase. Only 9 cycles were found with a normal luteal activity in the group with the devices releasing 200 μg/24 hl 3 of the subjects in this group had anovulation throughout the entire study, and 2 of them had prolonged anovulatory periods alternating with normal cycles.

Thirteen of the 14 biopsies taken with the 50 μg-releasing devices in situ after 6 and 10 weeks of exposure showed normal cyclic changes, whereas 11 of the 14 biopsies taken in the group exposed to a release rate of 200 μg/24 h indicated predecidual and atrophic changes. These were associated with a significantly diminished number of endometrial glands per microscopic field and with a significant increase in the number of days with bleeding and spotting. Hence, the increased frequency of intermenstrual bleeding may be associated with the predecidual and atrophic changes induced by the administration of NET.

No correlation was found between the individual plasma levels of NET and the following parameters: weight, height and ponderal index of the subjects, individual release rate of NET from the devices, effects on ovarian function, the appearance of the endometrium and the number of days with bleeding and spotting.

It is concluded that the vaginal rings studies provide a near zero order release of norethisterone and represent an ideal experimental model for the study of the effects of constant blood levels of contraceptive steroids.

INTRODUCTION

Since the pioneering studies of Mishell and coworkers (1,2), a considerable number of investigations have been reported on the vaginal administration of contraceptive steroids in relatively high, ovulation inhibiting doses (3-17). Vaginal devices releasing constant amounts of steroids appear to represent an attractive approach to contraception, since they are self-administered, long-acting, their presence in the vagina seems to cause little, if any, inconvenience and the exposure to the contraceptive steroid can be rapidly discontinued at any time by easy self-removal. In addition, vaginal administration of steroids results in an excellent absorption and avoids the “first passage effect” through the liver.

There is also another useful application for vaginal devices, namely to provide suitable pharmacodynamics models for the assessment of the likely effects of contraceptive steroids released at near zero order, for example from biodegradable implants. Such implants aim at eliminating the major fluctuation in the plasma levels of orally administered steroids, hoping that in this way some of the side effects can be reduced. It is also conceivable that a zero order release may considerably reduce the amount of steroid needed for contraception and thus the total steroid load to be metabolized. Were it possible to achieve constant plasma levels ofcontraceptive steroids following their release from vaginal devices, the above propositions could be tested experimentally.

Hence, the aim of the present study was to provide an in-depth assessment of the pharmacokinetic and pharmacodynamics effects of norethisterone (NET) released continuously over a period of 90 days from vaginal rings in constant small doses, which are not excepted to inhibit ovulation in the majority of subjects studied.

MATERIAL AND METHODS

Vaginal devices. These were fabricated by the Battelle Memorial Institute, Pacific Northwest Division (Richland, Wa, USA), under a contract from the World Health Organization. The devices were toroidal-shaped, with an outside diameter of 55.6 mm and a cross-section of 9.5 mm. They were fabricated entirely of Dow-Corning 382 Silastic Medical Grade Elastomer, a polydimenthylsiloxane silicone rubber. The details of fabrication and in vitro testing were reported elsewhere (18). The expected release rates were rates were 50 μg/24 h and 200 μg/24 h, respectively.

Clinical material. Fourteen apparently healthy women (aged 21 to 36 years) with a

a) 17α-ethinyl-17 β-hydroxy-4-estren-3-one

Minimum of 3 months history of regular menstrual periods (26 to 35 days) volunteered for this investigation. For a minimum of 3 months prior to the start of the study they did not use any steroidal contraceptives, or

intrauterine devices. Their hematological status was carefully checked before, during and immediately after the completion of the study. The volunteers were allocated randomly to the two types of devices.

Plan of study. Following an untreated (control) cycle, in which blood samples of 10 ml were withdrawn three times weekly (Mondays, Wednesdays and Fridays between 08.00 and 10.00 h) and endometrial biopsy specimen was taken on day 21-23 of the cycle (using a Randall curette without dilatation of the cervix and without anesthesia), the vaginal device were inserted on the 5th day of the next cycle and were left in situ for an average of 90 (range: 86-94) days. During the entire period of exposure to the devices, blood samples were withdrawn at the same frequency and in the same way as during the control cycle, and two endometrial biopsy specimens were taken during the 6th and 10th week of exposure, respectively. In connection with the endometrial biopsy, additional blood samples of 5.0 ml each were taken at hourly intervals during a period of 24 hours (25 samples) in order to assess the hour-to-hour variation in NET levels. Hence, the total volume of blood withdrawn from each volunteer during a period of 120 days amounted to 545 ml.
Because of the uncertainty as to the contraceptive efficacy of the small doses of NET released, the couples were advised to use a specified mechanical method of contraception (condoms). No pregnancies occurred.

The total exposure of the 7 women with the 50 μg-releasing devices was 634 days and of the 7 women with the 20μg-releasing devices 631 days. Upon termination of the study, the vaginal devices were removed and sent to the Battelle Memorial Institute for the estimation of residual content (i.e. release rate) of NET.

Steroid assays. The blood samples obtained by vacutainers were centrifuged within 20 minutes, frozen and stored at -20oC until processed. In each plasma sample, progesterone and estradiol were estimated by the radioimmunoassay method described by Aso at al. (19) and NET by the method of Bedolla-Tovar et al. (20).

Histological examination. The endometrial specimens were immediately fixed in Bouin's solution, embedded in paraffin, sectioned and stained with hematoxylin- eosin. The biopsies were evaluated with regard to the presence of irregular secretory changes, predecidual reaction and atrophic changes in the glands. In addition, the average number of endometrial glands per microscopic field (x400) was quantitated. Only glands having a perpendicular section were included in this study. For dating of the biopsies taken in the control cycle, the criteria described by Noyes etal. (21) were employed.

Classification of treatment cycles. This was based on the outcome of a series of recent studies conducted in Stockholm, in which daily hormone assays were carried out in the blood of 72 normally menstruating women throughout a complete cycle. The (geometric) mean length of these cycles was 29.7 days, with 90% tolerance limits (covering 90% of the population) with 90% probability at 26 and 35 days (Landgren, in manuscript. Ninety-eight per cent of these women had a preovulatory estradiol surge between 100 and 700pg/ml and a luteal estradiol maximum between 100 and 50 pg/ml. Furthermore, a study of these 72 cycles together with the data of 34 cycles published previously (22, 23) revealed that 94% of these 104 cycles exhibited a plasma progesterone level of 5.0ng/ml or more for a minimum of 5 days. Hence, in the present study, a cycle is classified as normal only if it fulfills the above criteria with regard to cycle length and peripheral estradiol and progesterone levels. Furthermore, anovulation with no luteal function is characterized by constant progesterone levels (as a rule below 1.5ng/ml) found in periods in which the mean value of the second part of the "cycle" (i.e. observation period) does not differ significantly from that found in the first part. Finally, "depressed" luteal function is characterized by significantly, but insufficiently elevated plasma progesterone levels (less than 5.0ng/ml for five days) during the second half of the observation period.

RESULTS

Pharmacokinetic observation. Figures 1 and 2 present some representatives examples of the study and indicate at the same time some of the problems posed by near zero order delivery systems. The data of Fig. 1 indicate rather high plasma levels of NET (around 1000pg/ml) associated with normal ovulatory cycles, as evidenced by the estradiol and progesterone levels. In the other subject (Fig. 2), the NET levels were invariably below 400pg/ml; nevertheless, ovulation was inhibited throughout the entire duration of the study. The data of Figs. 1 and 2 also indicated the virtual lack of any initial "burst" effect in NET levels following the insertion of the devices.

A statistically significant linear relationship was found in each of the 14 cases when the plasma levels of NET were plotted against the 90 days of exposure. The slope values an Y-intercepts of the individual regression lines, together with the estimated release rates of the individual devices, are indicated in Table I.

Although there is some overlap between the two groups concerning both the slope- and Y-intercept values, both differences are statistically significant ($p < 0.01$, respectively) both by the use of a parametric (t-test) and a non-parametric (Mann-Whitney test (26)) method of assessment. Hence, devices releasing 200μg/24 h give rise to higher plasma levels of NET than those releasing 50μg/24 h. Furthermore, the plasma levels of NET decline significantly faster in the former group. It follows from the regression coefficients that during the 90 days of study, the average plasma level of NET in the group with 50qg-releasing devices decline by 14%, and in the group with 200qg-releasing devices by 22%, corresponding to a daily decrease of 0.16% in the former and 0.24% in the latter group.

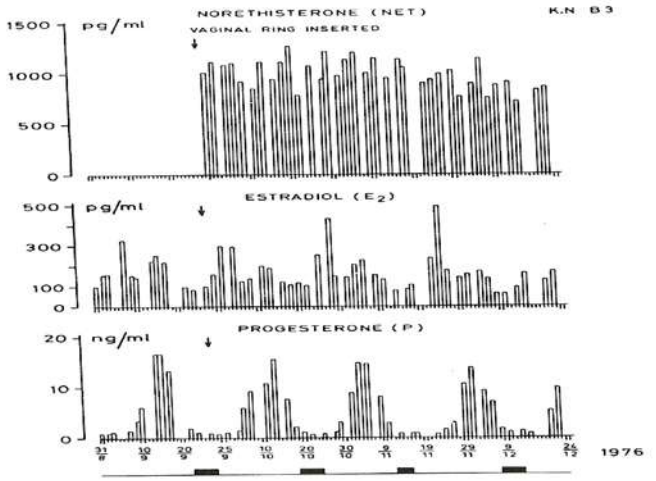

Fig1. 1. Systemic plasma levels of norethisterone, estradiol and progesterone in a subject before and during continuous exposure to norethisterone released from vaginal device at a rate of 360µg/24 h. Period of bleeding are indicated by filled bars.

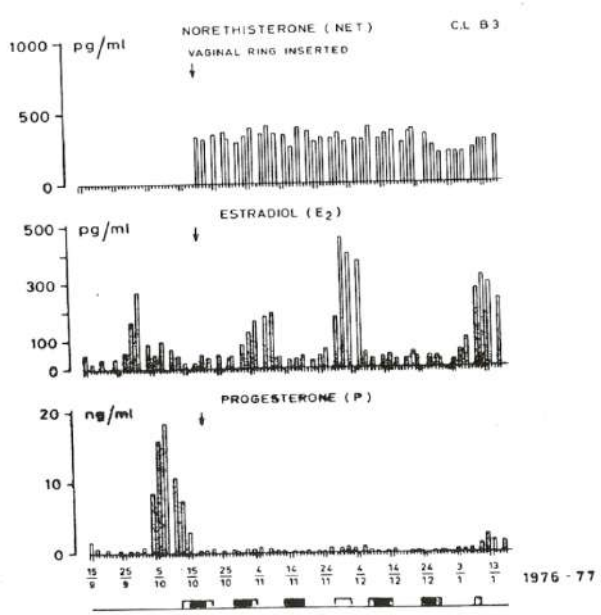

Fig.2. Systemic plasma levels of norethisterone, estradiol and progesterone in a subject before and during continuous exposure to norethisterone released from a vaginal device at a rate of 200µg/24 h. Periods of spotting are indicated by open bars, those of bleeding by filled bars.

Table I

Individual regression coefficients (slope and intercept values on the Y-axis) calculated from the regression lines of the plasma levels of norethisterone (pg/ml) measured during 90 days in 14 subjects with vaginal devices releasing norethisterone at a rate of 50 µg/24 h and 200 µg/24 h, respectively. The individual release rates estimated from the amount of norethisterone recovered from the devices at the end of the study are also indicated.

Expected release rate	Subject	Regression coefficient (slope)	Intercept (pg/ml)	Estimated release rate (qg/24 h)
	M.K.	-0.11	280	58	
	L.L.	-0.22	199	46	
	M.W.	-0.28	265	40	
50qg/24 h	W.M.	-0.42	149	53	
	M.G.	-0.74	430	50	
	B.S.	-0.76	327	57	
	A.W.	-2.12	466	26	
(Geom.) mean		- 0.44	23846		
95% Confidence limits		-0.18 to -1.08	194 to 411	35 to 59	
	A.K.	-0.71	525	130	
	M.H.	-0.98	699	240	
	C.L.	-1.01	361	200	
200 qg/ 24 h	B.S.	-1.32	535	40[c]	
	K.N.	-2.86	1115	170	
	B.A.	-2.99	727	200	
	B.W.	-3.84	1013	360	
(Geom.) mean		-1.63[d]	666163		
95% Confidence limits		-0.88 to -3.00	463 to 959	86 to 310	

a) These estimations were kindly carried out by the Battelle Memorial Institute, Pacific Northwest Division, Richland , Wa. , USA.

b) Arithmetic mean value: -0.66, S.E.M : 0.26

c) If this outlier is disregarded, a mean release rate of 206 (144-295) μg/24 h is obtained .

d) Arithmetic mean value : -1.96, S.E.M. : 0.47

Table II

Variation in plasma levels of norethisterone (NET) in 25 peripheral blood specimens obtained at hourly intervals from 14 women with vaginal devices releasing NET at different rates.

Release rate	Geom. Mean (pg/ml)	95% Confidence limits	Confidence limits as a percentage
	134	125 – 144	87 – 107
	182	174 – 189	92 – 104
50 qg	222	198 – 250	89 – 113
(7 subjects)	234	224 – 245	91 – 105
	234	217 – 252	93 – 108
	262	242 – 283	92 – 108
	363	347 – 379	92 – 104
	290	272 – 309	94 – 107
	431	411 – 452	95 – 105
200 qg	483	461 – 507	95 – 105
(7 subjects)	561	538 – 584	96 – 104
	578	553 – 605	91 – 105
	778	717 – 844	92 – 108
	831	796 – 868	92 – 104

Moreover, the data of Table I also indicate a mean release rate of 46 μg/24 h (with 95% confidence limits at 35 and 59 μg/24 h) for the devices expected to release 50 μg/24 h, and a release rate of 163 (86-310) for the 200 μg-releasing rings, with the proviso that if the outlier value of 40 μg/ 24 h in the high release group is disregarded, more likely mean release rate of 206 (144-295) μg/24 h may be obtained.

There was no correlation between the release rates and plasma levels, neither in the 50qg-release group (r=0.183), nor in the 200 μg-release group (r= 0.569; limit of significance at r=0.811). The exclusion of the outlier single value (40 μg/24 h) in the high release group did not change the significance of the correlation. Furthermore, the poor correlation was not improved after the values were adjested for differces in weight, height and ponderal index.

Does the vaginal device provide constant plasma levels of NET around the clock, or are there major differences between periods of activity and rest? In order to answer this question, the hour-to-hour changes in plasma levels were assessed during a 24-hour period, as shown in table II.

The data of Table II indicate a great uniformity of plasma NET levels; in none of the 14 subjects did the slope of the regression line – calculated from the 25 estimates around the clock (not shown in Table II) – differ significantly from zero, indicating that during this period a true zero order release was achieved.

Pharmacodynamics events. The events observed with the two types of release are summarized in Table III.

Table III

Incidence of pharmacodynamics events during 3subsequent cycles in 14 subjects with vaginal devices releasing norethisterone at different rates.

Events	Release rate/24 h	
	50qg (7 subjects)	200qg (7 subjects)
Ovulatory cycle	16	9
Cyclic follicular activity	3	9
With depressed luteal function		
Ovarian suppression	1	4
Total	20	22

A normal cycle length and a normal luteal phase were found in all 14 control cycle (not shown in Table III). Furthermore, there were a total of 16 cycles with normal luteal function among the 7 subjects during exposure to the 50qg-releasing devices; each of them exhibited a minimum of 2 such cycles. However, 6 of these 16 cycles were shorter than 26 days, and 5 of them were longer than 35 days. Moreover, the luteal function was significantly depressed in 3 cycles and absent in one.

Only 9 cycles were found with a normal luteal function in the group exposed to devices releasing 200μg/24 h; 3 of the 7 subjects in this group experienced anovulation throughout the entire study, 2 had regular ovulatory cycles, and in 2 of them prolonged anovulatory periods alternated with normal cycles. Moreover, a total of 9 instances with cyclic follicular activity without any luteal activity were observed in this group.

Another effect of the small doses of NET during exposure to a device releasing 50 μg/24 h of NET is indicated by the data of Fig.3; in this case, normal luteal phases were associated with abnormally elevated (> 800 pg/ml) preovulatory estradiol surges in each of 3 subsequent treatment cycles. A similarly elevated estradiol surge was also seen in another subject with a device releasing 200qg/24 h. As indicated before, in our normal material only one of 72 normally menstruating women exhibited a plasma estradiol level exceeding 700 pg/ml.

Table IV

Histological assessment of the endometrial biopsy specimens obtained from 14 women during a pretreatment (control) cycle and during the 6^{th} and 10^{th} week, respectively, with the norethisterone-releasing device insitu.

Endometrial Appearance		Release rate: 50 μg/24 h			Release rate: 200 μg/24 h	
	Pretreatment	6 weeks	10 weeks	Pretreatment	6 weeks	10 weeks
Normal cyclic	7	6	7	7	1	2
Predecidual	0	1	0	0	4	2
Atrophic	0	0	0	0	2	3

Endometrial morphology. The result of the histological examination are shown in Table IV.

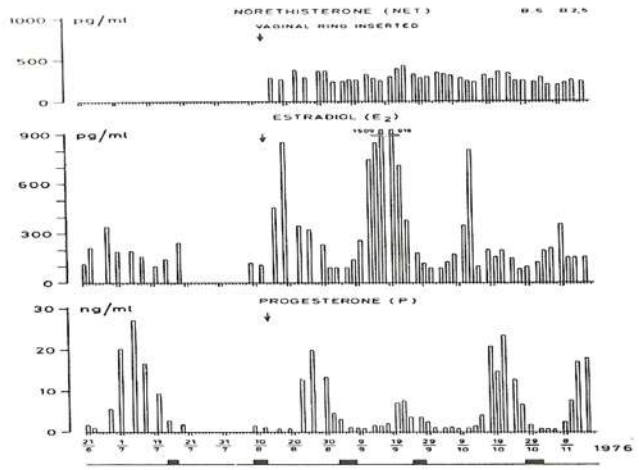

Fig.3. Systemic levels of norethisterone, estradiol and progesterone in a subject before and during continuous exposure to norethisterone released from a vaginal device at a rate of 57 μg/24 h. Periods of bleeding are indicated by filled bars.

All 14 pretreatment (control) biopsies and 13 out of 14 biopsies obtained in the 7 subjects during exposure to the devices releasing NET at a rate of 50μg/ 24 h indicated normal cyclic changes. On the other hand, only 3 of the 14 biopsies obtained in the subjects with the 200 μg-releasing devices in situ showed a normal cyclic change; 6 exhibited a predecidual reaction and 5 showed atrophic changes. Furthermore, there was a close agreement between the results of the histological dating of theendometrium and the classification of the day of the cycle on the basis of the plasma steroid levels in 14 of the double-blind comparisons made during the pretreatment period, in 13 out of 14 comparisons made during exposure to the devices releasing 50μg/24 h, but only in 7 of the comparisons made during exposure to 200 μg-releasing devices. In the latter group, predecidual or atrophic changes of the endometrium were found, sometime in the presence of hormonal changes indicating a normal ovulatory cycle, and sometimes in anovulatory cycles with no signs of cyclic estradiol or progesterone secretion

There was no correlation between the plasma levels of NET and the histological appearance of the endometrium; relatively high (> 1000 pg/ml), or relatively low (<400 pg/ml) plasma levels of NET were associated sometimes with a normal and sometimes with an atrophic endometrium.

In a attempt to quantitate the predecidual and atrophic changes of the endometrium, the number of glands per microscopic field was estimated. The results are presented in Table V.

Table V

Number (+- S.D.) of endometrial glands per microscopic field in 14 women before and 6 and 10 weeks after the insertion of vaginal rings releasing norethisterone at different rates.

Number of subjects	Release rate (g/24 h)	Control	6 weeks	10 weeks
7	50	3.1+-1.0	3.1+-1.0	3.9+-2.0
7	200	3.2+-0.8	1.6+-0.8	1.0+-0.7

The data of Table V that there was a significant decrease in the number of glands during exposure to the devices releasing 200 µg of NET, whereas devices releasing 50µg/ 24 h had no effect.

Bleeding and spotting. As indicated by the data of Table VI, the total number of days with bleeding and spotting was significantly higher in the subjects with devices releasing 200µgh/24 h than in those with the devices releasing 50µg/24 h. The significance of this difference ($p<0.01$) was ascertained both by a t- test for differences with unequal variances (25) and a Mann-Whitney test (26).

Table VI

Bleeding and spotting in 14 women with vaginal devices releasing norethisterone at different rates.

Release Rate/24 h	No. of subjects	Days with bleeding & spotting	Days of exposure	Average per Subject (+-S.D.)	Significance
50 qg	7	103	631	14.7 3.0	
200 qg	7	179	634	25.6 9.6	

DISCUSION

Perusal of the literature on vaginal rings releasing contraceptive steroids reveals that most, if not all, studies published address themselves to devices which release ovulation inhibiting, high doses of medroxyprogesterone acetate [b)] (1-6), norgestrel [c)] (7-11), or ethylnorgestrienone (R-2323) (12, 13, 14). In addition, a few papers deal with systems releasing chlormadinone acetate [e)] (15), or a combination of norgestrel with estradiol (16), or estradiol benzoate (17). It would appear that only one previous paper (7) reports on devices releasing NET. In this study, vagina rings containing 100 and 200 mg of NET were used; a high incidence of bleeding was observed, and ovulation occurred in about one-fourth of the treatment cycles. The estimated release rate was higher than 1500 μg/24 h.

a) 6α-methyl-17α-acetoxy-4-pregnene-3,20-dione
b) (±) 13β-ethyl-17α-ethyinyl-17β-hydroxy-4-gonen-3-one
c) (±) 13β-ethyl-17α-ethinyl-17β-hydroxy-4,9(10)-gonatrien-3one
d) 6chloro-17α-acetoxypregna-4,6-diene-3,20-dione

The present study was aimed at exploring the pharmacokinetic and pharmacodynamics properties of NET when released at a constant rate from vaginal devices in such small amounts which are unlikely to invariably inhibit ovulation. The data herein reported indicate that the devices do release almost constant amounts of NET for 90 days of longer, and the days of exposure during the entire span of the study. Furthermore, the data obtained on the hour-to hour variation of plasma levels indicate that by the use of the devices studied it is possible to obtain a true zero order release of NET. Hence, these devices represent an ideal experimental tool for studies on the effect of constant plasma levels of contraceptive steroids.

The data reported here also indicate that with devices of this type there is little, if any, initial “burst” effect. Such an effect usually manifests itself in considerably higher plasma levels during the first few days of the study (e.g.(4)), or longer (e.g. (24)), and represents a considerable drawback, since it greatly complicates the evaluation of the observed pharmacodynamics effects.

Statistically valid linear regression lines were obtained for the plasma levels of NET throughout the entire experiment in each subject. The mean regression coefficients obtained in the two groups (-0.44 and -1.63, respectively) indicate that the average daily decline in plasma NET levels was only 0.16% and 0.24%, respectively, and that thus a near zero order release of NET was achieved during the 90 days of the study. Although there was a considerable overlap and great individual variation in the plasma levels of NET, the (geometric) mean plasma level in subjects agreement with a previous report on norgestrel-releasing vaginal rings (11), no correlation was found between the individual release rates from the devices and the plasma levels of NET in the same subjects. Furthermore, the plasma levels of NET were not correlated to height, function, endometrial morphology or the frequency of bleeding and spotting. It would seem, therefore, that other factors, such as specific binding proteins in the circulation and in the endometrium may require a careful analysis in this respect.

In a recent study on the pharmacodynamics effects of the NET minipill (300μg daily), we have assessed the day-to-day changes in the plasma levels of progesterone and estradiol in 42 women during a control cycle and during the second treatment cycle. Using the same criteria for assessment as in the present study, a normal luteal function found in 40% of the subjects, 20% had a significantly depressed luteal found in 40% exhibited no luteal function whatsoever (Landren et al in manuscript). In the present study, 3 of the 7 subjects with the devices releasing NET at a rate of 200μg/ 24 h had anovulation throughout the entire study period, and 2 had anovulatory periods alternating with cycles with normal luteal function. Hence, it would appear –as first approximation – that the ovarian effect of a 300 μg NET minipill is roughly equivalent to that of 200μg of NET released continuously from a vaginal device during 24 hours.

The pharmacodynamics effect of the devices releasing 50μg/24h was different. The luteal function was significantly depressed or absent in 4 cycles, but there were 16 cycles with normal luteal function and a minimum of 2 such cycles in each subject. However, 11 of the 16 cycles with normal luteal function had a abnormal length. Hence, the first effect of NET when released in doses insufficient to inhibit ovulation is directed towards the

(incompletely comprehended) factors phase, in particular. This suggests that it might be rewarding to conduct an in-depth study on the plasma levels of variety of pituitary hormones during the follicular phase of cycles with a normal a luteal phase in women exposed to similarly small doses of progestogens which are insufficient to inhibit ovulation.

Whereas in 13 of the 14 endometrial biopsies taken in the group with the 50µg-releasing devices normal cyclic changes were observed, 11 of the 14 biopsies taken in the group with the 20µg-releasingdevices showed predecidual or atrophic changes. These changes were associated with a significant decrease in the number of endometrial glands and with a significant increase in the frequency of bleeding and spotting . On the other hand, no correlation was found between the endometrial changes observed and the circulating levels of NET, estradiol or progesterone. This seems to speak against the existence of a simple relationship between peripheral hormone levels (exogenous or endogenous) and intermenstrual bleeding in women using progestogen minipills for contraception. Thus, the present study pion to the necessity of conducting a series of studies at the endometrial level in order to identify the major factors responsible for intermenstrual bleeding in minipill users. It is felt that there is little prospect for progress in this field, unless the mechanism of intermenstrual bleeding is better understood.

Finally, it should be emphasized that the vaginal devices were excellently tolerated by all subjects. This fact, the safety data obtained in animal toxicological studies and the bleeding patterns observed seem to justify an exploratory study of the contraceptive efficacy of vaginal rings releasing NET at a rate of approximately 50µg/24 h. The frequency of bleeding and spotting seen with the rings releasing NET at a rate of 200µg/24 h seems to militate against the use of devices with this and higher release rate, unless a possible combination with an estrogen is considered (e.g. (16, 17)). However, such an approach will necessitate more information on the safety of estrogens released continuously into the vagina.

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Reprinted from: Contraception, vol 19, no 3/ 1979, 253-271

Studies on the biological and immunological properties of human follitropin: profiles of two international reference preparations and of an aqueous extract of pituitary glands after electrofocusing

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Abstract

Two international reference preparations, the First International Reference Preparation of Human Pituitary Gonadotrophins (FSH and LH/ICSH) for Bioassay (identified hereafter by its code no.: 69/1 04) and the First International Standard of Urinary FSH and LH (ICSH) for Bioassay (hereafter: hMG 1st IS) and an aqueous extract of human pituitary glands (hPE) were fractionated in triplicate by isoelectric focusing on sucrose density gradient (Ampholine, pH range 2.5-10.0). The FSH activity was monitored in each fraction by an in vitro bioassay, a radioimmunoassay and a receptor binding assay. Unfractionated 69/1 04 was used as standard in each assay system.

Compared with the hPE, the activity profiles of the 69/104 and hMG reference preparations were spread over a significantly wider pH range; the biological activity eluted in the pH range of 3.5-5.0 was of the order of 90% for hPE, 72% for 69/104 and less than 60% for hMG.

Major variations in biological to immunological *(B/I)* and bi010gical to receptor binding *(B/R)* activity ratios were observed in the individual electro focusing fractions. The *BII* ratios ranged from 0.8 to 2.2 (69/104), 1.0-5.7 (hMG) and 1.3-6.0 (hPE), respectively. The variation in the corresponding *BIR* ratios was: 0.5-1.7 (69/104), 0.58-4.0 (hMG) and 0.55-1.5 (hPE). When equal aliquots from each of the electro focusing fractions were combined and this ‘re-constituted' pool was compared with the starting material,

significant differences were observed in the *BII* ratios: 1.63 instead of 1.0 (69/104), 2.58 vs 2.34 (hMG) and 2.72 vs 1.32 (hPE). There was no significant change in the mean *BIR* ratios before and after electrofocusing: 1.0 vs 1.02 (69/104), 1.09 vs 1.02 (hMG) and 1.05 vs 1.04 (hPE).
The mean recovery of biological activity in each of the three reconstituted pools of the three preparations was 97% for 69/104, 88% for hMG and 98% for hPE, and the corresponding recoveries of receptor binding activities were 95%, 94% and 98%, respectively. In contrast, the mean recovery of immunological reactivity was only 55 % (69/104),80% (hMG) and 47% (hPE), respectively.
The reduction in the immunological reactivity of the 69/104 preparation without any apparent loss of biological or receptor binding activities following electrofocusing indicates that its immunoreactivity is unstable under mild experimental conditions, which do not influence its biological and receptor binding activities. Hence, whereas this preparation might possibly be suitable as a standard for in vitro bioassays and receptor binding assays, it is unsuitable as a standard for the radioimmunoassay of hFSH.

Previous studies with human luteinizing hormone (hLH) using an in vitro bioassay and radioimmunoassay procedures revealed that the 1st IRP of Human Pituitary Gonadotrophins (FSH and LHI ICSH) for Bioassay (code no. 69/104) exhibited ratios of biological activity to immunological re-activity *(B/I)* much lower than those of the plasma samples, unfractionated pituitary extracts or more extensively purified standard preparations such as Human Pituitary Luteinizing Hormone (LH (ICSH» for Immunoassay, code no. *68/40* (Bartfai et al. 1979; Balogh et al. 1979; Robertson & Diczfalusy 1977; Robertson et al. 1978).
The recent development of a sensitive and specific in vitro bioassay for hFSH (Van Damme et al1979) made it possible to examine the B/I and B/R (biological to receptor binding activity) ratios of different hFSH preparations. Indeed, in a recent study significant differences in B/I and B/R ratios were observed between various hFSH preparations of pituitary and urinary origin when the 69/104 preparation was used as a reference preparation (Marana et al. 1979b).

These findings called for an in-depth critical investigation of the biological and immunological behaviour of the various molecular species present in hFSH preparations of varying degree of purity.

In the present study the two international reference preparations of hFSH available at present (69/104 and hMG 1st IS) were chosen for fractionation using an isoelectrofocusing technique. In order to appraise a possible artifact formation during the purification of these preparations, an aqueous extract of pooled human pituitaries was also included in the plan of the study.

Materials and Methods

Abbreviations

B: biological activity, *B/I:* ratio of bioiogical activity and immunoreactivity, *B/R:* ratio of biological and receptor binding activities, BSA: bovine serum albumin, Hb: beef haemoglobin, hFSH: human follitropin (human follicle-stimulating hormone), hLH: human lutropin (human luteinizing hormone), hMG: human menopausal gonadotrophin; this abbreviation will be used throughout this paper to indicate the First International Standard of Urinary FSH and LH (ICSH) for Bioassay, hPE: human pituitary extract, I: immunological reactivity, IEF: isoelectro focusing, pI: isoelectric point, R: receptor binding activity, RBA: receptor binding assay, RIA: radioimmunoassay, 69/l04: code no. used to denote the First International Reference Preparation of Human Pituitary Gonadotrophins (FSH and LH/ICSH) for Bioassay, WHO: World Health Organization.

Reagents and preparations

Human pituitary gonadotrophins (1st IRP of Human Pituitary Gonadotrophins (FSH and LH/ICSH) for Bioassay (code no. 69/l04) and the 1st International Standard of Urinary FSH and LH (ICSH) for Bioassay (1st IS hMG) were obtained from the National Institute of Biological Standards and Control, London.

An extract of human pituitaries was prepared by homogenizing 10 human pituitaries in phosphosaline (pH 7.2) buffer containing 0.1 % bovine serum albumin (BSA); the homogenate was centrifuged at a speed of 100000 *g* for I h, the supernatant was aliquoted and snap frozen in a C02-ethanol mixture

after which it wasstored at -70°C until analyzed. These aliquots were used for IEF without further purification.
A highly purified hFSH preparation (human follitropin -lot no. 19426 Kabi Diagnostica, Stockholm) with a specific activity of 8340 (7860-8820) *IU/mg* (expressed in terms of the *69/104* standard) was used for iodination.
An anti hFSH-serum (anti-hFSH (WHO) serum) was provided in lyophylized form by the World Health Organization's Matched Reagents Programme.
The 69/l04 preparation was used as a reference preparation throughout the present study. The preparation was diluted in phosphate buffer (6.5 mM N32HP04, 1.4 mM KH2P04, 0.135 M NaCI pH 7.4) containing 1.0% BSA, snap frozen in C02-ethanol mixture and stored at -70°C in aliquots of 1.0 IUlml hFSH activity.

Electrofocusing procedure
Electrofocusing experiments for the three preparations were performed in triplicate on sucrose density gradient with carrier ampholytes (Ampholine, LKB-Produkter) of 2.5-10.0 pH range at 2.0% concentration using a 110 ml column (no. 8100, LKB-Produkter AB, Bromma, Sweden). Beef haemoglobin (Type I, Sigma Chemical Co.) was used as a marker protein. The mean pI of the Hb peak from 9 e1ectrofocusing experiments of 3 preparations was 7.85 ± 0.03. A detailed description of the procedure has been presented elsewhere (Robertson et al. 1977).

At the end of each electrofocusing run of 18 h, 0.8 ml fractions were collected (2117 Redirac Fraction Collector, LKB) and the pH of the fractions was measured directly at 4°C on a digital pH meter (PHM 63, Radiometer, Copenhagen). In order to remove ampholine and sucrose, the fractions were passed through Sephadex G-25 columns (9.0 x 1.5 em) at 4°C. The hFSH activity was then measured in each fraction employing an in vitro bioassay method, a radioimmunoassay procedure and a receptor binding assay technique. To assess the total recovery, aliquots from each fraction of every experiment were pooled together and assayed against the standard, using a multiple point parallel line assay design.
In vitro bioassayfor FSH

This method is based on the principle that when Sertoli cells from 10-day old rats are cultured and then incubated with graded doses of FSH in the presence of 19hydroxyandrostenedione, they convert the substrate into oestradiol-17~ in a dose dependent manner (Van Damme et al. 1979). The mean index of precision (X, Gaddum 1933) from 9 multiple point assays was 0.079, while the between-assay variation for the same set of experiments (n = 9) was 14%.

Radioimmunoassay (RIA) procedure

An equilibrium assay design was adopted (Marana et al. 1979b). The cross-reactivity and specificity of the WHOantiserum used in this study has been assessed previously (Marana et al. 1979a). The antiserum was used without absorption with hCG. Parallel response lines were obtained between the preparations and the standard (69/104). The mean index of precision (A.) from 9 multiple point assays was 0.031 while the between-assay variation was 7%

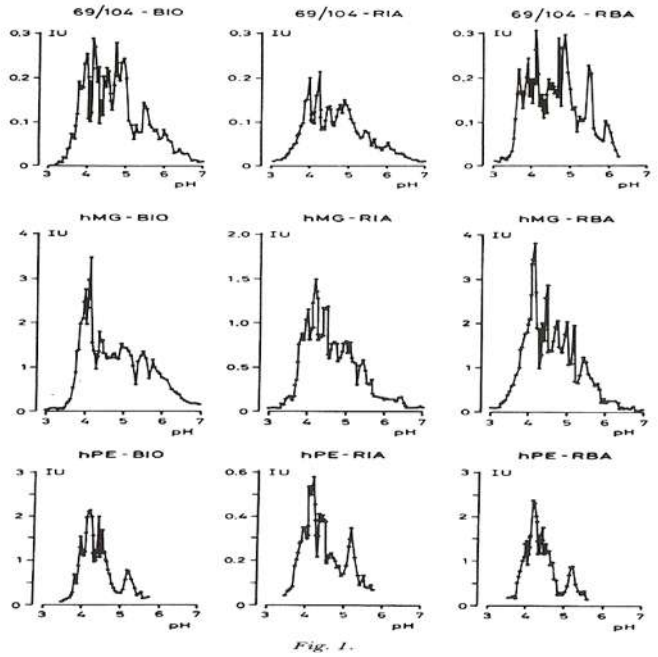

Fig. I.

Fig. I.

Electrofocusing profiles of 3 human follicle-stimulating hormone (hFSH) preparations. The hFSH activity in each was estimated by an in vitro bioassay (BIO), radioimmunoassay (RIA) and receptor binding assay

(RBA). 69/104: 1st IRP of Human Pituitary Gonadotrophins (FSH and LH/ICSH) for Bioassay, hMG: 1st IS of Urinary FSH and LH (lCSH) for Bioassay, and hPE: an aqueous extract of human pituitary glands. Unfractionated 69/104 was used as standard in all assay systems.

Receptor binding assay (RBA)

The radioreceptor assay method of Cheng (1975) using membrane preparations from adult bovine testes was followed during the course of this study. Iodination was carried out by the lactoperoxidase method (Thorell & Johansson 1971) with a slight modification as described by Marana et al.

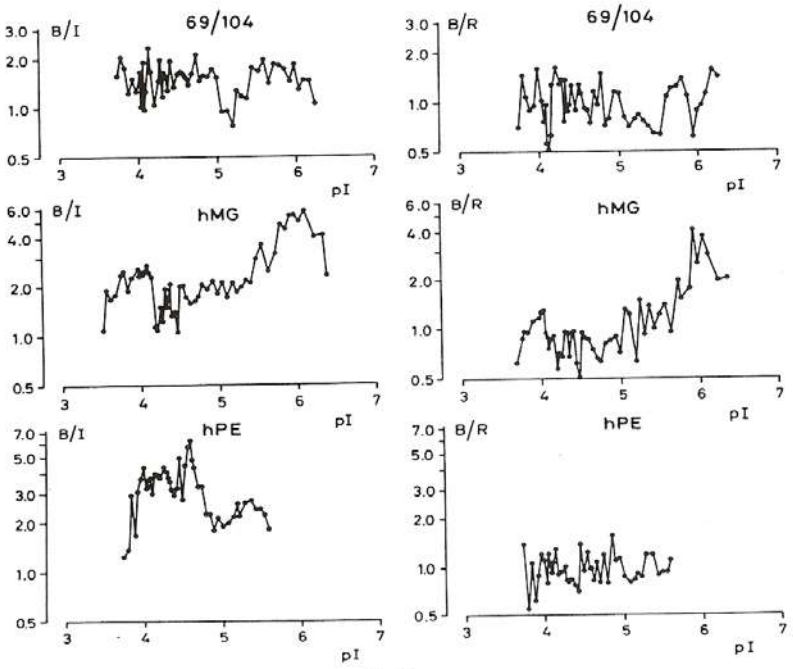

Fig. 2.

(I 979a). In order to remove the iodinated subunits from the tracer, it was purified by a high resolution gel filtration technique using Ultrogel AcA-54 (Suginami et al. 1978; Marana et al. 1979a). The mean index of precision (X) for nine 3+3 point assays was 0.038 while the coefficient of variation between-assays was 8.0%.

Results

The activity profiles of the three preparations, determined by different assay techniques, following electrofocusing are presented in Fig. 1. The hFSH

actIVIty In the case of 69/104 was localized between pI 3.5 and 6.5. No activity could be detected beyond this region. The pattern of the biological and receptor binding activities was found to be in close agreement; 7 out of 8 distinct peaks at .pI 3.73 ± 0.04, 3.93 ± 0.03, 4.08 ± 0.02, 4.42 ± 0.02, 4.74 ± 0.03, 5.03 ± 0.03 and 5.42 ± 0.04 were common in the two profiles. On the other hand, the immunological reactivity showed only 6 distinct peaks, of which 5 possessed pI values identical to those seen in the profile of the biologi· cal and receptor binding activities. The spread of the hMG profile was similar to that of the 69/l()l : preparation. The biological and receptor bindinf' activities showed a close correspondence. The peaks with pI values of 4.14 ± 0.02, 4.43 ± O .. 4.71 ± 0.04, 5.00 ± 0.02, 5.45 ± 0.03 and 5.79+ 0.05 were common in the two activity profiles. profiles of immunological reactivity showed at I two peaks with pI values of 5.11 ± 0.01 and 3.90±**0.02** which were not recognized by the bioassay or **the** receptor binding assay.

Compared to the 69/104 and hMG preparations, **the** hPE showed a narrower distribution of FSH, activity (pI 3.5 to 5.5). No activity was detected beyond pI 5.5 by any of the three assay systems. Peaks at 4.00 ± 0.02, 4.44 ± 0.02, 5.17 ± 0.01 and 5.50 ± 0.03 were common in the profiles of biological and receptor binding activities and immunological reactivity. However, two peaks in the immunological profile (at 4.09 ± 0.02 and 4.66 ± 0.05) could not be detected by the bioassay or the receptor binding assay techniques.

In all fractions of the three preparations, the *BII* and B/R ratios showed a wide variation when expressed in terms of the unfractionated 69/104 reference preparation (Fig. 2). The mean *BII* ratio for pooled fractions of 69/104 after IEF was 1.63 (range: 0.8-2.2), and the mean *BII* ratio for hMG after IEF (2.58) was slightly higher than that of the unfractionated hMG (2.34). The *BII* ratios of hMG were around 2.0 in all fractions, except in the region of decreased acidity (pI 5.90-6.40), where ratios as high as 5.0 were observed. Finally, the mean *BII* ratio of hPE after 1EF was 2.72 as compared to a ratio of 1.32 found prior to IEF. Also in this case, the fractions between pI 4.50 and 4.75 showed very high *BII* ratios exceeding 5.0.

The B/R ratios showed less variation than the BII ratios; the values were mostly around unity. The mean B/R ratios after electrofocusing of 69/104,

hMG and hPE were 1.02, 1.02 and 1.04, respectively. High B/R ratios (exceeding 2.0) were only found in the case of the hMG preparation in the relatively less acidic region (pI 5.90-6.40).

As indicated by the data of Table I, the mean recovery of the biological and the receptor binding activities of the *69/104* preparation in 3 electrofocusing experiments was 97 and 95%, respectively, while that of the immunological reactivity was only 55 %. The mean recovery of biological and receptor binding activities after electrofocusing of hMG was 88% and 94%, respectively and that of immunological reactivity was 80%. For hPE these recoveries were 98 % both for biological and receptor binding activities and 47% for immunoreactivity.

In order to assess the distribution of the different activities in the various pH areas, segments corresponding to 0.5 pH units were formed fromthe data of each experiment; the results in the form of histograms are presented in Fig. 3.

Table 1.

The potency of .three hFSH preparations *(IV* per ampoule) estimated by various assay techniques before and after lsoelectric focusing. All potency estimates and 9S% fiducial limits of error are expressed in terms of the 69/104 reference standard.

Pre-paration	Before isoelectrofocusing			After isoelectrofocusing[f]		
	In vitro bioassay	Radioimmuno-assay	Receptor binding assay	In vitro bioassay	Radioimmuno-assay	Receptor binding assay
69/140·	10d	IOd	10d	9.7	S.S	9.S
				(9.1-10.3)g	(S.I-6.0)g	(9.0-1O.0)g
hMC[b]	97.S	41.7	88.7	86.0	33.3	83.7
	(89.4-106)e	(40.3-43.2)e	(67.1-10I)e	(79.S-90.2)g	(31.7-3S.0)g	(80.3-90.2)g
hPEc	43.4	32.9	41.3	42.4	IS.6	40.6
	(41.0-46.2)g	(30.9-3S.3)g	(38.S-44.0)g	(39.7 4S.3)g	-(l2.9-18.3)g	(37.3-44.0)g

a First International Reference Preparation of Human Pituitary Gonadotrophins (FSH and LH/ICSH) for Bioassay.

b First International Standard ofVrinary FSH and LH (lCSH) for Bioassay.

c An unfractionated aqueous extract of 10 human pituitarespituitaries.

d According to the National Institute of Biological Standards and Control, London, each ampoule contains 10 IV FSH activity (bioassay). Since no potency estimates are established in terms of immunological reactivity or receptor binding activity, each ampoule was assumed to contain 10 IV hFSH.

• Assay results reported previously (Marana et al. 1979b). al. 1979b)

r Equal aliquots from each fraction were combined from each experiment to assess recoveries.

g Represents the mean and range of three electrofocusing experimentsexperexperiments.

A comparison of the three activities recovered following IEF of the 69/1 04 preparation indicated that segment 3 (pH 4.00-4.49) contained 33% of the total biological and receptor binding activities, whereas only 16% of the immunological reactivity was recovered in this segment *(P* < 0.01). Furthermore, with the exception of segment 1 (pH 3.25-3.50) the recovery of the immunological reactivity in all segments was significantly less *(P* <0.05 to *P* < 0.01) than those of biological and receptor binding activities. There was no significant difference in the recovery of the biological and receptor binding activities.

In case of hMG, segment 3 (pH 4.00-4.49) appeared to be the principal fraction, but with significantly less biological activity compared to both immunoreactivity and receptor binding activity *(P* < 0.01). On the other hand, the recovery of biologically active material from segments 6 and 7 (pH 5.5-6.5) was significantly more *(P* < 0.0 I) than the corresponding immunological reactivity or receptor binding activity.

Also, in the case of hPE, segment 3 (pH 4.04.49) contained more than half of the total biological and receptor binding activities was virtually the same in all the segments studied: however, in each segment significantly lower amounts of immunoreactivity (P<0.001 to P<0.05).

An analysis of the various activities present in the 3 preparations revealed that 90% of the biologically active FSH of the hPE was present in segments 2 to 4 (pH 3.5-5.0), whereas the corresponding figure for 69/l04 was 72% and that for hMG less than 60%. Significantly more bioactive FSH was recovered from segment 3 of the hPE (56%) than of 69/l04 (32%) and hMG (33%) *(P* < 0.01). On the other hand, the bioactivity recovered from segment 2 of the 69/l04 preparation was significantly higher than the corresponding activity present in the hMG and hPE preparations. However, in segments 6 and 7 (pH 5.5-6.5), significantly more biological activity was recovered from the 69/l04 and hMG preparations than from hPE *(P* < 0.01).

A comparative scrutiny of the immunoreactivity recovered from the three preparations revealed significant differences. More than 43% of the immunoreactivity recovered from the hMG preparation was present in segment 3 compared to 16% (69/l04, *P* < 0.01) and 20% (hPE, *P* < 0.01). Approximately 70% of the initial immunoreactivity of the hMG preparation

was located in segments 3 to 6 (pH 4.0-6.0) compared to 40% (69/104) and 37% (hPE).

As for the receptor binding activity, segment 3 represented 55% of the applied FSH activity in the case of hPE, 47% in hMG and only 30% in 69/104 *(P* < 0.01). On the other hand, in segment 2, there was significantly more receptor binding activity in 69/l04 compared to the hMG and hPE preparations *(P* < 0.05 and *P* < 0.01, respectively).

The data of Fig. 3 indicate in general a considerably broader distribution of various molecular species of FSH present in 69/l04 and hMG preparations in comparison to hPE.

Discussion

Several earlier studies, employing in vivo bioassays (Steelman & Pohley 1953), and various techniques of purification, have provided information aboutthe pI values of hFSH. Richerr (1971) estimated the pI value of purified hFSH to be between 3.36 and 5.55, while Saxena & Rathnam (1968) reported a pI of 4.25 after electrofocusing a purified human pituitary preparation. Bettendorf et al. (1968) suggested the existence of a protein with a pI of 5.0, which contained both FSH and LH activities, while a protein with a pI of 3.0 was said to contain only FSH activity.

The data reported in this paper indicate that indeed there are different molecular species of hFSH exhibiting pI values of the type reported before. In the different preparations studied, biologically active molecular species were found in the pH range of 3.5-5.5. However, there was a significant difference in the spread of these molecular species between the hPE and the two reference preparations of FSH, suggesting that factors such as chemical manipulations, metabolism, transport through kidney, storage and repeated freezing/ thawing, among others, may have caused physiological and/or artifactual changes in the chemical nature of the various molecular species. This impression was further strengthened by the discrepancy observed in the profile of the hFSH estimated by an in vitro bioassay or RBA in contrast to the RIA. Several peaks in the three preparations identified by both bioassay and RBA were absent from the profile of the immunoreactive material, and vice versa, there were immunoreactive peaks in all preparations which were not recognized by the bioassay and RBA techniques. These findings suggest that some of the biologically active hFSH was associated with little or no

immunoreactivity and that - in addition - the RIA system employed also detected some material with little or no biological activity.

An analysis of the B/I and B/R ratios of the various fractions revealed major differences. The average B/I ratios were significantly higher than unity for all the three preparations. Whereas the B/R ratios approached unity, the individual BJI ratios showed a wide fluctuation from values in the neighbourhood of unity to ratios as high as 4.0 or more. The B/R ratios showed less variation. An interesting exception was the material exhibiting a B/R ratio considerably higher than unity in the region of pH 5.7 to 6.3, which was present only in the hMG preparation.

The mean B/I ratios observed following electrofocusing were considerably higher than those reported by Marana et al. (1979b) especially in thecase of 69/104 (1.76) and hPE (2.72) preparations.

This strongly suggested that some immunological reactivity has been lost. Indeed, an analysis of the pool of the preparations, re-constituted after electrofocusing, revealed loss of approximately half of the immunoreactive material present in the 69/ I 04 and hPE preparations without any appreciable loss of biological activity, providing additional evidence that these preparations contained a quantity of ('dissociable') immunoreactivity not associated with biological activity. Whether this selective loss of the immunoreactive material in the case of hPE is attributable to the presence of free subunits, to any other immunoreactive constituents or conformational changes needs to be clarified. It is of considerable interest to note be clarified. It is of considerable interest to note the significantly higher recovery of immunoreactive material following fractionation of hMG. It appears possible that passage through the kidney, previous metabolism and/ or the methods of purification used in this case may have resulted in progressive desialylation of FSH and subsequent increase in immunoreactivity as reported by Vaitukaitis & Ross (1971).

An analysis of the data of Fig. 3 revealed that the material eluted in segment 3 (4.0-4.49) was the most important from the point of view of quantitative recovery. This was true for all three assay techniques. As much as 56% of the total biological activity of hPE was found in this fraction while the immunoreactivity was around 20%. A similar difference was observed in the case of the 69/104 preparation. Significantly more biological activity

was found in segment 2 following the electrofocusing of the 69/104 preparation compared to the other two preparations.

The data reported in this paper indicate that significant differences exist not only between the hMG and hPE preparations but also between the two preparations of human pituitary origin - the 69/104 standard and the hPE. The 69/104 preparation therefore cannot be considered as a true representative of human pituitary FSH as far as the composition of the various biologically active molecular species is concerned. Furthermore, the presence in this preparation of large quantities of 'dissociable' immunoreactive material renders this preparation unsuitable for use as a standard for quantitation of hFSH activity by radioimmunoassay procedures, since it is extremely unlikely that the hFSH present in pituitary preparations, plasma samples and other body fluids would invariably exhibit a constant B/I ratio.

For the time being this preparation appears to be suitable as a reference standard for in vitro for assays and receptor binding assays; its suitability for in vivo bioassays will depend on the differences, if any, in the relative half-lives of the various molecular species exhibiting different pI values. Also, whereas the presence of large quantities of hLH in this preparation does not seem to interfere with the validity of FSH assays by in vitro bioassa)'S and receptor binding assays, this may well be the case in certain types of in vivo bioassays. These considerations together with the data presented in this and in a previous paper (Marana et aI. 1979b) .strongly suggest that there is an urgent need for an hFSH preparation of high purity, homogeneity and stability for use as a reference standard in the measurement of hFSH activity by different assay techniques.

Acknowledgments

The authors are indebted to Mrs. B. Froysa and Mrs. C. Reuter for their expert technical assistance.

The hFSH standard preparations (69/104 and hMG 1st IS) were a gift from the National Institute of Biological Standards and Control, London. The expenses of this investigation were defrayed by grants from the Swedish Medical Research Council, the World Health Organization Special Programme in Human Reproduction and the AB Leo Research Foundation, Helsingborg, Sweden.

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Reprinted from: Acta Endocrinologica, no 97/ 1981, 157-165

In search of human dignity: reproductive health and healthy aging1

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1: Presented at the Eleventh Congress of the European Association of Gynaecolgists and Obstetricians, Budapest, Hungary, 19-22 June 1996

"Great men are they who see that spiritual is stronger than any material force; that thoughts rule the world" (Ralph Waldo Emerson [1].

1. Introduction

To deliver the first Gedeon Richter Memorial Lecture is a great privilege and I feel indebted to the organizing committee for this honour bestowed upon me. William Faulkner says somewhere that "the past is never dead, it is not even past" and my thoughts go back to another event, the Ernst Laqueur Memorial Lecture which I had the honour of delivering in Amsterdam, in 1969 [2].

There are remarkable similarities and remarkable differences in the life histories of Ernst Laqueur and Gedeon Richter, the founders of two important pharmaceutical companies, Organon and Richter. Both men saw a major future in hormone therapy; both of them had an exceptional vision, which was successfully converted into a mission and then into action, following the principle of Thomas Henry Huxley that "the great end of life is not knowledge but action" [3].

Gedeon Richter was some 8 years older than Ernst Laqueur and became the real pioneer in the field of organotherapy. When, in 1903, Gedeon Richer published his magnum opus, a monograph entitled "A legújabb organotherápiás gyógyszerek" (most recent organotherapeutic drugs), Ernst Laqueur was still a very young chemist, who just published his first scientific paper on the chemistry of casein, in the late 1920s. Laqueur became fascinated with the ovarian hormone and just barely lost the race on the isolation of the first estrogenic hormone to Edward Doisy with his Menformon. Gedeon Richter became interested very early in “the pill”, established collaboration with Ludwig Haberlandt of Innsbruck and hoped to market soon the first fertility regulating hormonal product, Infecundin, when the untimely death of Haberlandt in 1932 put an end to this endeaveur.

Had I lived in ancient Rome (and sometimes I wish I had), I should have characterized Gedeon Richter in the ‘Roman Way’: “Vir bonus, civis fortis, amicus fidelis”. He was a good man, a strong citizen and a faithful friend. He understood the importance of innovative ideas, that 'thoughts rule the world'. He was "a man of hope and forward-looking mind even to the last" [4], More than 250 years ago, Thomas Fuller said that "great hopes make

great men" [5], Gedeon Richter (Table 1) was a great man and I would like to pay tribute to his memory with this lecture.

2. Five revolutions

"They who live longest, see most", said Cervantes [6] and this author can state that he has seen a major part of the history of the 20th century. This includes many things; the devastating consequences of two world wars, the rise and fall of great ideas and great empires, an enormous amount of unnecessary human suffering, terror, cruelty and moral horrors. However, at the same time, I have seen more progress in science and technology than allscientists of all preceding periods in history. This should be obvious, since the size of science in manpower or in publications tends to double in each decade, or so. It progresses "in proportion to the mass of knowledge that is left to it by preceding generations, that is under the most ordinary circumstances in geometrical proportion" [7].

I have also seen a number of great revolutions, revolution defined as in the Oxford English Dictionary, "an instance of great change in affairs, or in some particular thing". Among those revolutions I was particularly impressed by five greatly intermingled ones, the revolutions in life expectancy, population, contraception, reproductive health and gender equity. This paper will address briefly those five revolutions, placing particular emphasis on the problems of reproductive health and healthy aging.

The 20th century was characterized, inter alia, by a spectacular rise in life expectancy; when I was born, worldwide life expectancy at birth was just above 40 years, today it exceeds 66 years [8]. During just about 40 years, female life expectancy at birth increased in the world’s most populous developing countries by 20-25 years, as indicated by the data of table1. [9]

The aging of humankind is projectd to continue during most 21st century and as indicated by the data of Table 2, the United Nations expects that global life expectancy at birth will stabilize at around 82 years, a hundred years from now, around the end of the next century. [10]

The 20th century was also characterized by a spectacular rise in world population. When I was born, the global population did not reach as yet 2 billion people; in less than two years time from now it will surpass 6 billion.

Indeed, the most recent projections of the United Nations indicate that a world population of 9 billion people may become a reality by the year 2035, indicated in Table 3 [11],

When did the governments of this world first recognize the existence of a population problem? The credit for this goes to the Government of India, which in 1952 established for the first time a national family planning programme. The example of India was followed others, first slowly and hesitantly and then rapid Thus in the early 1960s only seven governments provided family planning programmes, mainly, if not exclusively for the demographic rationale. However, the early 1980s over 120 governments supported such programmes and no longer only for the demographic rationale. By 1980, 55 developing country governments supported family

Table 1
Female life expectancy at birth (years)-selected countries

Country	Population[a] (millions)	Year 1950	Year 1992
China	1162	42	71
India	884	38	62
United States	253	72	80
Indonesia	184	38	62
Brazil	154	53	69
Japan	125	66	82
Bangladesh	114	35	56

Source: Un World Population Prospect (1984), World Development Report (1994)
[a]Mid 1992

Table 2: An aging humankind-estimated and projected life expectancy at birth UN medium variant projections

Year	*Life expectancy (years)*
1992	*66.4*
2000	*68.3*
2025	*74.5*
2050	*78.0*
2075	*80.5*
2100	*82.4*

Source: El-Bardy (1992), World Development Report (1993)

planning for the demographic rationale, but 65 provided support for the reproductive health and human rights rationale [12]. Today, the number of governments supporting family planning programmes directly, or indirectly, is close to 150.

Table 3

World's population-estimates and projections

Year	Population (Billions)
1965	3.34
1975	4.08
1985	4.85
1995	5.72
2005	6.59
2015	7.49
2025	8.29
2035	9.01

Source: United Nations, 1994

The quantum leap took place when at the United Nations International Conference in Population and Development (ICED) in Cairo, September 1994, where the overwhelming majority of governments agreed to shift the focus of population policies from a demographic to a quality-of-life imperative [13]. They recognized that a major part of health requirements of a population is related to reproductive ill-health. At last, it was understood that reproductive health is not only a fundamental huma right, but also a social and economic imperative. The day of the revolution in reproductive health arrived.

3.Reproductive health

The great importance of reproductive health is amply documented by some recent estimates of the number of people affected by reproductive ill-health worldwide [14-15].

The World Health Organization (WHO) estimates that some 120 million people around the world have unmet family planning needs, 85-110 million women have been subjected to genital mutilation; there are some 60-80

million infertile couples; some 20 million adults are living with HIV/AIDS and 2 million women have invasive cervical cancer.

There are each year an estimated 333 million new cases of sexually transmitted diseases, 20 million cases unsafe abortions and 20 million cases of severe maternal morbidity. The under-5 child mortality is estimated at 12.5 million and prenatal death at 7.2 million per annum. In addition, there are some 25 million infants with low birth weight annually, 2.8 million new cases of HIV-infection and 2 million new cases of female genital mutilation. Maternal mortality is estimated at 600 000 annually and there are 500 000 cases of cervical cancer each year.

Reproductive ill-health is a continuum, beginning with intrauterine malnutrition and accompanying our steps throughout adult life into senescence with problems caused by reproductive tract malignancies, or hormonally induced metabolis alterations, such as osteoporosis.

Based on the above and other data in reproductive ill-health, the major components of reproductive health care can be defined. In my own view, the most important ones are: family planning, maternat and new-born health, unwanted pregnancy, sexually transmited disease (including HIV/AIDS), malnutrition and anemia, infertility, reproductive tract infections, reproductive tract malignancies and adolescent reproductive health. This list differs somewhat from that published last year [16], in which I included as the first component 'the status of women', which is omitted from the present list. The reason for this is not that I ascribe less importance to this subject, but that I ascribe even more importance to it than I did last year. Today, I feel that gender equity is not a component of reproductive health, but an indispensable prerequisite for it. From this it also follows, that gender discrimination, harmful sexual practices and violence against women are essential components of gender equity and should be discussed in that context. Hence all these aspects will be considered under a separate heading: gender equity. Another difference between the present and past list is, that upon further reflection, I feel that 'environmental and occupational reproductive health' should not be considered in isolation, but rather as a component of paramount importance within environmental health.

Region	*Male*	*Female*
Sub-Saharan Africa	*33*	*53*
North Africa	*32*	*56*
Southern Asia	*37*	*63*
Latin America	*12*	*15*
Eastern Asia and Oceania	*9*	*24*
Developed	*2*	*2*

Source: The World's Women, 1995

4. Gender equity

It should be stated at the onset, that we are still very far from reaching gender equity in terms of nutrition, education, health services, human rights, income levels and personal security [17]. Only a few examples in literacy are shown in Table 4 [17].

The data convincingly indicate that there are marked gender differences in literacy in all developing regions of the world.

Other examples of gender discrimination: prenatal sex selection, infant health care, food allocation, infanticide and genital mutilation to mention a few. Examples of violence against women? What about battery, sexual abuse, rape, forced prostitution or human trafficking, a multimillion dollar 'industry' even in Europe today?

The United Nations Development Programme (UNDP) provides another dimension for appreciating the extent of gender disparity, by pointing out, that whereas women constitute 70% of the world's poor and 60% of its illiterate, women occupy only 10% of all parliamentary seats and only 6% of cabinet positions [18].

Not everyone is familiar with the concept of '30% threshold'; in 1990 the United Nations Commission on the Status of Women recommended such a 30% threshold of decision-making positions to be held by women. In parliamentary or cabinet representation only Denmark, Finland, the Netherlands, Norway, Seychelles and Sweden have crossed this threshold so far. Perhaps even more revealing is the situation with respect to the convention on the elimination of all forms of discrimination against women, which was approved by the United Nations as early as 1979. As of 1995, 41 member states of the United Nations still have not signed the Convention,

six signed it, but have not ratified and 43 ratified it but with a variety of reservations [18]. As Phraedus said in one of his fables almost 2000 years ago: "non semper ea sunt quae videntur"; things are not always what they seem..[19].

Klugman and Weiner's characterization of the situation in South Africa is also revealing: "Women's lives are framed within patriarchal assumptions and practices, so that both in the family and in society at large, women live in a terrain which is both defined and controlled by men" [20].

The ICPD (Cairo, 1994) emphasized that "the elimination of social and economic discrimination against women is a prerequisite for reducing poverty, promoting economic growth, and achieving sound population policies" [13]. Whether it is indeed a prerequisite or a very important desideratum, is only a matter of semantics, but it can be taken for granted that the urgent improvement of the political, social, economic and health status of women will have a very prominent place on the international agenda of the early 21st century. To achieve this without straining to the limit the social infrastructure of certain cultural settings, will be a challenge for the next generation in their endeavor to successfully complete this worldwide process, a process, which, in my opinion is irreversible.

Table 5: Estimated and projected percentage of fertile women using contraception

Region	*Year*		
	1960	*1983*	*2000*
Africa	*5*	*14*	*27*
South Asia	*7*	*34*	*52*
Latin America	*13*	*56*	*67*
East Asia	*14*	*74*	*78*

Source: WHO/FHE/FPP/EB94

5. The contraceptive revolution

This is one of the 'success-stories' of our time. When I was born, few, if any, modern methods of contraceptive were available. In the early 1960s the number of contraceptive users in all developing countries was some 31 million; by 1990, more than 380 million people used contraception in the developing world [21]. The estimated andprojected percentage of fertile

women using contraceptives in different regions of the developing world is shown in Table 5 [22].

It is projected that by the year 200, more than 560 million people will use contraceptive methods; 510 million of them will use modern methods. Tubectomy will remain the quantitatively most important method (207 million acceptors), followed by intrauterine devices (125 million), oral contraceptives (75 million), vasectomy(4 million), condom (35 million) and injectable contitives (21 million). In addition, some 57 million people will use traditional methods [23],

What is behind these figures? Perhaps the greatest cultural and social change of our time, the contraceptive revolution. Contraception has come to stay; it became a way of life of the 20th century.

6. Maternal health and ill-health

Whereas the contraceptive revolution represents a success-story, maternal health and unwanted pregnancies do not. Indeed, maternal health provides a good pie for the modern myth of progress, or rather the lack of it. An estimated 50 million of the annual world total of 200 million pregnancies are interrupted, 30 million are in need of emergency obstetrical care, millions of deliveries are unattended by trained personnel and 12 million deliveries result in post-partum disabilities, e.g. obstetrical fistulae, severe anaemia, infertility pelvic inflammatory disease etc. [14].

WHO recently revised its previous estimate of annual maternal deaths worldwide from 500 000 to 586 000 [24]; there is no equity in sight in the various parts the world as yet, as shown by the data of Table 6.

Table 6

Maternal deaths per 100 000 live births-selected countries Country	Maternal mortality ratio
Sweden	7
USA	12
Hungary	30
Russian Federation	75
China	95
Romania	130
India	570
Indonesia	650
Nigeria	1000
Somalia	1600

Source: WHO, 1996

The data of Table 6 indicates maternal mortality (maternal deaths per 100000 live births) selected countries varied between 7 and 1600. These: marked differencesre-emphasize the importance of four crucial elements to prevent maternal mortality [25]: (a) enable women to regulate their infertility ;(b) provide natal care; (c) make basic emergency obstretical care available to all;and (d) ensure that every woman in childbirth is attended by trained persons.

Although abortion statistics are notoriously uncertain, the most likely estimates indicate that some 40 – 60 million pregnancies are terminated each year. From the public health point of view, the fundamental problemis not whether an abortion was legal, or illegal, but whether it was safe, or unsafe. Unsafe abortion is defined as a procedure for terminating an unwanted pregnancy either by persons lacking the necessary skills or in an environment lacking minimal medical standards, or both. Estimates by WHO indicate that in 1990, some 20.5 million unsafe abortions resulted in 67000 death, mostly in Asia (39000 deaths), Africa (21000 deaths) and Latin America (5500deaths).

It is established that abortion laws influence maternal health; in countries where abortion is illegal, women resort to clandestine, often unsafe abortions at high risk to their health. Therefore legislation on abortion has a significant impact on women's health. Whereas the laws of 173 countries accept the indication for abortion to save the life of woman, only 119 accept the indication to preserve her physical health and only 95 to preserve her mental health. Rape or incest is accepted by 81 countries, fetal impairment by 78 economic or social indications by 56 countries.

There is a marked difference in the legislation of developing and developed countries with respect to the grounds on which abortion is permitted. While 86% of developing countries permit abortion to save the life of a woman, only 23% accept rape or incest, 22% foetal impairment and 8% economic and social reasons. Corresponding percentage in the legislations of developed countries are 94%, 86%, 83% and 81%, respectively. [31,32]

7.Infertility and sexually transmitted diseases

Infertility is not a life-threatening condition, but its impact on the mental and social well – being of couples may be devastating and in many cultural settings it may result in serious social consequences, like divorce, ostracism. Estimates by WHO put the number of infertile couples worldwide at 60 – 80 million.

In a recent review attention was called to the spectacular progress in assisted reproduction and in the management of infertility that has taken place during past two decades. It was emphasized, that the high cost of many of these procedures excludes the possibility to offer them as a public health service in the developing world; hence it contributes to the widening gap between haves and have-nots in our two-track society.

Studies conducted by WHO strongly suggest that tubal factors may represent the most important cause of infertility, at least in Africa [33], which underlines the importance of prevention of sexually transmitted disease.

Recent estimates by WHO indicate that the annual total of new cases of sexually transmitted diseases is higher than it was believed previously (250 million) and that it exceeds 330 million cases. There are some 89 million cases of chlamydia and 62 million of gonorrhoea! infections and 12 million new cases of infectious syphilis, the latter with a 10-15 fold rise in several Eastern-European countries since 1991. In addition, there are some 170 million new cases of trichomoniasis and a considerable number of other sexually transmitted infections, such as genital- and human papilloma virus, genital herpes, chancroid etc. [14],

Some of these infections are associated with a high prevalence of subsequent infertility, others, like human papilloma virus (particularly types 16 and 18 and probably also types 31 and 33) are involved in the pathogenesis of cervical cancer. Furthermore, sexually transmitted diseases if left untreated increase the risk of HIV-infection by 300-400% [34].

What is the current situation with regard to HIV/ AIDS? WHO estimates that by late 1994 there were some 18 million HIV-infected individuals around the world, among those 11 million in sub-Saharan Africa, 3 million in South and South-East Asia, 2 million in Latin America and the Caribbean and more than 1 million in North America. Conservatively, WHO projects

Table 7
Population size (millions) with and without AIDS for 15 sub-Saharan African countries

h AIDS	*Without AIDS*	*Percentage difference*
.6	*161.6*	-
.4	*189.8*	*-0.2*
.2	*223.4*	*-1.0*
.6	*260.8*	*-2.4*
.8	*303.4*	*-3.8*

Source: United Nations, 1994.

that by the year 2000, a world total of 30-40 million men, women and children will have been infected with HIV, 90% of them in the developing world. The cumulative number of AIDS cases by that time is expected to reach 12-18 million [35],

Whereas sexually transmitted diseases increase the risk of HIV-infection, HIV increases the risk of tuberculosis; WHO points out that in 1995 more people died from tuberculosis (some three million) than in any other years in history. Tuberculosis is the principal killer of HIV-positive people and kills more women than all causes of maternal mortality combined.[36]

There was been much debate and a fair amount of controversy as to whether the HIV/AIDS pandemic will have an impact on population size. Recent estimates and projections by the United Nations indicate, that at least in sub-Saharan Africa a decrease in population size can be expected as indicate by the data of Table 7. [37]

8. Infant mortality and reproductive tract malignancies

Because of limitations in time and space, these two problem areas will only be touched upon briefly. Each year approximately 50 million human beings die, 39 million in the developing and 11 million in the developed world. Of the 50 million, an estimated 12.7 million deaths occur under the age of 5, the great majority of those (12.5 million) in developing countries. The most important causes of death are acute respiratory infection (4.3 million), diarrhoea (3.2 million), perinatal causes (3.0 million) and malaria (0.8 million).

Some three million infants die each year in the first week of their life, following poorly managed pregnancies and deliveries. Infant mortality rates

in 1990 ranged between 5/1000 live births (Finland, Sweden) and 160/1000 livebirths (Sierra Leone) and under-5 mortality in 1992 for males varied between 7 (Ireland, Singapore) and 283 (Mozambique) per 1000 live births [9].

WHO projects that the number of deaths from malignancies by the year 2000 will be 7.1 million; 3.2 million women and 3.9 million men. Of these 7.1 million can- cer-deaths, 4.3 million are expected to occur in the developing and 2.8 million in the developed world. Although, as indicated in Table 8 gastrointestinal tract malignancies are responsible for most cancer-deaths, the five most important reproductive tract malignancies kill almost 1 million persons each year [15].

Table 8

Worldwide mortality from some malignant neoplasms in 1993

Malignant neoplasm	*Deaths (thousands)*
Trachea, bronchus and lung	1035
Stomach	734
Colon and rectum	468
Mouth and oropharynx	458

Source: WHO, 1995

It is expected that with the rapid aging of the world population, the quantitative importance of reproductive tract malignancies will further increase.

9. The road to improved reproductive health.

Good reproductive health depends on a multitude factors, for instance economic situation, education, employment, living conditions, family environment, social relations, traditional values and legislation. The importance of reproductive health is further underlined by the fact that it is a fundamental human right, which requires therefore a holistic approach, gender equity and the highest ethical standards.

It is the goal of the United Nations and the ambit of all member states that all individuals should h access to reproductive health care, regardless of income, ethnicity, sex and age.

Some basic indicators related to reproductive health worldwide are shown in Table 9; it is obvious from data that all indicators are closely correlated to economic situation of the countries [15].

Worldwide poverty is undoubtedly the most important factor interfering with a rapid improvement of reproductive health of the human race. WHO stresses that "poverty is the most widespread, pervasive i intractable disease in the world today" [28], Taking i account that 20% of the world population is living absolute poverty [38] and that among the some member states of WHO there are only 25 counties classified as 'developed market economies' (including number of small countries, like Iceland, Luxembourg Monaco and San Marino), it is not too difficult to the magnitude of the problem.

However, nothing would be more erroneous than assume that without solving the monumental problem of worldwide poverty, little, or nothing can be done improve reproductive health. What about legislation for instance? It has certainly a major impact on reproductive health by deciding on the age at marriage, of consent, minimum age of school-leaving and employment, education on sexuality and reproduction health, information on and access to family planning prevention of unsafe abortions, management of at action complications, prenatal care, delivery care ; postnatal care, or diagnosis and management of se ally transmitted diseases. Why don't we listen a more to Arnold Toynbee?. 'The positive advice I would five in very general terms is that the human race is far more able to deal with technology, than it is in dealing with itself'.

Since the implementation of reproductive health programmes is the sovereign right of every member state, it must consistent with national laws and development policies, with full respect to the cultural, ethical and religious values of the country. However, it must also conformity with the universally recognized human rights! Furthermore, the provision of appropriate reactive health services must be 'all-embracing' in the sense that it should include information, education, felling, prevention, detection, management, care rehabilitation. All this is in conformity with Principe of the Final Document of 1CPD (Cairo, 1994): everyone has the right to the enjoyment of the highest able standard of physical and mental health" [13].

Table 9:Some basic indicators

Some basic indicators Indicator	Developed world		Developing world	
	Developed Market economies	Economies in transition	Developing countries(other than LDCs)	Least developed countries (LDCs)
Gross National Product (US$ per capita)	22262	1992	1043	200
Adult literacy rate (women)	98	98	64	38
Under-5 mortality rate(per 1000 live births)	9	29	75	156
Maternal mortality ratio (per 100000 live births)	13	60	350	1050
Institutional delivery (percentage live births)	98	95	41	20
Unsafe abortion (per 1000 women, aged 15-49 years)	1	22	15	26

Source: WHO, 1996

How important then is reproductive health? Indeed, very important. It is so important, that seven of the ten set by WHO for the period 1996-2001 are directly relevant to it. In order to reach those ten goals, 25 specific targets have been set; 17 of those are wholly or partially within the area of reproductive health [40]. Reproductive health is universal, developmental and inter-generational. It is also neglected, because of its intimate nature and complex cultural and social ramification. It is the quintessence of general health and health is the quintessence of all human development.

10.Healthy aging

The last part of this review returns to the first revolution mentioned at the onset, the revolution in life expectancy, which has dramatically increased in the 20th century. The issue is not only the greatly increased life expectancy at birth; there is a genuine lengthening of our life-span, as indicated by recent estimates of an increased life expectancy at the age of 60 years. The human race is growing and aging rapidly.

Aging and the elderly represent an area of multiple definition: biological, social, economical and chronological. Particularly the social and economic definitions are highly variable, reflecting the changing perceptions of society as to its actual needs of work-force and thus retirement from it. The seemingly simplest, the chronological definition is not so simple, since it is a function of the expected life-span and of the adjustment of definitions accordingly. Originally, the elderly was defined by the United Nations as those aged 60 and over; however, in view of the rapidly increasing life expectancy this age limit was raised to 65 years, which still causes problems in the comparison of available statistical data, since some governments and international organizations switch to the new definition with a certain time-lag. In this review, the definition adopted by WHO in more recent publications will be used [15] and distinction will be made between the 'elderly' (people aged 65 years and over) and the 'old old' (those aged 80 years and over).

When I was born, the elderly population of the world consisted of less than 100 million people; by 1950 it reached 127 million out of a global population of 2.5 billion, or 5% of the total. Then the aging process accelerated and by 1995 the elderly population reached 370 million out of a total population of 5.7 billion, or 6.5% [15]. Obviously, the above average percentage is based on very disparate figures, as indicated in Table in 10.

Thirteen percent of the population of the developed world (159 million out of 1214 million) in 1995 consisted of elderly people; the corresponding percentage in the last developed countries was only 3%. It should also be noted that life expectancy at birth is still much less in the least developed countries than in the developed world and that the life expectancy of women is much higher than that of men in the developed world.

And the future? It is projected by WHO that during the next 2-3 decades, the most rapid increase in the elderly population will take place in the developing countries in general and in the Asian region in particular, as shown by the data of Table 11.

Whereas the elderly population of Sweden is expected to increase by 33% during the period 1990- 2025, the elderly of the big Asian countries is projected to increase by 200-400% [42],

Table 10
Projected increase in the elderly population between 1990 and 2025 for selected countries

Population (millions)	*823*	*391*	*3898*	*589*
Elderly population (millions)	*115*	*44*	*193*	*18*
Elderly population (percentage of to tal)	*14*	*11*	*5*	*3*
Life expectancy at birth (years)	*77*	*69*	*66*	*52*
Female advantage in life expectancy (years)	*6.4*	*9.7*	*3*	*2*
Gross National Product (USS per	*23 262*	*1992*	*1043*	*200*

Source: WHO, 1996

What about the 'old old'? They constitute the fastest growing population in most countries of the world [43]; as shown by the data of Table 12, the percentage of the 'old old' will double in Europe and treble in Asia between 1990 and 2025.

Table 11: Projected increase in the elderly population between 1990 and 2025 for selected countriea

Country	Percentage increase
Indonesia	414
Thailand	337
India	242
China	220
Bangladesh	219
Australia	137
UK	45
Sweden	33

Long-term projections are always 'tricky' since, for obvious reasons they invariably carry a great amount of uncertainty; however, they hint at an evolution, which is not unlikely to happen. The United Nations medium variant projections suggest that the 21st century will witness a very rapid worldwide increase in the proportion of the elderly population, as shown by the figures of Table 13 [10]. Indeed, the projection suggests that by the end of the 21st century, the world's 'old old' population will be 6.6% of the total.

Table 12:Estimated and projected population aged 80 years and over

Region	Percentage of population		
	Year		
	1990	2010	2025
Sub-Saharan Africa	0.3	0.3	0.4
Latin America	0.8	1.2	1.8
Asia	0.6	1.2	1.8
North America	2.8	4.0	4.6
Europe	3.2	4.9	6.4

Source: WHO, 1995

Why is aging such a difficult social problem?Because the last years of life are accompanied 1 increase in disability and sickness, with particularly high demands on social and health services. There major debate in the developed world as to the carrying capacity of modern society in this respect and hardly a secret that most, if not all, developed countries are in the process of significantly reducing their and health budgets.

In many developing countries, the majority of surviving to old age face a longer life of economic deprivation, with little, if any, social support. Because of the gradually increasing gender gap in life expectancy (isn't it slightly ironic, calling it officially male advantage?') the majority of the elderly w women experiencing greater burdens than men in of disability and morbidity, including the most] pervasive of all human diseases: poverty.

The classical areas of concern in relation t elderly include i.a. cardiovascular and cerebrovascular disease, malignant neoplasms, chronic respirator ease, neuropsychiatric disease and a variety of agerelated metabolic alterations, for instance same organ and musculoskeletal changes, diabetes and endocrine and nutritional diseases and oral ill-health.

Table 13.

Projected percentage of population aged 65 years and over and 80 nd over, UN medium variant projections

Aged 65 and over	Aged 80 and over'
6.8	*1.1*
9.7	*1.6*
14.0	*3.0*
18.9	*4.8*
21.6	*6.6*

El-Badry, 1992.led in the 65 and over group.

Available information on worldwide mortality even more morbidity is scanty. Nevertheless, some : data compiled by WHO may provide an idea as : magnitude of the problem. Worldwide mortality from 1993 are shown in Table 14.

The most important causes of death in the developed are: diseases of the circulatory system (5.1 million s), malignant neoplasms (2.4 million) and chronic respiratory tract diseases (0.9 million). In the developing world infectious and parasitic diseases cause more 16 million deaths, diseases of the circulatory sys- 1.1 million and malignant neoplasms, 3.5 million s. The total of all deaths amounts to some 50 m; 11 million in the developed and 39 million in developing world.

Concerning estimates of morbidity, WHO in collaboration with the World Bank estimated recently the world wide burden of disease [29].As an example, the percentage distribution of the disease burden of the y population of developing countries is shown in ; 15.

Table 14

Percentage distribution of causes of death in 1993

Cause	Developed countries (11 million)	Developing countries (39 million)
Infectious and parasitic diseases	1.2	41.4
Diseases of the circulatory system	46.7	10.7
Malignant neoplasms	21.6	8.9
External causes	7.5	7.9
Perinatal and neonatal causes	0.7	7.9
Chronic respiratory tract disease	7.8	5.0
Maternal causes	-	1.3
Other and unknown causes	14.5	16.8

Table 15
Percentage distribution of the disease burden in the elderly population of developing countries, 1990

Disease	*Female"*	*Male"*
Cerebrovascular diseases	*16.5*	*13.8*
Ischemic heart disease	*11.6*	*11.7*
Malignant neoplasms	*10.4*	*14.5*
Chronic obstructive pulmonary	*8.1*	*9.6*
Alzheimer and other dementias	*4.8*	*4.1*
Respiratory infections	*4.6*	*4.0*
Peri -endo- and myocarditis	*3.6*	*3.6*
Diabetes mellitus	*2.4*	*1.5*
Tuberculosis	*1.9*	*4.0*
Cirrhosis	*1.2*	*2.1*

Source: World Development Report, 1993. [a] Aged 60 years and over.

It appears from the data of Table 15, that the most important causes of disease burden in this population were: diseases of the circulatory system, malignant neoplasms and chronic obstructive pulmonary disease [29].
A major problem, that must be mentioned in connection with aging, is the increasing isolation of the elderly. Among the most important causes contributing to this isolation are: incontinence (urinary and fecal), impaired vision and hearing, reduced mobility, falls and fear of falls and oral ill-health. The quantitative aspects of these and related problems have been discussed in several WHO publications [43-45].

11. Aging and ethics

The soaring world population in general and its elderly population in particular, will undoubtedly raise major social, economic and ethical issues; indeed it may strain to the limit the ability of health, social and even political infrastructures of many countries to cope with this new revolution, the revolution of the elderly, of the 'old old' and of the disabled. Because of the increasing gender difference in life expectancy, the majority of

theelderly will be women, particularly poor and frequently in poor health and the medico-ethical problems caused will go far beyond the ability of the health professionals of the next century to resolve.

Any serious discussion of future health policy for the elderly usually starts with economical aspects and then in view of the magnitude of the challenge shifts to ethical considerations, opening an almost endless frontier of ethical inquiry into our present-frequently conflicting-premise: what should be the balance of resource allocation among and between generations? This then leads to the individual versus community premise: what should be the balance between family and government obligations in providing care for the elderly? What about the premise of equity: should there be limits of health care for the elderly and if so, who will decide over life or death? Really, where do we stand as to the sanctity of life premise: what about the human rights of the disabled, poor or demented elderly? What is our present position as to the fundamental ethical dilemma of euthanasia? Last, but not least, I miss a general debate on the sustainability premise: how could the elderly contribute to the maintenance of a costly social fabric and participate more effectively in determining their own fate and welfare? [46].

12. Epilogue

More than 300 years ago, Blaise Pascal remarked: "man is equally incapable of seeing the nothingness from which he emerges and the infinity in which he is engulfed" [47]. It may well be so, however, what Pascal couldn't foresee was that the time will come when humankind will undertake a global effort to improve the human condition in general and the health condition of every human being, in particular. Pascal couldn't foresee that the day would come when the governments of the Earth will agree that "all individuals should have access to basic health care, regardless of income, ethnicity, sex, age or disability" [13] and that the international debate would no longer be centered on the question whether or not the above goal can be realized, but rather on the ways and means; on the when and how. And it can happen, if we let it happen. Never before in history has humankind had so many resources, so much knowledge and such powerful technologies at

its disposal. It can be done, if we decide that it should be done. The decision is no longer medical, but rather political.

The classical question, so often asked by the new generation of gynaecologists, is ….." but what can I do?". Probably much more than they think. Publilius Syrus, the former Persian slave who became a celebrated Roman philosopher, wrote 2000 years ago: "nobody knows what he can do till he tries" [48], A great deal can be done, like influencing public opinion. The historian-philosopher, Eric Hobsbawn in his book 'The age of extremes' observes, that: in the 1990s public opinion became 'inescapable'. However, it is not constant, it is changing and can be changed. Hence, the message of today is the same as it was at the beginning of the century, when the economist J.M. Keynes suggested to set in motion "those forces of instruction and man of onethousand-and-one quotations". The assertion of imagination, the unveiling of illusion, the dissipation of hate, the enlargement and instruction of men's hearts and minds must be the means". [49]

Too close, may I recall that at a meeting the other day I was introduced by the chairman of the session as "a man of one thousand-and-one quotations". Perhaps he was right.... in contrast, Ludwig Wittgenstein, the greatest logician of our time, appears to be a man of a single quotation. His magnum opus, the Tractatus LogicoPhilosophicus has a single quotation, his motto from Kurnberger: "and whatever a man knows, whatever is not mere rumbling and roaring that he has heard, can be said in three words" [50].

If so, what should be the three-world summary of this Gedeon Richter Memorial Lecture? Perhaps science humility and hope.

Why science? Because, as George Sarton said "the acquisition, systématisation and dissemination of positive knowledge are the only human activities, which ar truly cumulative and progressive. In fact, progress had no definite and unquestionable meaning in other field than in the field of science" [51].

Why humility? Because, as T.S. Eliot wrote "the only wisdom we can hope to acquire is the wisdom of humility: "humility is endless" [52],

Why hope? Because I am convinced that there is also another wisdom we can hope to acquire and that is the wisdom of hope, all of us are prisoners of

hope, the quintessence of the human condition. As Martin Luther said in one of his table-talks "the essence of a man not what he is or does, but in his hope" [53].

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Reprinted from: European Journal of Obstetrics & Gynecology and Reproductive Biology 71 /1997, 123-133

From the contraceptive to the anthropocentric revolution
(Gregory Pincus in memoriam)

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ABSTRACT

The author has seen more progress in science and technology than all scientists of all preceding periods together since the dawn of history and has witnessed a number of powerful revolutions (e.g. scientific, information, postindustrial, globalization, environmental, contraceptive, reproductive health, gender equity and demographic) that have profoundly changed the world and our perception of it. The contraceptive revolution started off this irreversible process with spectacular results. By 1990, contraception had become a global lifestyle for more than 900 million couples. It also resulted in subsequent revolutions in reproductive health and gender equity in a new world with a dramatically changed population structure with fewer and fewer children and more and more elderly.

These revolutions will assist humankind in the 21st century in decol1Structing the deterministic world view of past centuries and replacing it with a science-driven anthropocentric world view.

KEY WORDS:

New realities, Contraception, Reproductive health, Population size, Population structure, Institutional reform, Anthropocentric world view.

'Be not deceived. Revolutions don't go backward.' (Abraham Lincoln 1856)

A RENDEZVOUS WITH NEW REALITIES

From windshields to windmills

"At certain periods of history it is only poetry that is capable of dealing with reality' says Joseph Brodsky and I am convinced that he is right, except I suspect that history consists exclusively of such periods.

In fact, the presumed essence of reality could have made life complicated during the past few millennia for those who really tried to reflect on it. In the view of Xenophanes of Colophon, the Eleatic philosopher (circa 370-473 BC), 'Pure truth hath no man seen nor e'er shall know' and, some 2000 years later, Erasmus of Rotterdam remarks that 'All things in life are so multifaceted, contradictory and obscure that we can never be sure about the truth . Wise words, but where do they take us' Fortunately for us, Blaise Pascal some 160 years later in the 17th century, emphasizes that 'It is not certain that everything is uncertain·'. Certainly not! There are some realities, old ones and new ones, and this article will deal with some new realities. One of our fundamental problems seems to be that the world is rapidly changing around us, but our perception of those changes is lagging behind. As the Brundtland Commission stated, 'The Lite of change is outstripping the ability of scientific disciplines and our current capabilities to assess and advise".

As I see it, the wind of new realities is blowing with increasing strength; some people will try to construct progressive windshields and, those who are more clever, new types of windmills. However, not much will be achieved without some vision of the future. But what is vision? Perhaps the amalgamation of rational projections with instinctive perceptions. Is vision then really that important' 'Without vision the people perish', said President Roosevelt. However, I am profoundly convinced that only those with some knowledge of the past can have a vision of the future. In Winston Churchill's words, 'The longer you can look back, the further you can look forward.

From magicians to the dry cleaner and beyond

When our modern chronology started 2000 years ago, the global population was around 300 million people; in 1920, when I was born, it was still below two billion (1800 million), but today it exceeds six billion[6] Hence, in my lifetime, I have seen the birth of another two worlds, equal in numbers, needs, aspirations, hopes and dreams, and I have seen three generations in action. Schopenhauer says that 'the man who sees two or three generations is like someone who sits in a conjurer's booth at a fair, and sees the tricks two or three times. They are meant to be seen only once'7. I have lived through half a century in which humankind was involved in heavy filtration with the Apocalypse, in the increasing shadow of atomic and hydrogen bombs, and I have witnessed a century in which some 200 million human beings were killed by other 'human' beings on the orders of political magicians, a senseless process that is still ongoing and on a large scale. It would appear that rationalism is - at least as yet - a tool of limited usefulness as far as humankind is concerned; Ludwig Wittgenstein is credited with saying that, from time to time, certain words have to be purified and cleaned before they can be used again. Is *Homo sapiens* perhaps one of those?

However, before rushing to the dry cleaners, let me also say that, in my lifetime, I have also seen more progress in science than all scientists of all preceding periods have together achieved since the dawn of history. This should not be too surprising to my fellow scientists, since 'science progresses in proportion to the mass of knowledge that is left to it by preceding generations, that is under the most ordinary circumstances in geometrical proportion.

Those ten revolutions: how many are there?

The word 'revolution' has several meanings: it can be used to describe any movement around an axis, the movement of celestial bodies, or even the complete overthrow of a government, or to characterize a 'great change in affairs, or things'. Such 'great changes' are frequently associated with fundamental changes in our perception of the world and ourselves, and I am using the term in this context.

My generation has been a most privileged one; we have not only witnessed, but also participated in, at least ten powerful revolutions that have

profoundly changed our world and our world view. Others may prefer a different 'menu'; like most things in life, our 'favorite' revolution can be a matter of taste and, *de gustibus et coloribus lion est disputadum*, one should never discuss taste and colors, as the medieval scholastic philosophy stated. Furthermore, in a world where the amount of new scientific information doubles every 7-8 years, all of us can be accused of omission, but rarely of commission, in the selection of our 'crucially important revolutions'. [n fact, there may be no end to their number. However, the dictum of George Orwell, that 'All animals are equal, but some animals are more equal than others"), has also some relevance to our selection process.

The present author would like the reader to consider the following incomplete list of revolutions of our time: the scientific, technological, information, postindustrial, globalization, environmental, contraceptive, reproductive health, gender equity and demographic. This list is a slight expansion (with the information revolution) of the nine revolutions described in previous papers[1n.11]; however, the present article adds an additional dimension to these in the form of an 11 the revolution, which [consider to be the most powerful and most fundamental of all: the anthropocentric revolution, to be discussed below.

What is the common denominator in these revolutions' It is indicated by the motto of this paper from Abraham Lincoln: 'Be not decided. Revolutions don't go backward'. Maybe something for the staunch opponents of contraception to reflect upon? At any rate, these revolutions either did not exist or only had a very limited impact prior to the second half of the 20th century. In fact, the past 30 years represent a continuous chain of revolutions, perhaps the most important period in our history, when global identify became a reality rather than just a philosophical concept, bringing to humankind - for the first time - a holistic view of the world. The past 30 years represent the first age since the dawn of civilization that dared to establish the United Nations (UN) with its important specialized agencies, such as the World Health Organization (WHO), and felt it both feasible and mandatory to provide large-scale assistance in any comer of the world to those stricken by famine or other natural catastrophes.

This review will briefly consider the revolutions in contraception (the first revolution of all that gave impetus to many others) and reproductive health, together with the demographic and anthropocentric revolutions.

THE CONTRACEPTIVE REVOLUTION: A PASSPORT INTO THE FUTURE

A few milestones of progress

There is a plethora of information published on the history of family planning in general, and of the 'pill' and intrauterine devices in particular. The present author also felt justified on several occasions to review different aspects of the contraceptive revolution, given the fact that, like Dean Acheson[16], he was also 'present at the creation', witnessing 'the birth of the pill' at the Laurentian Hormone Conference in 1956[17] It can be the easiest task, or the most difficult one, to prepare reviews of this sort, depending on the approach to the problem: straightforward chronology of the events or an attempt to analyze the history of the leading ideas. With regard to the contraceptive revolution, the issue was further complicated by the apparent conflict of the 'leading ideas' of the 1950 and 1930s with a general confusion and mix-up of demographic concerns with reproductive health and fundamental human rights.

If I were asked to give only three milestones of the early history of contraception, I would probably indicate the years 1995, 1959 nad 1960. In 19*55,* at the Congress of the International Planned Parenthood Federation (IPPF) in Tokyo, Gregory Pincus reported, for the first time in history, that huge doses of orally administered progesterone inhibit ovulation in women. Then in 1959 , the USA Food and Drug Administration approved the first oral contraceptive, Enovid"', consisting of 8.830 µg norethynodrel and 15µg mestranol[14] - an enormous dose today, isn't it' Francis Bacon said that 'As the births of living creatures at first are ill-shapen, so are all innovations, which are the births of time); it is always easy to be critical in hindsight, but just imagine what would have happened if many pregnancies had occurred, because of the administration of insufficient doses. My third milestone is the year 19*60,* when the first second-generation lI1trauterine device, the

Margulies spiral, was introduced, to be followed 2 years later by the Lippes loop [2]".

Let me also try to illustrate the progress of ideas with three milestones:

(1) The year 19*52:* the Government of India, for the first time in history, established a national family planning program;

(2) 1962: following incredibly bitter and heated debates, the United Nations authorized its specialized agencies with resolution UN 18.38 to give advice, upon request, to member states on population issues[21];

(3) 1965: the World Health Assembly, with resolution WHA18.49, requested the Direct Or-General to establish a program in the area of human reproduction[21].

These two resolutions provided much-needed international legitimacy and general recognition to the issues of population, family planning and contraception.

1 am profoundly convinced that the introduction of steroidal contraception was a major revolution, scientifically, medically, socially and ethically, and I agree with D. Ewen Cameron, who, in a letter to *The New York Times,* stated more than 30 years ago that 'Few contributions to medical knowledge have done so much to bring to women everywhere a sense of worth and digniry[22].

In fact, if I were asked to characterize the progress in our ethical approach to human fertility by two historical quotes, I would quote Napoleon Bonaparte and Sir Dugald Baird - rather strange bedfellows in the history of contraception, aren't they? In a letter to Gaspard Gourgaurd from St Helena in 1815, Napoleon remarks that 'Women are nothing but machines for producing children' and in a paper published 150 years later. Sir Dugald Baird emphasizes that 'To freedom of speech and worship and freedom from want and fear - listed by President Roosevelt in the 1940s - a fifth one, "freedom from the tyranny of excessive fertility" should be added'[21.]

Revolution or way of life?

'When an idea corresponds to the necessity of the epoch, it ceases to belong to those who invented it and it becomes stronger than those who are in charge of it', wrote Jean Monnet in his Memoires (1978), referring to the European Union, but the sentence describes at least as well, if not better, the contraceptive revolution. Around 1960, the world-wide number of

Table 1 Average prevalence of specific contraceptive methods in the world, in less developed regions and in more developed regions. From reference 25

	Prevalence of use (%)		
Method	*World*	*Less developed regions*	*More developed regions*
All methods	57	53	70
Female sterilization	18	21	8
Male sterilization	4	4	7
Pill	8	6	17
Injectable	1	2	0.1
Intrauterine device	12	14	5
Condom	5	2	14
Vaginal barrier methods	1	0.2	2
Rhythm	3	2	6
Withdrawal	4	2	12
Other methods	1	1	1

contraceptive users was probably less than 30 million; today it exceeds 900 million[24], posing the question whether or not it is still a revolution, or just the normal way of life for a billion couples? What kind of methods are people using? The average prevalence of specific contraceptive methods, based on couples with the woman of reproductive age (around 1990), is shown in Table 1.

The data indicate major regional differences: the prevalence of female sterilization, intrauterine devicesand injectable contraceptives is considerably higher in developing countries, and the of oral contraceptives, condoms and particularly conventional methods (barrier methods, rhythm, withdrawal, etc.) is higher in the developed countries.

Also of interest to us are the policies of governments towards access to contraceptive methods; the latest data (from 1996) are presented in Table 2[2]. Of the 179 countries listed, 142 provided direct support for family planning services through government-operated facilities (e.g. hospitals, health centers, fieldworkers) and 13 provided indirect support (e.g. grants to non-governmental organizations that provide family planning information or

services). Of the 22 countries providing no support, nine are in the developed regions (including Bulgaria, Greece, France, Germany and Switzerland). Two States, the Holy See and Saudi Arabia, have an official policy of limiting access to contraception.

Contraception-21: an unfinished agenda

Contraception has come to stay; it is more than likely that it will be the way of life for many generations to come. As Fathalla states, 'What we are witnessing is a major evolutionary jump that is science-mediated, rather than brutally imposed by Nature,26. The issue is, however, whether there is an unmet need for a second contraceptive technology revolution and many of us believe that this is still the case. Why? Because an estimated 120-150 million women who want to limit or space their pregnancies are still without the means to do so effectively-[7] and because men still do not fully share the contraceptive burden with women.

Recognizing this unmet need, a woman-centered agenda for contraceptive research and development (Contraception-21) was adopted by the Rockefeller Foundation and its partner foundations in 1993[28], and was subsequently endorsed by a major study of the Institute of Medicine of the US National Academy-'.

Table 2 Government policies on providing access to contraceptive methods in the world, in developing regions and in developed regions. From reference 25

Number of countries with policy

		Number of countries with policy	
Policy	World	Developing regions	Developed Regions
Limited Access	2	1	1
No support	22	13	9
Indirect support	13	9	4
Direct support	142	112	30
Total	179	135	44

The woman-centered agenda

Three major areas of unmet need were selected for action:

(1) Expanding male contraceptive choices and responsibility;

(2) Protection against sexually transmitted infections;

(3) 'Retro-active' contraception.

For such a program to have an impact, the participation of both the scientific and industrial communities is essential. This is being realized via a variety of mechanisms such as:

(1) The AMPPA Network (Application of Molecular Pharmacology for Post-Testicular Activity), a global collaborative effort with the Ernst Schering Research Foundation (a non-profit subsidiary of

(3) A Network for Research on Vaginal Microbicides in collaboration with the Population Council, New York.

Other important mechanisms are:

The Consortium for Industrial Collaboration in Contraceptive Research and Development for promoting public/private sector partnerships in the search for new leads in the three priority areas of the woman-centered agenda. The Consortium is currently supported by several large US-based foundations and the UNFPA.

(2) Collaborative research and development on mifepristone to reduce unwanted pregnancies and recourse to abortion. The Rockefeller Foundation supports the CONCEPT Foundation (based in Bangkok) to bolster the efforts of Chinese research and foster new levels of collaboration with industry. The World Health Organization Special Programm in Human Reproduction provides technical assistance to the clinical institutions involved.

The Contraception-21 program of the Rockefeller Foundation is a pioneering example of a new approach to global health problems in general, and contraceptive development in particular. As the turn of the century approaches, is becomes increasingly obvious that there is a need for new types of partnerships between the private and public sectors and it is gratifying to know that the World Health Organization is prepared to assume a leadership role in the health revolution. In fact, in 1995, the World Health Assembly called for a global consultative process that would involve

the widest range of partners to develop a new global health policy for the 21st century.

Gregory Pincus and the contraceptive revolution

Since the present author had the privilege of working h 'Goody' Pincus since the late 1950s, it is not unusual that he still reflects a great deal on the historical ; played by Pincus. Was he aware of the fact, when presented his (perhaps somewhat dry) contribution at the Congress of the IPPF in Tokyo in 1955[18], that he initiated one of the greatest revolutions of the 20th century? Did he ever imagine that, before the end of century, contraception would be a self-evident way life for almost one billion fellow women and men? Did he ever consider that the contraceptive revolution would inevitably usher into existence the revolution reproductive health, which, in turn, would give rise another major intellectual-political revolution, that in gender equity? Probably not, or at least not in these terms. Although . . . who knows? It may be worthwhile to remember how contemporary scientists assessed his contributions soon er his premature death in 1967: 'Pincus was a master what we term "applied science", he was the creator d innovator of a new field - the conscious use of science to effect social change in the interest of man d civilization"". Perhaps, after all, he did foresee, more than we think, the future social benefits of contraception. However, he had to move slowly and carefully. Contraception was extremely controversial and it had many fierce opponents at that time, and the idea of contraception as a new lifestyle of large populations was (and still is) both disturbing and painful several sections of our multifaceted and incredibly complex society.In retrospect, 1 strongly feel that we still do not appreciate sufficiently the fundamental significance of the contraceptive revolution for our subsequent intetellectual progress. In fact, this was the very beginning.

The wisdom of hindsight

Contraception is an extremely important constituent of reproductive health, but it is not the only one, and, in retrospect, it appears almost incredible that we could accept, in a restricted manner, the contraceptive revolution without considering it in a much broader perspective. But such were the times and

the public debate of the 1960s was overshadowed by demographic concerns. Hence, it was not immediately recognized in the public debate at that time that reproductive health is not only a fundamental human right for all, but it is also a social and economic imperative. Again, with the wisdom of hindsight, it becomes so easy to see all this today; all we have to do is to consider some estimates of reproductive ill health.[31-35]

Reproductive ill health.

An estimated 120-150 million women who want to limit or space their pregnancies are still without the means to do so effectively2. The global number of women subjected forcefully to genital mutilation exceeds 100 million. Isn't it interesting? Did those who carry out the mutilation ever hear about the advice given by Isocrates (436—338 ik:) to Nicocles, King of Cyprian Salamis, almost 2500 years ago? 'Do not do to others what angers you if done to you by others.' Indeed, how would our fanatic 'mutilators' react to our kind suggestion to submit themselves to a similar type of friendly surgery? Human nature was not meant to be simple.

Moreover, there are some 60-80 million infertile couples around the world and 4.4% of all deaths each year are caused by human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS). The world-wide prevalence of reproductive tract malignancies is also impressive: WHO estimates that there are some eight million cases of (female) breast cancer, four million cases of cervical cancer, 3.5 million cases of prostatic cancer, 1.7 million cases of ovarian cancer and 1.5 million cases of cancer of corpus uteri,32.

In 1997, 590 000 children aged under 15 years became infected with HIV, and the annual number of new cases of sexually transmitted diseases exceeded 330million'[4]. Of the 182 million pregnancies occurring every year, an estimated 36% are unplanned and 20% will end in abortion"'. In fact, in 1995, an estimated 26 million legal and 20 million illegal abortions were performed, corresponding to a world-wide abortion rate of 35 per 1000 women aged 15-44 years". The number of cases of preventable severe maternal morbidity exceeds 20 million annually. In 1990, the world-wide maternal mortality was around 430 per 100 000, with an enormous country-to-country variation: 1700 per 100 000 in Afghanistan and 7 per 100 000 in

Spain, and life expectancy at birth for women in 1998 varied between 39 years (Sierra Leone) and 83 years (Japan)".

The importance of reproductive health

How important then is reproductive health[5] Very important: when, in 1994, the International Conference on Population and Development in Cairo assessed available data on reproductive ill health, the Program of Action was subsequently adopted by as many as 178 countries'". In fact, it is so important that seven often of the principal goals set by WHO for the period 1996- 2001 are directly relevant to reproductive health'[9].

THE DEMOGRAPHIC REVOLUTION

How much is too much?

When did the demographic revolution begin? In my view, this happened in 1960 when the global population reached the 'magic' three billion mark, a fact that generated much more heat than light in the international debate. The media talked about an overpopulated world with 'standing room only' and the most celebrated (perhaps even somewhat masochistic?) prophets of'doom and gloom' predicted that the carrying capacity of the Earth (believed to be around four billion people at that time) would soon be reached. This was the time for the frequent preparation of long-term projections of the size of future populations. Based on different assumptions about total fertility rate, the global population was projected to stabilize by the end of the century anywhere between eight and 15 billion people, or even at a much higher level (22-25 billion) according to the worst scenario.

Table3: Percentage of elderly (aged 65 years and over) in the world, in Europe and in Germany during the period 1950-2050. From reference 6

Year	*World*	*Europe*	*Germany*
1950	5.2	8.2	9.7
1975	5.6	11.4	14 8
2000	7.1	14.6	16.4
2025	10.0	20.2	23.4
2050	15.1	25.8	28.4

This was the time when President Kennedy warned that 'unless man halts population growth, population: growth will halt man'. This was the time of enormous Western political pressure on developing countries t' adopt national family planning policies; in 1952, only the Government of India supported such a policy. D the year 1974, 55% and, by 1996, 90% of all developing country governments supported family planning programs". This was the time for setting nation: contraceptive targets for the number of acceptors this, had to be reached. There was a lot of discussion about numbers, rates and target populations, perhaps more than about the fundamental health aspects ofvoluntary contraception.

Aging rapidly and growing rapidly

In 1853, in his journal, Henry David Thoreau remark that 'A man is wise with the wisdom of his time only; and ignorant with its ignorance', and my generation was happily ignorant about the enormous complexity of a new demographic reality in which humankind both growing rapidly and aging rapidly. According the latest figures, it is projected that the world population will reach seven billion in 2013, eight billion in 2028 and nine billion in in 2054. However, the future population structure will be markedly different from that of yesterday or even of today, since the percentage of elderly (aged 65 years and over) will be much higher, as indicated in Table 3.

The data of Table 3 include the most populous member of the European Union, Germany. However, the percentage of the elderly in 2050 will be even higher

Table 4 The largest oldest-old (80 years and over) populations in 1998 and 2050. From reference 6

	1998		*2050*
Country	*Population 1millions)*	*Country*	*Population 1millions)*
China	10.5	China	100
USA	8.6	India	47
India	5.7	USA	27
Japan	4.3	Japan	12
Germany	3.1	Indonesia	10

in several European countries, including Spain (36.9%) and Italy (34.9%)[5].
A special group among the elderly, those aged 80 years and over (called the 'oldest old') is expected to grow at a particularly fast rate: their global number today is 66 million, but, by the year 2050, is projected to increase to 370 million. The fastest increase in this special group will be exhibited by the centenarians; their present number of 100 000 is expected to increase to 2.2 million'. The largest oldest-old populations in 1998 and 2050 are indicated in Table 4.
The United Nations[6] also project that, by the year 2050, there will be 14 countries with more than 10% of the oldest old in their populations, nine of them in Europe: Italy, Spain, Switzerland, Greece, Austria, Germany, the Netherlands, Sweden and Belgium. The highest proportion of oldest old (14%) is projected for Italy. Needless to say, the number of women in these age groups will considerably exceed that of men. How 'considerably'? In 1998, the world-wide number of females per 100 males among those aged 55-59 years was 103; among those aged 80—89 years it was 181, among those aged 90—99 years it was 287 and among the centenarians of the world 388".

The tendency to oversimplification

In his Study of Histore (1946), Arnold Toynbee talks about that tendency to oversimplification which the human mind displays in all its activities. I am convinced that he was right. First we were mesmerized by the populatiom growth, then we became concerned about aging populations; but what about ou children and their relation to the elderly of today? Estimates

Table 5 Percentage of children (aged 15 years and below) in the world, in Europe and in Germany during the period 1950-2050. From reference 6

Year	*World*	*Europe*	*Germany*
1950	34.4	26.2	23.2
1975	36.9	23.7	21.5
2000	30.0	17.6	15.5
2025	24.*3*	15.3	13.3
2050	20.5	16.2	13.4

Table 6 Ratios of children to elderly (calculated from the data of Tables 3 and 5) in the world, in Europe and in Germany during the period 1950-2050

	Children : elderly ratio		
Year	*World*	*Europe*	*Germany*
1950	6.6	3.2	2.4
1975	6.6	2.1	1.5
2000	4.2	1.2	0.9
2025	2 4	0 8	06
2050	1 4	0.6	0.4

aged 15 years and below) in the world, in Europe and in Germany during the period 1950-2050 are presented in Table 5".

The declining trend in the percentage ot children world-wide, and particularly in Europe, is unmistakable. However, the major changes in population structure are perhaps best noted if the ratio of children to elderly are calculated from the data of Tables 3 and 5, as presented in Table 6.

If the medium variant projections of the United Nations materialize, then, by the year 2050, there will be close to two elderly persons tor every child in Europe. In fact, our present global population pyramid is no longer what it used to be; both bottom ends are curtailed because of the substantially reduced number of children aged 5 years and below, as indicated in Figure 1. The dark peak area represents the population aged 80 years and over.

However, by the year 2050, the classical population pyramid of" the world will no longer be classical, not even a pyramid, but rather a barrel-like structure as depicted in Figure 2.

The big perhaps: fertility

What is at the root of all the above changes is an unexpectedly rapid decline in total fertility rate per woman that occurred duringjust a few decades of the second half of the 20th century.

Broadly speaking, these rates can be approximated by the number of children a woman will have in her lifetime. Until recently, it was more or

less generally accepted that 2.1 children per woman corresponded to the replacement level of fertility that would be sufficient to keep a population constant. As ilustrated by data Table 7, total fertility rates declined rather dramatically between 1970 and 1990, a period of just 20 years.
European fertility rates were still above replacement levels in 1970; 20 years later, these figures were approaching the lowest rates in the world. If the UN medium variant projections of fertility rates materialize, substantial geopolitical consequences can be expected: for instance, in 1998,
the population of Europe was 729 million and that of Africa 749 million. On the basis of fertility rate estimates and projections, the UN project that, by the year 2050, the population of Europe will decline to 628 million and that of Africa will increase to 1766 million'[1].

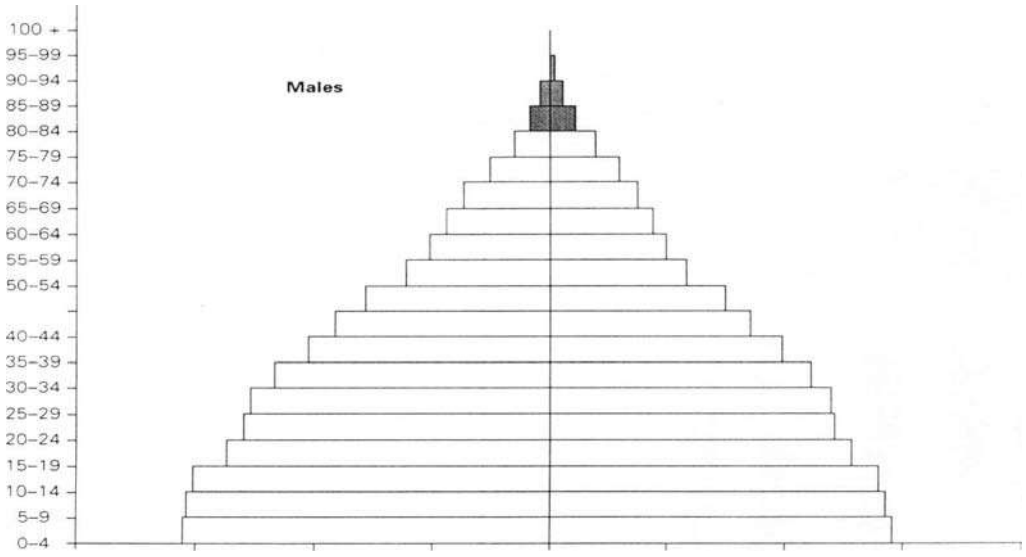

Figure1: The world population pyramid in1998. Shared area at the top indicates the proportion of population aged 80 years and over. From reference 6

Demographic processes are generally believed to be governed by significant biological regularities and behavioral inertia; therefore, the rapidly declining fertility rates shown in Table 7 came as a big surprise. However, nothing would be more erroneous than to assume that we are dealing with a classical manifestation of what the malicious sometimes call Eurosclerosis since declining fertility is a global phenomenon; in fact,

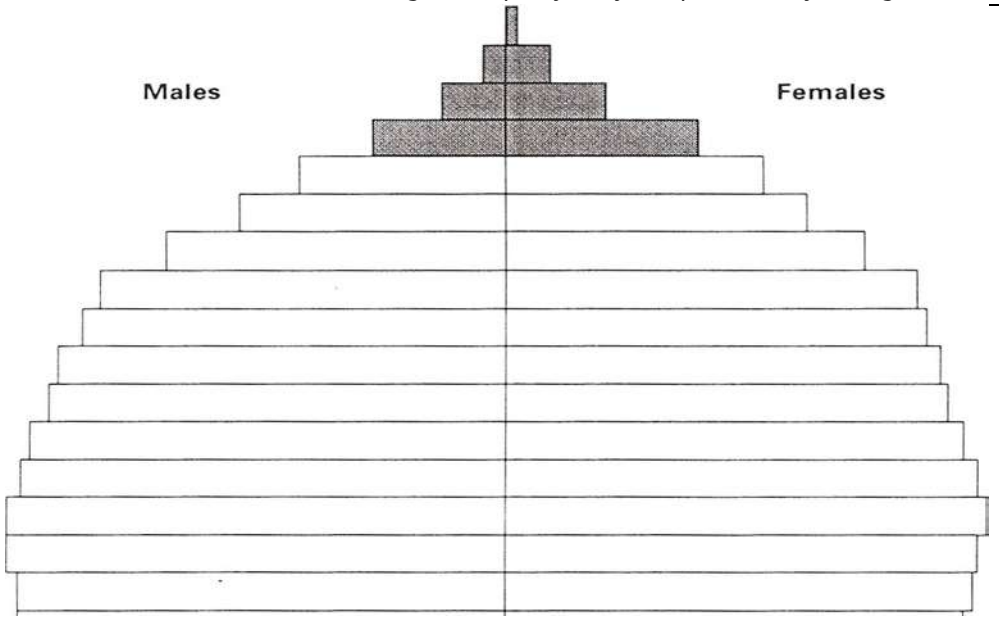

Figure2: the world population pyramid as projected for 2050. Medium variant projection. Shaded area at the top indicates the projected proportion of population aged 80 and over. From reference 6

Europe is in good company. In 1955, only 0.1% of the global population was at or below the replacement level ot fertility; by 1975, the figure increased to 21% and by 1995 to 45%, and it is projected that, by the year 2025, the figure will be 76%M. Hence, the conclusion appears to be inescapable that never before in history have birth rates fallen so far, so fast and so low for so long all around the world, and that the implications of these demographic changes - for good or for ill - are as yet unclear. For the time being, we have no theory to suggest how such low global fertility levels could ever reach replacement levels again .

THE NAME OF THE GAME: INSTITUTIONAL REFORM

Should we reform our forefathers?

Unfortunately (or perhaps fortunately?), we cannot reform our forefathers; however, we could (and should) reform our outworn institution, several of which are the admirable creations of our forefathers, but by now rather petrified and outdated for meeting the exigencies of the new realities.

In fact, most of our institutions are catering for a population structure (with many children and fewelderly) chat does not exist any longer. Any specific

examples? What about our contemporary systems of social security, health and socioeconomic policy, and their financing? What about present approaches to education, housing and marketing? Or even the concept of the nation state? Is it still sacrilegious to think that it is too small to do the big things, but too big to do the important small things? At any rate, it is not too difficult to predict that the greatest impediment to the implementation of greatly needed reforms will be our legendary political inertia. The expression 'too little, too late' is not only a frequently usedused political argument, but it is also possibly our way of life.Only two examples can briefly be discussed here: social security and health care.

Table7 Total fertility rate per woman (replacement level of fertility: 2.1 children per woman) in the world, in Europe and in Germany during the period 1960-90. From reference 6

Year	*World*	*Europe*	*Germany*
1960	495	2.56	249
1970	448	2.14	1.64
1980	358	187	146
1990	296	157	1.30

Social security

In a nutshell, the problem that calls for a reform is the imbalance between the increasing number of beneficiaries and the decreasing number of contributors, owing to the changing population structure. The situation will be much more complicated in those countries where the government has assumed exclusive responsibility for the provision of pensions. The implicit pension debt of some countries already exceeds the gross domestic product: Uruguay, 296%; Hungary, 213%; Brazil, 187%; and the Ukraine, 141%. Not even the most ambitious government believes funds exceeding 200% of the gross domestic product can be collected by increased taxation; hence, much political ingenuity will be required to find a viable long-term solution.

Health care

In 1965, some 52 million human beings died around the world; 34% of these were aged 5 years or less and 19% were aged 75 years and over. WHO

projects that, in the year 2025, some 65 million people will die, only 8% of these being below the age of 5 years and 53% being at or above 75 years of age[33]. Hence, the health trends and disease burden will markedly change during future decades. WHO projects that, by the year 2020, the health trends will be dominated by the aging of the global population, the HIV epidemic, tobacco-related mortality and declining child. WHO also

Table 8 The burden of disease (selected causes, 1998) in high-income and middle-income countries. From reference 35

Number of cases millions)

Disease	*High-income countries*	*Low- and middle-income countries*
Infectious and parasitic	3.0	321.0
Neuropsychiatrie	25.4	134.0
Cardiovascular	19.5	123.5
Neoplasms	17.1	67.7
HIV/AIDS	1.0	70.0

HIV, human immunodeficiency virus: AIDS, acquired immunodeficiency syndrome

projects that, by 2020, the leading causes of disease will be heart disease, depression and road traffic accidents^. In fact, neuropsychiatry diseases today are already the number-two contributor to the disease burden in developed countries and the number-three contributor in developing countries, as illustrated by the data of Table 8[35].

For reasons that are obvious, age-related diseases will also increase and WHO expects a major rise in the prevalence of a number of age-related metabolic alterations such as osteoporosis, atherosclerosis and particularly diabetes, musculoskeletal changes and even so-called 'male problems' (benign prostatic hypertrophy, erectile dysfunction, etc.). There is, however, an important paradigm shift from a mainly biomedical model of healthy aging towards one that includes and emphasizes social and behavioral aspects, recognizing the necessity of paying more attention to those factors

influencing the quality of life of the elderly, such as incontinence, impaired vision, impaired hearing, reduced mobility, falls and oral ill health.
If I were asked to set an agenda for health research for an aginghumankind, my suggestion would be to prevent the preventable and postpone what cannot be prevented with regard to frailty, disability, immobility and dependence. It would be to maintain the social function of the elderly, reduce gender inequalities and socioeconomic differentials and ensure under all circumstances human dignity of the elderly.

A Freudian omission: poverty?

I suddenly realize that 1 have omitted mentioning the most important disease of all afflicting humankind: poverty. Perhaps a subconscious sense of guilt? But is poverty really a disease? Very much so! WHO stated this in no uncertain terms on the occasion of the Cairo conference: 'Poverty is the most widespread, pervasive and intractable disease in the world today'4-1.
Retrospect is a very strange approach to human affairs, maybe even 'unphysiological'. Why? Because, in 1960, in his nomination acceptance speech, President Kennedy said the following: 'The world is very different now. For man holds in his mortal hands the power to abolish all forms of human poverty and all forms of human life'. Fortunately, humankind did not make any use of the power to abolish all forms of human life, but unfortunately, did not use it to abolish poverty either. We agree, that, from the technological point of view, it could be done; never before has humankind had so many resources, so much knowledge and such powerful technologies at its disposal. Hence, the elimination of poverty - like our common future - is an ethical category, because we can choose it ourselves. What might then be the psychological diagnosis of our seemingly (or not only seemingly) hypocritical behavior? As the economist, Professor Galbraith suggested, 'People of privilege will always risk their complete destruction rather than surrender any material part of their advantage"0. Of course, nations also consist ot people and as such are subject to 'Juvenal's law', stated in his Satire No. 14: Crcscit amor nummi quantum ipsa pcaniia crescit (The love of money grows as the money itself grows).
In all fairness, however, it should also be said that our political inertia to eradicate poverty is effectively assisted, not only by the ubiquitous and

destructive violence, but also by Mother Nature creating a seemingly endless chain of natural disasters requiring large-scale international interventions all over the world.

In strict quantitative terms, so much can be said that, in spite of all our international efforts, the number of people living in absolute poverty did not diminish during the past 15 years. According to the estimates and projections prepared by the World Bank in 1992, the number of poor in all developing countries in 1985 was 1051 million, in 1990 it was 1133 million and for the year 2000 it was projected to be 1107[44]. These figures do not include the people living under the poverty line in the former USSR or in the former Yugoslavia and, for reasons that are obvious, the 1992 projections could not take into account the recent impact of devastating civil (and not so civil) wars and natural catastrophes in Africa, Central America and East European countries. Hence, the above figures depict a highly optimistic projection for theyear 2000; therefore, it is more than likely that humankindwill enter the 21st century with more than a billion people (some 20% of the total population) living in absolute poverty.

THE ANTHROPOCENTRIC REVOLUTION

A new world view

Anthropocentric revolution - what a strange name, what does it mean? Let me try to explain. In just a couple of months' time, humankind will enter a new epoch: the 21st century. What kind of a new epoch will that be? Tartarus, Elysian, or a fair (or unfair) mixture of the two? Nobody knows. However, I feel that to every new epoch in history belongs a new spiritual, ethical and philosophical content and that the new world view of the 21st century will be 'anthropocentric'. During hundreds of thousands, it not millions, of years, nature was shaping humankind; today. Homo sapiens is shaping nature and sets the boundaries between the artificial and the natural. Contraception, assisted reproduction and genetic manipulation are just early examples of this trend. These and other new realities have brought with them a new philosophy that deconstructs the deterministic world view and replaces it with a science-driven anthropocentric approach.

What is a deterministic world view? It was very well characterized by Hugo Grotius: 'The law of nature is so unalterable that it cannot be changed by God Himself41. It could equally well have been said by Isaac Newton or even Albert Einstein. The anthropocentric view is superbly characterized by Jurgen Mittelstrass: We are talking our evolution into our own scientific hands in a consistent manner unthought of by earlier societies[46.] To me this sentence indicates the quintessence of the anthropocentric revolution. Does it mean that we consider science omnipotent? Certainly not, on the contrary! ‘Science is a dialogue between mankind and nature, the results of which have been unpredictable', says Ilya Prigogine47. In fact, human knowledge is imperfect and it will remain imperfect forever. However, it is indefinitely perfectible and research is indefinitely perfecting human knowledge. The fundamental problem is something else: will humankind be fit to exercise wisely its enormous power? 'Homo sapiens has, by way of scientific research, appropriated much of the power that rested in the hands of God and the question arises as to whether humanity is sufficiently capable of handling that power in a responsible manner'4*. There is no problem whatsoever with science, the problem is with those irrational elements in human nature. In the words of Mencken, 'Themost costly of all follies is to believe passionately m the palpably not true. It is the chief occupation of mankind'.

How sapiens is Homo sapiens?

Scientific knowledge perse is neither good nor evil - it is just an instrument (ancilla). In the times of Hugo Grotius, in fact over many centuries, science was considered to be exclusively ancilla theologiae, the instrument of theology; today, it is frequently considered as ancilla pecuniae, a money-making instrument, but its real role is, and always will be, to serve as ancilla humanilatis, humankind's instrument to improve the human condition. Here enters wisdom into the picture, which can be defined as the application of scientific knowledge to the benefit ofhumankind. The answer to the question: how sapiens is Homo sapiens, will be of paramount importance torn our common future. It is obvious, however, that wisdom cannot be equated with science and science cannot be equated with scientists, who are human beings exhibiting the entire spectrum of human behavior. Albert

Einstein said that 'Raffiniert ist der Herr Gott, aber boshaft ist er nicht' (God is subtle, but he is not bloody-minded; in Einstein's own translation: God is slick, but he ain't mean)1". Will we need a journey of 1000 miles to get there?

A journey of 1000 miles

Modern science is much criticized these days for its inability to provide simple (so-called yes or no) answers to the complex questions posed by our time. In fact, a depressing number of people show a palpable disenchantment with science - perhaps some of them have even lost faith in science. These people feel that they must 'belong and believe' and therefore they are looking elsewhere for the 'answers' (and promises) that, in their view, science cannot deliver. These are golden times for the various sects spreading the gospels of pseudo-science,para-science and even anti-science4". I think that the political impact of these societal manifestations should not be underestimated; they may even seriously interfere with research funding.

However, scientists should try to view the contemporary history of such pseudo-intellectual movements in a much broader historical context. Two short statements may perhaps serve as starting points for such reflections, the first from Mencken: 'Men become civilized, not in proportion to their willingness to believe, but in proportion to their readiness to doubt'1' and the second from Snow: 'The scientific revolution is the only method by which most people can gain the primal things (years of life, freedom from hunger, survival for children)'1-. At the end of the day, people will understand how science differs from all these movements, provided that scientists incessantly try to educate their fellow men and women. However, never underestimate their wisdom; don't we share - all of us - the infinite wisdom of hope?A final comment: a couple of years ago, I remarked that 'To put it bluntly and in a simplistic fashion, it is rather easy to become a pessimist when reflecting on human behavior and an optimist when reflecting on the progress achieved by humankind'[1] \ However, human behavior is extremely complex and any simplistic philosophy runs the risk masterfully expressed by Jean-Paul Sartre in *Les Mots* (1964): 'Comme tous les songe-creux, je

confondis le désenchantement avec la vérité' (Like all dreamers, I mistook disenchantment for truth). Furthermore, human behavior is not destiny; it can be changed with time and circumstances. Perhaps we need a little bit more time to develop further in this context? As Bertrand Russell said in 1961, 'In a biological sense, Man, the latest of species, is still an infant. No limit can be set to what he may achieve in the future'.

EPILOGUE

In 1970, in an article in Der Spiegel, Bertrand de Jouvenel remarked that 'Year by year we are becoming letter equipped to accomplish the things we are things tor. But what are we actually striving for?' Veil, we - medical scientists - think we know very cell what we are striving for. 'Over a billion people all enter the 21st century', says Dr. Gro Harlem Brundtland, the Director-General ofWHO, 'without having benefited from the health revolution: their lives from main short and scarred by disease'^. Isn't it a worthy bjective to improve their lot? Can't we also fully underwrite another statement by Dr. Brundtland? 'Wecan combat ill-health. We can do our part to combat poverty and suffering. Nothing in life as I see it has more meaning'[34]. Furthermore, we have a tremendous advantage in the nature of our scientific knowledge that is truly cumulative and progressive and can continuously be transferred from one generation of investigators to the next. As Lucius Apuleius (2nd century ad) said *'singillatim mortales, cunctim pcrpctui'* (as individuals we are mortal beings, but together we represent perpetuity).

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Reprinted from: The European Journal of Contraception and Reproductive Health Care; no4 / 1999; 187-201

The demographic revolution

EGON DICZFALUSY

Pure truth hath no man seen
*nor e'er shall know (*Xenophanes of Colophon*)*

Ten non-violent revolutions are considered, characteristic for the second half of the 20th century that profoundly changed our world and our perception of it. Perhaps the most important of those, the demographic revolution, resulted in unprecedented changes both in population size and structure. An attempt is made to analyze the 'big perhaps', some of the incompletely comprehended economical, medical, social, ethical and political consequences of present and projected demographic changes.

The future of futurology

There are two types of futurologists; those who cannot predict the future and those who know they cannot predict the future, I belong to the latter category. How then can we consider our common future? Well following Gabriel Garcia Marqu6z: Como no es posible predecir el futuro, Hay que inventarlo; since it is not possible to predict the future, it has to be invented. The question is: how?

There is a Latin proverb from the 16th century, tempora mutantur et nos mutamur in illis, times change and we change with them too1, and few of us would argue with it. The problem is, however, the rate of change. The changes around us are accelerating, whereas our perception of these changes and of their consequences is lagging behind. As the World Commission on Environment and Development stated: 'The rate of change is outstripping the ability of scientific disciplines and our current capabilities to assess and advice'2. In times of such rapid changes it becomes important to have a vision of the future, but I suspect that only those with some knowledge of the past can have a vision of the future. Not everyone agrees with this view, Napoleon3 felt that only 'the stupid speak of the past, the wise of the present

and fools of the future (a fine example of 'imperial arrogance'?). However, since both wisdom and foolishness are accepted constituents of human nature, let us consider briefly the past before embarking on a discussion of our common future

Chiaroscuro for the privileged

Es bueno vivir muchopara ver mucho (It is good to live long in order to see much) says Sancho Panza in Don Quixote4 and I have seen most of the history of the 20th century. When I was born, the population of the world was less than 2 billion people, in 1999, it will exceed 6 billion. Hence, in my lifetime, I have seen the birth of another two worlds, equal in numbers, needs, aspirations, hopes and dreams. I have seen much, perhaps even too much... Schopenhauer remarks that 'the man who sees two or three generations is like someone who sits in a conjurer's booth at a fair, and sees the tricks two or three times; they are meant to be seen only once'5. I have seen the tricks of the 'political magicians' of three generations. I have seen their heavy flirtation with the apocalypse under the shadow of the hydrogen bomb and I have seen them exercising two powerful determinants of human destiny, the arrogance of power and the arrogance of ignorance. Henry Kissinger said in an interview in 1975: 'intelligence is not all that important in the exercise of power, and is often, in point of fact useless'. Sancho Panza continues El que larga vida vive mucho mal ha dapassar (those who live long also pass through much evil) and I have seen a fair (or unfair?) amount of it; I have lived in a century, in which some 200 million human beings were killed by other human beings, on orders of 'political magicians'. Like Winston, the hero of George Orwell6, I also understood HOW and didn't understand WHY. Like so many of my contemporaries, I have seen plenty of other dark aspects of what we call 'human nature', be it aggression, barbarity, terror, cruelty, hypocrisy, naked cynicism, moral and intellectual corruption and the worst of all, indifference.

However, my world view - like that of Alexander Pope - perhaps best reflects Rembrandt's perception of the chiaroscuro. Just think for a moment of his Nightwatch, painted in 1642. I wonder sometimes, whether Alexander Pope ever knew of it? Chronologically, it would not seem to be impossible. Pope's magnum opus An Essay on Man' was written around 1734. Anyhow,

the central piece of Pope's philosophy, 'there must be shadows, if there should be light' appeals to me very much, since I always felt that I have seen an incredible amount of light! In fact, I belong to the most privileged generation of human beings that ever existed on this Earth! Why? Because in my lifetime I have seen more progress in science than all scientists of all preceding periods together, since the dawn of history. This should not be too surprising to my fellow-scientists. As Friedrich Engels stated: 'science progresses in proportion to the mass of knowledge that is left to it by preceding generations, that is under the most ordinary circumstances in geometrical proportion'7.

Ten revolutions

The word revolution has several meanings, it can be used to describe any movement round an axis, the movement of certain celestial bodies, or even the complete overthrow of a government, but the term can also be used to describe a 'great change in affairs, or things'. Such 'great changes' are frequently associated with fundamental changes in our perceptions, and I am using the term in this context.

I am convinced that our generation has been a most privileged one, since it not only witnessed, but also participated in at least ten powerful revolutions that have profoundly changed our world and our world view. Others may prefer a different 'menu'; like most things in life, our 'favorite' revolution can be a matter of taste and de gustibus non est disputandum, ooe should never discuss taste.

These revolutions either did not exist, or only had a very limited impact prior to the second half of the 20th century. In fact, this period represents a continuous chain of events and achievements usually described as the 'first time in history'. Thus, our time was - according to Arnold Toynbee - 'the first age since the dawn of civilization in which people have dared to think it practicable to make the benefits of civilization available to the whole human race'8. The 20th century was the first time in history whenhumankind dared to establish a United Nations (UN) with its important specialized agencies (like the World Health Organization) and hundreds of international professional and academic associations (like the Academia Europaea). This was the first time in history, when global identity became a reality rather

than a philosophical concept, resulting eventually in the environmental revolution. This was the time, when the worldwide success of the contraceptive revolution - with some 900 million contraceptive users at present - inevitably ushered into existence the revolution in reproductive health, which, in turn, gave rise to another major intellectual-technical revolution, that in gender equality.
However, without considering the most powerful of all of them, the demographic revolution (with its unprecedented changes in the size and the structure of human populations), it is hardly possible to assess the human Condition of today.

A new departure, 1960

The global population reached the 'magic' 3 billion mark in 1960, generating more heat than light; a worldwide concern and, needless to say, lot of disagreements seasoning the emotional international debate. The media talked about an overpopulated world with 'standing room only' and it was predicted that the carrying capacity of the world (believed to be around 4 billion people at that time) would soon be reached. This was the time for the frequent preparation of long-term projections of the size of future populations. Based on different assumptions about total fertility rate, the global population was projected to stabilize by the end of the century anywhere between 8 and 15 billion people, or even at a much higher level according to the "worst case scenario'. This was the time when President Kennedy warned that 'unless man halts population growth, population growth will halt man'. This was also the time of bitter controversies in the United Nations General Assembly between the Scandinavian countries, spearheaded by Sweden, and the Latin American proxies of the Vatican, led by Argentina, as to whether the Specialized Agencies of the United Nations should be permitted to give advice - upon request - to Member States on population issues. Finally, the ice was broken in 1962 with the UN resolution 18.38, which provided the necessary authorization 9.
Three years later, in 1965, the World Health Assembly passed a resolution WHA18.49, requesting the Director-General to establish a Programme in the area of Human Reproduction; it was accomplished the same year9. Pari passu with the awakening of the international community, a rapidly

increasing number of national governments decided to provide support IB family planning programmes. It was the Government of India that - for the first time in 1 - established a national family programme in 1952. This was soon followed by other, so that by 1974, 55% of the governments of the world provided family planning care and, by 1991, 96% of all governments of the world did so10. Whereas the initial rationale was undoubtedly a demographic concern, by the early 1980s the majority of developing country governments underlined that their rationale for supporting family planning was reproductive health and human rights. By 1990, worldwide contraceptive prevalence was 57%, it was highest (79%) in Eastern Asia and lowest (18%) in Africa".

Standing room only?

In the 'Study of History', Arnold Toynbee talks about' ... that tendency to over-simplification which the human mind displays in all its activities'12 and - in retrospect - our approach to population issues was no exception. Quite a few governments were taken by surprise to learn that population growth cannot be manipulated without markedly altering population structure as well. Concern about ageing populationsbecame a favorite topic for the international debate in the late 1980s. However, led again by the principle of over-simplification, it was believed initially that it would only affect developed countries. It is recognized today that we are dealing with a global phenomenon. However, before discussing numbers, rates and percentages, it may be useful to consider briefly another manifestation of our beloved over-simplification, the dilemma of unjustified generalizations.

The limits of generalization

Erasmus in his Encomium moriae (1509) had emphasized 'the urge of the human mind for analogy and generalization' and scientific progress would be very slow indeed without some generalizations. Just like many of our theories, generalizations are rarely true, or false; rather, they are more or less useful. In fact, some generalizations can be very fruitful, irrespective of whether or not they are 'true', provided that their limitations are borne in mind. Hence, the term 'developing countries' represents a very useful generalization, provided it is recognized that developing countries differ

from each other at least as much as they differ from developed countries. In fact, all of them are unique in certain aspects. Montaigne said in Les essais (1580) 'The most universal quality is diversity'. Even the generalization that I have employed, that 'Humankind is growing rapidly and ageing rapidly' is a useful one, provided we remember the exceptions and marked differences that exist between various regions. For instance, the population of Europe is declining and the population of Africa shows little, if any ageing, at least not yet.

The tyranny of numbers; elderly populations

Humankind is ageing rapidly; but how rapidly? The UN estimates and medium variant projections of the percentage of elderly (aged 65 and over) in the world, in Europe and in Italy (with the probably most rapidly ageing population in the world) are indicated in Table 1 In 1950, 5.2% of the global population and 8.2% of that of Europe was elderly; today 7% of the world population and 14.6% of the European population is elderly. It is projected that, by the year 2050, more than 15% of the world population will be elderly, and for Europe and for Italy 25.8% and 35.7%, respectively13. The population of many developing countries is

Table 1. Percentage of the elderly (aged 65 years and over)
Estimates and medium-variant projections

Year	World	Europe	Italy
1950	5.2	8.2	8.3
1975	5.6	11.4	12.0
2000	7.1	14.6	17.7
2025	10.0	20.2	25.6
2050	15.1	25.8	35.7

Source: World Population Prospects: The 1996 Revision (Ref: 13)

ageing very rapidly. Whereas it took Sweden 84 years to double its elderly population (from 7 to 14%), in China it took only 30 years, and the Republic of Korea only 18 years. The reasons for the rapidpopulation ageing are multifactorial; one of them is the increase in life expectancy at birth. As an example, in Europe in 1950 it was 68.5 years for women and 63.4 years for

men, (gender difference 5.1 years). Today, it is 77.3 years for women and 69.3 years for men, with a difference of 8.0 years and, by 2050, it is projected to be 83.3 years for women and 77.1 years for men. However, life expectancy is increasing at all ages, including even the 'oldest old'. The fastest growth is exhibited by the centenarians, their number in the world is expected to double in every decade14. In 1950, there were only 200 people aged 100 years and over in France. By the year 2000, their number is expected to rise to 8500, and by 2050 it is projected to reach 150 000 - a 750-fold increase in 100 years 10.

Fernando Pessoa says that 'We can only see what we have already seen'; perhaps so. A rapidly growing elderly population represents an unprecedented and fundamentally new feature in our history, no wonder, that few governments are prepared - as yet - to grasp the likely economical, medical, social, ethical and political consequences

The elderly of tomorrow: our children

A 'brave new world' with 15, or 20% elderly (the latter in case the low-variant projection of the UN materialises11) will certainly represent a fundamentally new demographic reality; however, the challenge will be compounded by the fact that the increasing number of elderly will be associated with a decreasing number of children. Estimates and medium-variant projections of the percentage of children (aged 15 years or less) in the world, in Europe and in Italy, are presented in Table 213.

In 1975, the worldwide percentage of children was close to 37%; in Europe it was somewhat less than 24%. By the year 2000, these figures will decline to 30% and 17.6%, respectively and it is projected that, by 2050, the global figure will decline to 20.5% and the Italian figure to 12.4%! From the data of Tables 2 and 3, a ratio of children to the elderly can be calculated, as shown in Table 4.

The name of the game: fertility rates

The major determinant behind the changes indicated above, is the total fertility rate per woman. Broadly speaking these rates can be approximated by the number of children a woman will have in her lifetime. Until recently it was generally accepted that 2.1 children per woman corresponded to the

Table 2. Percentage of children (aged 15 years or less)

Year	World	Europe	Italy
1950	34.4	26.2	26.3
1975	36.9	23.7	24.2
2000	30.0	17.6	14.2
2025	24.3	15.3	11.4
2050	20.5	16.2	12.4

Source: World Population Prospects: The 1996 Revision (Ref. 13)

Table 3. Ratio of children to the elderly

Year	World	Europe	Italy
1950	6.6	3.2	3.2
1975	6.6	2.1	2.0
2000	4.2	1.2	0.8
2025	2.4	0.8	0.4
2050	1.4	0.6	0.3

In 1950, this ratio was 6.6 worldwide and 3.2 in Europe; it is projected that, by 2050, the global ratio will be 1.4, the European ratio 0.6, and the Italian one only 0.3. If this projection does materialize, then in 2050 there may be three elderly people, or even more for every child in Italy.

Table 4. Total fertility rate per woman (Replacement level: 2.1 children per woman)

Year	World	Europe	Italy
1975	4.48	2.14	2.28
1980	3.92	1.97	1.92
1985	3.58	1.87	1.55
1990	3.36	1.83	1.35
1995	2.96	1.57	1.24

Source: World Population Prospects: The 1996 Revision (Ref.: 13).

replacement level of fertility. Since population size is increasing not only by births, but by survival at old age as well, it is possible that the true replacement level of fertility in the future might be somewhat less. As illustrated by the data of Table 4, total fertility rates declined rather dramatically between 1975 and 1995, just in a period of 20 years.

European and Italian fertility rates were still above replacement level in 1975; 20 years later these figures were much below replacement level, the Italian figures being among the lowest in the world. Demographic processes are believed to be characterized by significant biological regularities and behavioral inertia, thus the rapidly declining fertility rates shown above came as a surprise. The impression of a really rapid rate of change is further strengthened by an inspection of subsequent revisions by the UN of the projections of future fertility rates. The medium variant projection for Italy in the year 2000 (1.27) - published in 1994 - is lower than the low variant projection (1.54) published in 1984 and the medium variant projection (1.19) published just 2 years later, in 1996, is lower than the low projection (1.22) from 1994. Tempora mutantur... However, nothing would be more erroneous than to assume that we are dealing with a manifestation of what the malicious sometimes calls Eurosclerosis, since the declining fertility is a global phenomenon. In 1955, only 0.1% of the global population was at or below replacement level of fertility, by 1975, the figure increased to 21.0% and by 1995, to 45%, and it is projected that by the year 2025, the figure will be 76%10. Some regions, for example Africa (3.28), or Pakistan (2.59) are projected to have fertility rates higher than replacement level in 2025, but no longer in 2050. Hence, the conclusion appears to be inescapable that never before in history have birth-rates fallen so far, so fast, and so low for so long all around the world and that the implications of these demographic changes - for good and for ill - are as yet unclear. For the time being, we have no theory to suggest how such low global fertility levels could ever reach replacement levels again. A humble advice to governments: beware of simplistic solutions, like generous family allowances, in our very complex world, they may, or may not have any impact. What is urgently needed, is then more research!

Vladimir Nabokov says that 'what can be controlled is never completely real, what is real can never be completely controlled'. The demographic changes of our time are certainly real, humankind is growing older.

Can we afford longevity?

A few years ago, I read an official report containing a somewhat provocative question: longevity has been our quest for ages; now that we

have found it, can we afford it?'15. Perhaps the question is not only provocative, but even a bit silly, for what is the alternative? Like our masochistic prophets of gloom anddoom, the report seemed to be better in depicting, a hopeless future rather than in suggesting constructive solutions. Of course we can afford longevity! Only a few countries spend more than 10% of their GDP on health today, perhaps not Africa, but the world can afford spending more! What is perhaps more important, is to remember what Edmund Burke said 200 years ago: 'You can never plan the future by the past,16. Why not? Because - as the poet says' ... life goes not backward nor tarries with yesterday,17. Why is it then so difficult for certain governments to grasp the conclusions of the World Commission on Environment and Development?' It is frustrating the attempts of political and economic institutions, which evolved in a different, more fragmented world, to adapt and cope'2. What is urgently required, is the introduction of institutional reforms and to demythologize ageing.

Should we reform our forefathers?

How could we? We can't even reform our contemporaries. However, we could (and should) reform our contemporary institutions, several of which we have inherited from our forefathers. Most of our institutions are catering for a population structure (with many children and few elderly) that does not exist any longer. Any specific examples? What about social security, health and socio-economic policy and the financing of such services? What about our housing and educational systems? What about inter-generational conflict, a favorite topic of our prophets of doom and gloom, is it a myth, or reality? Our current financial and commercial routines in a new global reality would also benefit from a fresh critical look. In fact, there is a need for a variety of institutional reforms. However, it is not too difficult to predict that the greatest impediment to the implementation of greatly needed reforms will be our political inertia. Rapid adjustment to unexpected and/or inevitable changes is not the greatest virtue of contemporary societies. The expression 'too little, too late' is not only a frequently used political argument, maybe it is our way of life.

Several of our present institutions were the admirable creations of our forefathers, like the pension systems originally introduced by Bismarck

around 1880 and rapidly adopted by other countries. However, the increasing number of beneficiaries accompanied by a decreasing number of contributors will make a reform in the pension systems of several countries inevitable. The situation will be much more complicated in those countries, where the government has assumed exclusive responsibility for the provision of pension. The implicit pension debt of selected countries in this category is indicated in Table 518

Not even the most ambitious government believes that they can collect funds exceeding 200% of the GDP by increased taxation. However, sooner, or later the day must come when a solution will be found. But how?

Demythologizing ageing

Survival into older age is not only a challenge; it is perhaps the greatest achievement of the 20th century. However, 'increased longevity without quality of life is an empty prize' says Gro Harlem Brundtland, the Director-General of WHO - and continues: 'And ageing is to be celebrated. But in order to make it worth celebrating we' need to ensure quality of life as we age. This will be the greatest challenge for public health in the next century and the contribution of the World Health Organization will be indispensable'19. In fact, there are already some positive developments. In Europe, longevity is increasing more rapidly than the cost of health care, disability rates in the USA and in Germany are declining and according to Ursula.

Table 5. Implicit pension debt as a percentage of gross domestic product (selected countries)

Uruguay:	296
Hungary:	213
Brazil:	187
Ukraine:	141
Turkey:	72
China:	63
Peru:	37

Source: World Bank. World Devel-opment Report 1997 (Ref. 18).

Lehr - 79% of men and 74% of women aged 80 and over can still manage their daily life in Germany20.

At the threshold of the 21st century it has become obvious to an increasing number of governments that a variety of medical problems related to ageing can only be solved by research; however, they should also recognize that a quantum leap in research support will be required in order to prevent the preventable and postpone the inevitable. The fundamental research issue - with major implications for our national economies - will be to find cost-effective ways of preventing or postponing disability, immobility and dependence. A quantum leap in socio-economic research will also be needed to ensure dignity and maintain social function in diverse cultural and economic settings. In his Satire No. 11, Juvenal remarked that Morte magis metuenda senectus\ old age is more to be feared than death. Is this still the correct description of our inevitable destiny? It used to be, but does not need to be any longer today, when the conditio humana is rapidly changing and we can intervene more and more in these changes - including our own biological foundations - thanks to progress in science!

The limits of science

In 1963, de Solla Price remarked that 'Using any reasonable definition of a scientist, we can say that between 80 and 90 per cent of all the scientists that have ever lived are alive now. Now, depending on what one measures and how, the crude size of science in manpower or in publications tends to double within a period of 10 to 15 years.'21 Impressive, isn't it? Yes, except, that today it is believed that the amount of new scientific information doubles every 5-6 years! It seems rather safe to predict that in the 21st century medical science will make tremendous progress in relieving suffering and improving the human condition in general, and the condition of the frail and disabled elderly, in particular. Do we have then any limits at all to what science can achieve in the future? Of course, we have. 'One thing I have learned in along life' - said Einstein' is that all our science, measured against reality, is primitive and childlike - and yet it is the most precious thing we have'22. We should admit - willingly and with humility - that human knowledge is imperfect and that it will be imperfect forever. However, it is indefinitely perfectible!

What is then the main limiting factor to scientific progress? It seems to be extrinsic, rather than intrinsic, it consists of the political determinants of the

conditio humana\ the same determinants, which - under different conditions - can also provide a most powerful stimulus for rapid progress. Is this really so strange? Not more than human nature itself... In his Nomination Acceptance Speech, in 1960, Kennedy said: 'The world is very different now. For man holds in his mortal hands the power to abolish all forms of human poverty and all forms of human life'. Fortunately, during the past 40 years, or so, Homo Sapiens did not make any use of the power to abolish all forms of human life, but unfortunately, did not use it to abolish poverty either. In 1998, just as in 1960, poverty remains the root of a vast number of ethical challenges in the care of the elderly. As Callahan stated 'Given the rising proportions of the elderly in the population and the increasingly effective and expensive technologies that will be applicable to them, we are at the edge of a new and endless frontier of ethical inquiry'23.

With the advent of the 21st century, ethical issues are also likely to assume a crucial role in many other scientific disciplines. With the exception of the large number of scientists employed by the armament industries, most of us - at the other side of the fence - would wholeheartedly support the views expressed by Einstein 'The creations of our mind shall be a blessing and not a curse to mankind'24, but how could this be ensured in the brave new world of the 21st century? I feel that Stig Stromholm identified the real issue - power - by reminding us that 'Scientific research today, as distinct from most research work in the past, means the wielding of more or less power over one's fellow men'25, and one cannot help wondering together with Juvenal (Satire No. 10): Quis custodiet ipsos custodesl, who will control the controllers? Should it be again the political establishment, with its somewhat less than perfect track-record? Could not man develop a different system that would enable the institutions of the international scientific community to be an integral part of the necessary control mechanisms? Upon further reflection, I find even this issue much more complex, since science is also subject to the inherent uncertainty of the human condition and, as Ilya Prigogine26 formulated it, 'science is a dialogue between mankind and nature, the results of which have been unpredictable'.

Probably this is the most difficult issue we have to consider in the present context. Can our poets help? 'The only wisdom we can hope to acquire is the wisdom of humility' says T. S. Eliot27, whereas Kahlil Gibran states that '...

in truth we are neither wise nor foolish. We are green leaves upon the tree of life, and life itself is beyond wisdom and surely beyond foolishness'28. Beautiful poetry, but where does it lead us in our world today, with some 30 million refugees? Really, what is then the problem with us? Maybe that identified by Henri Bergson: 'Homo sapiens, the only creature endowed with reason, is also the only creature to pin its existence on things unreasonable'29. Henry Mencken's 'diagnosis' reflects post-World War II. sentiments: 'The most costly of all follies is to believe passionately in the palpably not true. It is the chief occupation of mankind'30. Surprisingly enough, Bertrand Russell presents the problem in almost brutal language, perhaps because it is written in 1951: '... the human race becomes divided into rival groups of fanatics, each group firmly persuaded that its own brand of nonsense is sacred truth, while the other side's is damnable heresy'31.
Primo Levi defines the major challenge confronting Homo Sapiens as 'the bestial vice of hatred' 32.
All this is true and the opposite is also true. In their ambition to extract the quintessence of the chiaroscuro of human nature, these philosophies have managed to eliminate the chiaro, leaving us only with the oscuro. Post tenebras lux, darkness will be followed by light, is one of the tenets of the Western philosophy; but where is that light? Sure enough, human nature may be bad, but not indefinitely and exclusively so! 'Men are cruel, but Man is kind' says Rabindranath Tagore33 and a philosophy ignoring human generosity, genuine goodwill and the profound commitment to improve the human condition is somewhat naive - to say the least - and it is certainly unsuitable to assist us in meeting the challenges of the 21st century. Why do we not try something more productive and visionary? What about our bitterly criticized, toothless, hopelessly politicized and deeply divided international organizations? All of us have been greatly disappointed with them, at least on certain occasions and there are few, if any, among the almost 200 member states, that do not wish to reform them. To reform them, yes, but to abolish them, certainly not! I have never seen such a suggestion. Why not? Because it is generally recognized that they are greatly needed, indeed, they have come to stay. In a historical perspective, the UN with its 50 years of existence is still a new-born, with a bright future. 'A journey of thousand miles must begin with a single step', says a Chinese proverb and -

for the first time in its history - humankind possesses a network of international instruments with an enormous potential for a structured coexistence of all governments of this world. Why do we not give them more time to further develop and improve? And the long-term perspective? It is even more encouraging. It was formulated by an ageing Bertrand Russell 'In a biological sense, Man, the latest of species, is still an infant. No limit can be set to what he may achieve in the future'34. And that future belongs to those scientists, who believe in the beauty of their dreams.

That journey of thousand miles

In just one more year and we will enter the third millennium. I am convinced that it will be celebrated with all kinds of manifestations, such as lots of fireworks and other useful investments into our common future. However, not even the skeptics can deny that the turn of the millennium will be a historical milestone for humankind in its start of a new journey of thousands and thousands of miles.

What will man carry with him for this new journey from the 20th century? An incredible amount of scientific and technological knowledge together with a vast amount of powerful resources never possessed before since the dawn of human history. Alas, humankind will also carry a number of old and new diseases. Let me mention two of those, since they are rarely, if ever, considered in our textbooks of pathology; poverty and the so-called Don Quixote disease. Is poverty really a disease? 'It is the most widespread, pervasive and intractable disease in the world today', says the World Health Organization35. But what is the Don Quixote disease? The term was coined by Feodor Dostoevsky, to describe 'a passionate search for reality'. Since 'human kind cannot bear very much reality'36, we try many approaches to it, not only the scientific, but also religious, cultural, ethical, economical, ecological, socio-critical, philosophical and, of course, the political approach 37. Although any and all of these approaches can infinitely enrich human existence, their material contribution to the improvement of the human condition is not equal, and 'the scientific revolution is the only method by which people can gain the primal things (years of life, freedom from hunger, survival for children)'38. I hope therefore, that Homo Sapiens will also carry with them into the third millennium a more universal

appreciation of the - as yet incompletely comprehended - potential of science for improving the quality of life of the entire human race.

A new worldview

Finally, man will also bring with him into our common future a new - rather anthropocentric worldview, a philosophy that deconstructs the 'old' deterministic worldview, but also rejects the 'modern' view of the 'prophets of gloom and doom' based on the randomness, futility and absurdity of human existence in an arbitrary universe of pure chance. Man will carry with him a new science - the science of Prigogine - which finally reflects the complexity of the real world, 'a new science which considers us and our creativity as part of a fundamental movement at all levels of nature'26. Indeed, a new science is making its entry into a new world, the world of tomorrow, a science that 'tries ceaselessly to extend the frontiers of the knowable and the valuable, to transcend the givenness of things, to imagine a new and better world' 39

The three last words, or perhaps even four?

There is a new generation of young scientists around us and since their souls are dwelling in the palace of tomorrow rather than in our old-fashioned house of yesterday, they may much prefer another, dry, strict and to the point approach to scientific presentations rather than the present one, 'stuffed with boring classical quotations'. They may like more a formal, logical approach, like that of Ludwig Wittgenstein (indeed, there is not a single quotation in his Tractatus logico-philosophicus). However, it is perhaps a slight surprise, that even the Tractatus starts with a motto, a motto from Kiirnberger; '... und alles, was man weiss, nicht bloss rauschen und brausen gehört hat, lässt sich in drei Worten sagen'. Whatever a man knows, whatever is not mere rumbling and roaring that he has heard, can be said in three words.40. If this is so, then what should be the three word summary of this paper? Although I really tried, it proved to be too difficult for me to arrive at a three word summary. However, I think I can manage with four words, that might perhaps also serve as a tentative and humble recommendation for our common future: Science, Dignity, Tolerance and Hope. In my view, what will be needed by Homo Sapiens in the next

century, is the universal acceptance of the scientific approach to solve problems in a rational fashion, together with the universal recognition of human dignity and the universal acceptance of the principle of tolerance in international, inter-racial and inter-cultural relations. And the last one? It is perhaps the most essential for every human being, the infinite wisdom of hope.

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Reprinted from: European Review, Vol. 7; No. 2, 263-276 (1999)

Voyage into our common future: from futurophobia to futurophilia

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This paper was presented at the Plenary Opening Lecture at the 2nd World Congress on Aging Male, Geneva, February 2000

"L'optimiste et le pessimiste ne s'opposent que sur ce qui n'est pas."
Paul Valéry

THE FUTURE OF THE PAST

The motto of this paper from Paul Valéry under¬lines the most fundamental element of the human condition: uncertainty, the particular Leitmotiv of our time. However, Valéry further challenges us by asking: 'Are you not the future of all memories stored within you? The future of the past". Hence, all of us represent the future of the past, but with markedly different perspectives. As an Arabian proverb says: 'When a man is young he writes songs; grown up, he speaks in proverbs; in old age he preaches pessimism'. I will certainly not do that; I am not a pessimist. I am not an optimist either; I am simply confused. However, to be confused today is something rather respectable; 1 have heard it said that if you are not confused in these modern times, you are not well informed.

I am confused because the changes around us are increasing in magnitude and are accelerating, but our perception of these changes and of their likely consequences is lagging more and more behind. As I see it, 'The wind of new realities is blowing with increasing strength. It is up to us to decide whether we prefer protective windscreens or new types of windmills'2. However, to pursue either of these two options requires a vision of the future, that strange melange of rational projections and instinctive perceptions, and I am convinced that only those with some knowledge of the past can have a vision of the future. Why? Because, in a certain sense — as

William Faulkner said - the past is never dead; it is not even past. Whether we like it or not, we have to live with the consequences of that past.

WHEN WILL THE PAST DIE?

It is estimated that when our modern chronology started, exactly 2000 years ago, the global population was around 300 million people, a number that did not increase during the first millennium3. It appears from the data of Table 1 that the first 1000 million was reached only in 1804 and the second in 1927[4].

Table 1: Growth of the world population during the past two millenia. From reference 4

Year	Word population (millions)
0	300
1000	310
1500	500
1804	1000
1927	2000
1960	3000
1974	4000
1987	5000
1999	6000

When I was born, in 1920, the world population was 1860 million people. Last year, we passed the 6000 million mark. Hence, in my lifetime, I have seen the birth of another two worlds, equal in numbers, needs, aspirations, hopes and dreams. 'El que larga viva vive mucho mal ha de pasar' (Those who live long also pass through much evil), says Cervantes in Don Quijote5 and 1 have seen a fair (or rather unfair) amount of it. I have seen the devastating consequences of two world wars, the dangerous flirtation with apocalypse by the superpowers of the time and the most enthusiastic, almost religious, embrace by a vast number of people of pseudointellectual ideologies, exposing millions and millions of fellow men and women to incredible cruelty, moral horrors, indescribable suffering and prema¬ture death.

I have been around for the major part (80%) of the 20th century, a historical period in which more than 200 million human beings were killed by so-called human beings, on the orders of political visionaries, the architects of 'modern ideologies'. Around 37 million deaths occurred in wars; some 7 million in civil wars and 30 million in international wars, but approximately 170 million people were killed by governments, such as those of the Soviet Union (62 million), China (45 million), Germany (21 million) and Japan (8 million)6.

Ludwig Wittgenstein says that from time to time certain words have to be purified and cleaned before they can be used again. Do we feel that Homo sapiens is one of those words? However, before we ascribe a major historical role to our distinguished dry-cleaners, let us also reflect for a moment on some real achievements by Homo sapiens in the 20th century. In fact, I have witnessed a dozen revolutions that fundamentally changed our world and our world-view. To a large extent, they represent rather recent developments taking place in the second half of the 20th century, after humankind had awakened from the nightmare of the Second World War. They are shown in Table 2.

REVOLUTIONS DO NOT GO BACKWARD

According to George Seldes7, Abraham Lincoln, in a speech on 19 May 1856, said 'Be not deceived. Revolutions do not go backward'. Although he

Table 2 A dozen revolutions

Scientific Technological
Information
Post-industrial
Global identity
Rising expectations
Environmental
Contraceptive
Reproductive health
Gender equity
Anthropocentric
Demographic

may have had a more restrictive interpretation of the word 'revolution' than I do, I strongly believe that his assessment can be extended to all the revolutions indicated in Table 2, first and foremost, the scientific revolution. In my lifetime I have seen more progress in science and technology than that achieved by all scientists of all preceding periods since the dawn of history. This is easy to understand in a world where the amount of new information doubles every 6—8 years. A far-reaching consequence of the scientific and technological revolutions is represented by the information and post-industrial revolutions; it is hard to deny that they have already changed our world, although we have only seen the very beginning of these changes. It is also easy to see how the information revolution ushered into existence the revolution of rising expectations.

The revolution in global identity is also a product of our time, which — for the first time in history - brought a holistic view of the world and led to the establishment of a large variety of international organizations, the most important and comprehensive being the United Nations (UN), with its numerous specialized agencies such as the World Health Organization, the High Commissioner for Refugees, the World Bank, etc. Indeed, as Arnold Toynbee remarked, our time is 'the first age since the dawn of civilization in which people have dared to think it practicable to make the benefits of civilization available to the whole human race'[8]. On the other hand, the fashionable concept of globalization is a rather broad and confusing one, encompassing anything from the transfer of science and technology to developing countries to monopolistic market dominance and exploitation by transnational corporations of uncertain power structure, and more often than not seems to entice confrontational interpretations.

The environmental revolution is also a recent departure in the history of humankind, with the recognition that the waste-absorbing capacity of Mother Nature is finite and that environmental degradation cannot be isolated and restricted within narrow national boundaries. Was it 2400 years ago that Isocrates advised Nicocles, the king of Cyprian Salamis: 'Do not do to others what angers you if done to you by others?' If any enlightened governments of our time would also be students of Isocrates, they seem to be 'slow learners', to use the terminology of Charles Darwin. The

contraceptive revolution was among the very first of our time that inevitably triggered off the revolutions in reproductive health and gender equity. I have covered these three revolutions in several recent reviews[9-11] and will not discuss them here. The same applies to the anthropocentric revolution[11,12]. The present paper will focus on the demographic revolution and some of its anticipated consequences, following up two previous reviews published in this journal13'14.

THE DEMOGRAPHIC REVOLUTION

When did the demographic revolution start? I would suggest that it happened in 1960. This was the year when humankind reached and passed the 3000 million mark (Table 3), causing a lot of anxiety and heated emotional debate, with the usual mixture of extreme views as to the future size of the global population and the carrying capacity of the Earth.
The media told us that there would soon be 'standing room only' and the celebrated prophets of gloom and doom of the time assured us that the

Table 3 The global population between 1950 and 1990.From reference 4

Year	Global population (milions)
1950	2521
1960	3022
1970	3697
1980	4440
1990	5266

Earth could not possibly support more than 5000 million people. Even President John F. Kennedy sounded alarmist: 'Unless man halts population growth, population growth will halt man'. An incredible amount of pressure was put on the governments of the developing countries by the Western world to adopt and support family planning programs. The first national family planning program was instituted by India in 1952 and was soon followed by many developing countries. By 1996, some 90% of all developing countries supported family planning4. However - and in all fairness - by this time, the overwhelming majority of developing country governments stated that their rationale for providing support to family

planning programs was human rights and reproductive health, rather than demographic considerations.

GROWING RAPIDLY AND AGING RAPIDLY

'Old age is the most unexpected of all the things that happen to a man', wrote Leon Trotsky in his Diary in Exile in 1935, but what should one say when it happens to the entire global population? And what should one say when this global aging happens simultaneously with a very rapid global growth? This is a fundamentally new situation in the history of humankind. The data of Tables 1 and 3 convey very well the impression of an unprecedented growth of the world population during the past 50 years. Will this growth continue during the next 50 years in an unchanged manner? Yes and no. The medium variant projections of the UN are shown in Table 4.
Based on The 1998 Revision4, it is projected that, between now and the year 2054, the global population will increase by some 50%, from 6000 to 9000 million people; the projection for the year

Table 4 Medium variant projections of the global populations for the next 50 years. From reference 4

Year	Global population (millions)
1999	6000
2013	7000
2028	8000
2054	9000

2050 is 8900 million. In this context, a moment of reflection might not be out of place; my first review presented at the First World Congress'3, which was based on The 1994 Revision, projected for the year 2050 a global population of 10 000 million, while my second review, which was published a few months later14 and was based on The 1996 Revision, projected 9400 million people. Thus, within just 4 years, the UN projection for the global population of the year 2050 was revised downward by 1100 million people. Needless to say, demographic projections do not represent destiny, not even predictions. They should only be considered as illustrations of what can happen given reasonable assumptions. However, it is easy to see that, in our rapidly changing world, 'reasonable assumptions' may also be subject to

more rapid changes than they were in the past. Changes in assumptions will result in changing projections.

Moreover, there will be a rather fundamental difference in the population structure of the 9000 million people of the mid-21st century compared to that of the 6000 million people of today. The projected increase of the elderly population (aged 65 years and over) is presented in Table 5.

As late as 1975, the percentage of elderly persons in the world was 5.7%; in China it was only 4.4% and in Europe 11.4%. Even today, it is less than 7% in the world, including China. However, the UN projects that, by 2050, the percentage of elderly persons will be 16.4% world-wide, 22.6% in China and 27.6% in Europe. In fact, in certain European countries (Greece, Italy and Spain), it is projected to be as high as 34.3-36.9%.

The population aged 80 years and over (the oldest old) is expected to increase even faster

Table 5 Estimates and projections of the percentage of elderly persons (aged 65 years and over) in the world, Europe and China. From reference 4

Percentage of elderly

Year	*World*	*Europe*	*China*
1950	5.2	8.2	4.5
1975	5.7	11.4	4.4
2000	6.9	14.7	6.8
2025	10.4	21.0	13.2
2050	16.4	27.6	22.6

(Table 6.) Approximately 66 million people, or 1.1% of the global population, are oldest old today. It is projected that, by the year 2050, 370 million people, or as much as 4.2% of humankind, will be oldest old. Hence, between today and the year 2050, the number of oldest-old persons is expected to increase 5.6-fold; however, whereas the number of octogenarians (80—89 years) is projected to increase some 5.3-fold and that of nonagenarians (90—99 years) almost eight-fold, the number of centenarians (aged 100 years and over) is projected to increase 22-fold, from 100 000 to 2.2 million.

he ratio of females to 100 males is expected to increase with age; at the moment, it is 103 in the age bracket 55-59 years, 181 in the age bracket 80-89 years, 287 between 90 and 99 years, and as high as 388 among centenarians4. However, there are marked regional variations hidden behind the above averages, and the European and American profiles appear to differ from those reported from South-East Asia and Africa, as shown in Table 7.
The largest oldest-old populations of today are in China (10.5 million), the USA (8.6 million), India (5.7 million), Japan (4.3 million) and Germany (3.1 million); it is projected that, by 2050, their numbers will increase to 100 million in

Table 6: estimates and medium variant projections of the elderly and oldest- old population of the world. From reference 4

Year	*Population (millions)*	*Percentage of population > 65 years*	*Percentage of population > 80 years*
2000	6055	6.9	1.1
2025	7824	10.4	1.9
2050	8909	16.4	4.2

Table 7: number of men per 100 women by region. From reference 36

Region	*65-69*	*70-74*	*75-79*	*80+*
Europe	79	65	55	42
The Americas	85	79	72	58
South-East Asia	95	94	93	105
Africa	89	88	86	83

China, 47 million in India, 27 million in the USA and 12 million in Japan. Indonesia will replace Germany as the fifth in 'the league', with 10 million oldest old.
What about the 'Club of 14'? Somewhat jokingly, I have classified 14 countries as members of this exclusive Club. The UN projects that, by 2050, 14 countries will have more than 10% of the oldest old in their population;

in addition to China, Japan, Hong Kong, Macau and Singapore, there will be nine European members of the Club :Italy, Spain, Switzerland, Greece, Austria, Germany, the Netherlands, Sweden and Belgium. The highest proportion of oldest old is projected for Italy (14%).

YEARS TO LIFE?

What is in the background behind the rapid growth of elderly populations is an unprecedented increase in life expectancy. Estimates and projections of life expectancy at birth world-wide and in selected regions are presented in Table 8. In 1950, the world-wide life expectancy at birth was 46.5 years; in China it was less than 41 years and in Europe it was around 66 years, a difference of some 25 years! The world-wide life expectancy at birth today is higher than it was in Europe in 1950, and in China it exceeds 71 years. Hence, during the past 50 years, life expectancy at birth in China has increased by 30 years. For the year 2040, the UN projects a life expectancy at birth of 76 years for the global population and 79 and 80 years for China and Europe, respectively, suggesting that, during the next 40 years, the regional differences will diminish and eventually disappear.

Table 8 Estimates and projections of life expectancy at birth (both sexes combined) in the world, Europe and China. From reference 4

Year	*World*	*Europe*	*China*
1950	46.5	66.2	40.8
1975	59.7	71.2	65.3
2000	66.5	74.1	71.2
2025	73.1	78.1	76.3
2040	76.0	80.1	78.7

However, for the time being, these regional differences are still very marked, disturbingly so, as indicated by the data of Table 9. Life expectancy at birth today is highest in Japan may expected to live 40 years longer than a man born in Mozambique, and the average Japanese woman may look forward to a life 45 years longer than a woman in Mozambique.

Table 9.Life expectancy at birth in the year 2000 in selected countries. From reference 4

Life expectancy (years)

Country	*Men*	*Women*
Japan	77.2	83.3
France	75.0	82.5
USA	74.2	80.6
China	69.1	73.5
Russian Federation	61.3	73.2
Thailand	66.3	72.7
India	64.9	66.9
Nigeria	49.0	51.6
South Africa	45.2	48.6
Mozambique	37.5	38.8

The estimation of life expectancy at 65 years assumes a greater and greater importance, because it also increases rapidly. During the past 50 years, it increased by 8.4 years and 7.6 years for women in Japan and France, respectively. Some recent estimates of the highest life expectancies at the age of 65 years are shown in Table 10.

However, the process does not stop there; for the time being, standard life expectancies for men

Table 10.Life expectancy at 65 years of age in 1998 in selected countries. From reference 37

Country	Men	Women
Japan	16.4	21.0
France	16.2	21.1
Spain	15.8	19.8
Italy	15.7	19.6
USA	15.3	18.3
Mexico	15.3	17.0
Russian Federation	10.9	15.0

and women at 75 years of age are 10.2 and 12.3 years, and at 85years of age 5.2 and 6.2 years, respectively15.

AN ENDANGERED SPECIES: OUR GREAT-GRAND CHILDREN

The approach of our generation to the complexities of demographic issues is a good example for the thesis of ArnoldToynbee16 when he points to '. . . that tendency to over-simplification which the human mind displays in all its activities'. Erasmus calls it 'the urge of the human mind for analogy and generalisation'17. First we were obsessed with the challenge of the 'population explosion', then we shifted our concern to the problems of global aging, and only now do we start to grasp the future consequences of a rapid fertilitydecline . . . what will happen to our children and grandchildren?

Recent estimates and projections4 of the percentage of children (persons aged 14 years or less) world-wide and in selected regions of the world are presented in Table 11. As recently as 1975, the world-wide percentage of children was around 37% and in China it exceeded 39%. Today, it is less than 30% world-wide and less than 25% in China. According to the medium variant projections of the UN, by the year 2050, the global percentage of children will be below 20%; in China this figure will be only 16.3% and in Europe it will be one of the lowest in the world, 14.4%. What will be the consequences of this, as far as the elderly population is concerned? One consequence, a change in the ratio of children to elderly persons, is shown in Table 12. The data of Table 12, which were obtained simply by dividing the figures of Table 11 by those ofTable 5, indicate that just 25 years ago, in 1975, there were 6.5 times more children than elderly persons in the world and nine times more in China. It is projected that, by the year 2050 the global ratio of children to elderly persons will be only 1.2, whereas the ratio in China will be 0.7 and in Europe 0.5, where there will be twice as many elderly persons as children. A rapidly changing population structure (with many elderly persons and fewer and fewer children) represents an unprecedented and fundamentally new feature in our history; no wonder, then, that few governments are prepared - as yet - to grasp the likely economic, social, ethical and political consequences.As Arnold Toynbee pointed out, 'The human race is far more able at technology than in dealing with itself ... in religion, politics and social relations'8.

Table 11 Estimates and projections of the percentage of children (aged 14 years or less) in the world, Europe and China. From reference 4

	Percentage of children		
Year	World	Europe	China
1950	34.3	26.2	33.6
1975	36.9	23.7	39.5
2000	29.7	17.5	24.9
2025	23.4	14.7	18.3
2050	19.7	14.4	16.3

THE NAME OF THE GAME: FERTILITY

The major determinant behind the changes indicated above is an unprecedented decline in total fertility rate per woman, which took place recently over a few decades. The total fertility rate represents the number of children that would be born to a woman if she were to live to the end of her childbearing years and were to bear children at each age at prevailing age-specific fertility rates. Broadly speaking, these rates can be approximated satisfactorily by the number of children a woman will have in her lifetime. Until recently, it was more or less generally accepted that 2.1 children

Table 12 Ratios of children to elderly persons in the world, Europe and China. Calculated from the data of Tables 5 and 11

	Ratio of children : elderly		
Year	World	Europe	China
1950	6.6	3.2	7.5
1975	6.5	2.1	9.0
2000	4.3	1.2	3.7
2025	2.3	0.7	1.4
2050	1.2	0.5	0.7

per woman corresponded to the replacement level of fertility to keep a population constant. As illustrated by the data of Table 13, total fertility

rates declined dramatically between 1965 and 1995, a period of just 30 years.

The total fertility rate dropped below the replacement level in Europe in 1975 and in China in 1995, signaling a future decline in the number of people living in these areas. If the UN medium-term fertility projections materialize, the population of Europe will diminish between now and the year 2050 by some 100 million people, from the present 729 million to 626 million. In fact, by 1995, the total fertility rate in Europe was among the lowest in the world, and in several European countries (e.g. Greece: 1.28; Italy: 1.20; Spain: 1.15) it was extremely low.

Another challenge on the horizon is represented by the correlation between educational level and fertility. According to a recent fertility survey by the National Statistical Institute of Spain, in 1999 the total fertility rate of Spain was 1.07; it was 3.19 among illiterate women, 1.37 among those who had completed secondary school and 0.77 among those with a university degree.

However, before assuming that we are dealing with a manifestation of what the malicious sometimes calls 'Eurosclerosis', let us remind ourselves that we are in excellent company, since fertility rates are rapidly declining all over the world. In 1955, only 0.1% of the global population was at or below the replacement level of fertility; this figure increased to 21.0% by 1975 and to 45% by 1995, and it is projected that by the year 2025, the figure

Table 13 Total fertility rate per woman between 1965 and 1995 in the world, Europe and China (replacement level of fertility: 2.1 children per woman). From reference 4

		Fertility rate per woman	
Year	World	Europe	China
1965	4.91	2.36	6.06
1975	3.92	1.97	3.32
1985	3.34	1.83	2.46
1995	2.71	1.42	1.80

will be 76% of a global population exceeding 8 billion people4. The conclusion appears to be inescapable that never before in history have birth rates fallen so far, so fast and so low for so long all around the world. Is humankind at a new historical crossroad? Maybe so. At any rate, the

implications of these demographic changes -for good or for ill - are as yet unclear.

PLANNING THE FUTURE BY THE PAST

More than 200 years ago, in a letter to a member of the National Assembly, Edmund Burke wrote that: 'You can never plan the future by the past' and then he added: 'To innovate is not to reform'. What a pity that he could not send the same letter by e-mail to our contemporary politicians. There is a major need for institutional reform, since - due to the demographic changes described above - many of our contemporary institutions are catering for a population structure (with many children and few elderly) that does not exist any longer. Many of our institutions were the admirable creations of our forefathers and served us very well for more than a century, but not any longer. As The Prophet of Khalil Gibran says: 'Life goes not backward nor tarries with yesterday'18.

Hence, the time has now come to reform many institutions, for instance social security, health care, socioeconomic policy and the financing of such services. Our housing and educational systems and current financial and commercial routines also require a fresh critical look, not to mention our hierarchical, pyramid-like bureaucratic administrative structures, which were believed for millennia to represent the most rational way to organize and manage human co-existence. Last, but not least, what about the sacrosanct concept of the nation state, for which it is more and more difficult to adapt and cope with a world of new global realities?

It is not too difficult to predict that the greatest impediment to the implementation of greatly needed reforms will be political inertia. 'Rapid adjustment to unexpected and/or inevitable changes is not the greatest virtue of contemporary societies. The expression 'too little, too late' is not only a frequently used political argument, maybe it is our way of life'12.

LIFE TO YEARS WITH DIGNITY

In a world that will soon be inhabited by some 1.5 billion elderly persons, there will be a pressing need for reform in health and social care. The World

Health Organization (WHO) estimates that, in 1965, about 48 million human beings died world-wide: 34% of them were aged 5 years or less and 19% were 75 years of age or older. WHO projects that, in the year 2025, some 65 million people will die: only 8% of them will be below the age 5 years and 53% will be at or above 75 years of age19. In fact, health trends and the disease burden will markedly change during the next few decades. By the year 2020, health trends will be dominated by the aging of the global population, the humanLIFE TO YEARS WITH DIGNITY

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In the world of the mid-21st century, with more than 16% elderly persons in the global population, age-related diseases, e.g. cardiovascular diseases and neoplasms, will certainly increase in importance. WHO also expects a major rise in the prevalence of a number of age-related metabolic alterations such as osteoporosis, atherosclerosis and, in particular, diabetes, musculoskeletal degenerative changes and so-called 'male problems' (prostatic cancer, benign prostatic hypertrophy, erectile dysfunction, etc.). It is particularly emphasized by WHO that much more attention must be paid to those factors that influence the quality of life of the elderly, such as incontinence, impaired vision and impaired hearing, reduced mobility, falls and fear of falls, and oral ill-health.

It becomes more and more obvious to an increasing number of governments that many, if not all, of the challenges related to the health problems of an aging humankind can only be solved by research; what they fail to appreciate - as yet - is that a quantum leap in research support will be required to prevent the preventable and postpone the inevitable.

What kind of research are we talking about? I would like to attempt to summarize the research agenda under four 'How' headings:

(1) How to prevent or postpone frailty, disability, immobility and dependence;

(2) How to maintain the social function of the elderly;

(3) How to reduce gender inequalities and socioeconomic differentials;

(4) How to ensure the human dignity of elderly people.

To me, all these areas are extremely important, but the last one is of fundamental importance. The first sentence of the Constitution of the Federal Republic of Germany says that: 'die Würde des Menschen ist unantastbar' (human dignity is unassailable) (1. Grundgesetz, Artikel 1., Satz 1), and I would like to see this sentence incorporated into the Constitution of every single member state of the UN. What is human dignity? Emanuel Kant says: 'What is over everything else, what has no equivalent, that is dignity'. To me, it embraces not only the 'nine pillars' with sufficient food, potable water, shelter, sanitation, health care, a healthyenvironment, education, employment and personal security10, but also a society without racism, sexism and agism. Agism? Yes. Ann Bowling wrote recently that 'agism in clinical medicine and health policy reflects the agism evident in wider society. Legislation may be required to end agism in society'21.

DIGNITY AND POVERTY

One of the greatest enemies of humankind is mankind and one of the greatest enemies of human dignity is poverty. What is poverty? It is a disease; one of the worst diseases of humankind. Is it really a disease? Many of us feel so. WHO stated this in no uncertain terms: 'Poverty is the most widespread, pervasive and intractable disease in the world today'22. It has been with us since the dawn of history and lip service has been duly paid to

its eradication by well-meaning politicians all over the world. In 1960, in his Nomination Acceptance Speech, John F. Kennedy said: 'The world is very different now. For man holds in his mortal hands the power to abolish all forms of human poverty and all forms of human life'. Fortunately, during these past 40 years, man did not abolish all forms of human life, but, unfortunately, did not abolish human poverty either.

How much poverty is, in the world today? There are various methods to measure it, none of them without some conceptual and technical problems and limitations. At any rate, the latest data of the World Bank23, shown in Table 14, might convey a rather representative picture. The data indicate the percentage of national population in the largest developing countries below international poverty lines (< 1 and < 2 purchasing power parity dollars per day). More than 700 million Indian and 280 million Chinese citizens live on less than 1 purchasing power parity dollar today, in addition to a considerable number of our fellow men and women living in other developing countries.

The data of Table 14 may help to place into proper perspective those of Table 15; the latter table indicates the 1990 assessment by the World Bank24 of the size of the population in selected developing countries without access to health services. It would appear that there are not only new realities, but also some very old ones in the world around us today. Dr Gro Harlem Brundtland, the Director-General of WHO, remarked last year: 'Over a billion people will enter the 21st century without having benefited from the health revolution: their lives remain short and scarred by disease'25.

What can be done in a world in which the World Bank projects that, in 30 years' time, the gross domestic product per capita in the developed world will reach around US$45 000, whereas in Sub-Saharan Africa and Asia it will not exceed a few hundred dollars? Is then resignation the name of the game? Certainly not! President Kennedy was right: Homo sapiens holds in his hands the power to abolish all forms of poverty. It may happen, if we let it happen. In fact, never before has humankind had so many resources, so'much knowledge, such powerful technologies and a UN organization with so

Table 14 Percentage of the population below international poverty lines in selected countries. From reference 23

Country	*Population (millions)*	*Percentage with < 1 ppp dollar/day*	*Percentage with < 2 ppp dollar/day*
China	1278	*22*	58
India	1014	53	89
Indonesia	212	12	59
Brazil	170	24	44
Nigeria	111	31	60
Ethiopia	63	46	89
Thailand	61	< 2	24

PPP, purchasing power parity

Table 15 Total population and population without access to health services in selected countries around 1990. From reference 24

Country	*Population (millions)*	*Population without access to health services (millions)*
China	1178	118
India	898	133
Indonesia	187	38
Pakistan	123	58
Bangladesh	115	62
Nigeria	105	35

many special agencies experienced in combating poverty. So, why don't we try? Will it be difficult? Yes, very much so. Perhaps impossible? No! The famous polar discoverer and later Nobel Laureate (Peace Prize), Fridtjof Nansen, the creator of the Nansen Pass who, in 1920—1922, on behalf of the League of Nations, succeeded in repatriating some half-a-million prisoners of war, is credited with saying: 'Never stop because you are afraid — you are never so likely to be wrong. Never keep a line of retreat: it is a wretched invention. The difficult is what takes a little time; the impossible is what takes a little longer'26.

THE PEDESTALS OF REASON AND CONFUSION

One of the principal proponents of the concept of transmodernity, the philosopher Marc Luyckx, stated recently that: 'In the 20th century, the pedestal of Reason has been eroded by experience that scientific discovery and technical innovation can lead not only to miracles of change but also to unprecedented dirt, damage, and disease'27.

The process of erosion was not confined to the pedestal of Reason; after Hiroshima and Nagasaki,the pedestal of Science was also eroded and some of those disenchanted with science and its misuse and abuse sought refuge in 'modern spiritual movements' such as pseudoscience, parascience or even antiscience28. Their attitude seems to reflect what J.-P.Sartre verbalized so well in Les Mots (1964): 'Comme tous les songe-oreux, je confondis le désenchantement avec la vérité' ('Like all dream¬ers, I mistook disenchantment for truth'). Probably the issue is much more fundamental, since - at least in certain situations — all of us are subconscious, if not conscious, worshippers of the Great Irrational. This was masterfully expressed — exactly 100 years before Sartre - by Fyodor Dostoyevsky in his Notes from the Underground (1864): 'the formula "two and two make five" is not without its attractions'.

To believe passionately would seem to be very typical of what we sometimes call human nature. It has caused many problems in the past and is not unlikely to continue to do so in the future. The great American polyhistor of the 20th century, Henry Louis Mencken, characterized our predicament by saying that: 'The most costly of all follies is to believe passionately in the palpably not true. It is the chief occupation of mankind'29. If so, it makes it somewhat easier to grasp that the most passionate believers (or rather non-believers?) turned completely away from science and became the champions of anti-science, claiming that science failed to produce any benefits for humankind, a catalyst for disaster and a threat to peace28.

Perhaps the 20th century has eroded some old pedestals, but also created some new ones, such as the great pedestal for Confusion. Anti-science is certainly a very strong candidate to be placed there. It is easy to see that

nothing is wrong with science, but that there may be something deeply wrong with those who abuse it. Scientific knowledge per se is neither good nor evil; it is just an instrument, ancilla. In medieval times, it was ancilla theologiae which was used only to strengthen or modify certain theological theorems. In the super- materialistic atmosphere of our times, many people believe that it is mainly ancilla pecuniae, a money-making instrument. However, there is within us an ethical imperative that dictates that it should become ancilla humanitatis, a major instrument to be used to improve the human condition. At the end of the day, the believers of pseudoscience, parascience and various fashionable isms of our time will find out that 'the scientific revolution is the only method by which most people can gain the primal things (years of life, freedom from hunger, survival for children)'30.

KNOWLEDGE AND WISDOM

In one of his immortal poems, T.S. Eliot asks: 'Where is the Life we have lost in living? Where is the wisdom we have lost in knowledge? Where is the knowledge we have lost in information?'31. Eliot identifies one of the disturbing problems of the 20th century, since information is not knowledge unless it is systematized and critically evaluated, and knowledge is not wisdom unless it is applied to the benefit of humankind to improve the human condition. Therefore, the fundamental issue in the assessment of our common future will be to try to provide a reasonable answer (if there is one) to the question: how sapiens is Homo sapiens?

What makes it so difficult to give an answer is the fact that, to a certain extent, Homo sapiens is an undefined entity consisting of many subspecies. What about Homo dogmaticus, Homo fanaticus or Homo bellicosus? Do they have a common denominator? Perhaps what Primo Levi called the 'bestial vice of hatred'32. Is there a universal remedy for it? Can it perhaps be eradicated in the coming centuries? But how? As Albert Einstein formulated the problem, 'How is it possible to control man's mental evolution so as to make him proof against the psychoses of hate and destructiveness?'33. The sceptics feel that little can be done about human nature, but they forget that human nature is not destiny; it is subject to evolutionary forces and can be changed by time and circumstances. They

also miss an important prospective formulated by Bertrand Russell: 'In a biological sense, Man, the latest of species, is still an infant. No limit can be set to what he may achieve in the future'34.

FROM FUTUROPHOBIA TO FUTUROPHILIA

Homo sapiens has just entered the 21st century and tries to look forward into a future that is just as uncertain as the present. From the 20th century, he has carried along what I have called the seven obstacles on humankind's golden road to Samarkand: population growth, aging of populations, environmental degradation, global unemployment, poor health, persistent poverty and intra-species aggression35. However, Homo sapiens has also carried with him the infinite wisdom of hope and an incredibly powerful scientific and technical knowledge, the amount of which is expected to double every 6-7 years, perhaps even faster during the 21st century. It appears, therefore, that there is plenty of reason for an optimistic futurophilia, since Homo sapiens possesses a virtually unlimited intellectual andtechnical potential to meet the challenges of the new century, together with those of the past one, like the 'seven obstacles' indicated above.

In meeting the challenges of the 21st century in general, and of an aging world population in particular, medical research and the medical profession will play an even more important role than it did in the 20th century. What, then, should our message be to the coming generations of physicians and medical scientists? For this, I would like to paraphrase a statement by Béla Bartok, one of the great composers of the 20th century and the greatest Hungarian composer, a statement that I have seen in a Hungarian publication. Bartôk said: 'If I would ever cross myself, I would say this: "In the name of nature, science and art"'. My own message to our colleagues is similar, but still very different - I would rather say: 'In the name of human dignity, science and charity', or, in Latin 7« nomine dignitatis, scientiae et caritatis.

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Reprinted from: The Aging Male, no 1/ 2000, 37-48

The aging male and developed countries in the 21st century

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Key words: DEMOGRAPHY, ESTIMATES AND PROJECTIONS, AGING POPULATIONS, POPULATION STRUCTURE, CONTRACEPTION, FERTILITY

"It is bad enough to know the past; it would be intolerable to know the future."

Somerset Maugham

We cannot reform our forefathers, but we can always quote them. Bernard Shaw said, 'It's all that the young can do for the old, to shock them and keep them up to date'. If so, what can the old do for the young? Exactly the same and this is what 1 intend to do here. 1 will present many figures, estimates and projections but avoid predictions, always reminding myself that estimates are estimates, projections are uncertain, predictions are fairy tales and humility is endless. I will also remind myself of Albert Einstein's dictum, that whoever undertakes to set himself up as a judge of truth and knowledge is shipwrecked by the laughter of the gods.

Some historical estimates of world population are shown in Table 1, beginning in the year AD14. I his was an important point in history, being the year in which the Emperor Augustus died and was succeeded by Tiberius. The world population was around 250 million, of whom 37 million were living in Europe. Survival for individuals and for nations was difficult in those times, and 1000 years later the world population was virtually the same, with the European population being, if anything, smaller. Then things started to move. By the year 1500, the world population had reached more than 400 million, by 1750 more than 700 million, and by the beginning of

the 20th century it was almost 1.7 billion, of whom 280 million people were living in Europe and 500 million in China[1,2].

Table 1 (millions). Historical estimates of world Population Data from references of world 1 and 2

Year *(AD)*	*World*	*Europe*	China
14	256	37	73
1000	280	32	60
1500	427	62	100
1750	731	102	207
1901	I668	284	500

The wish to control fertility is probably as old as human kind. Two years before the end of the 19th century, Sigmund Freud wrote a paper in the *Wiener Klinische Rundschau*[3] in which he said. 'Theoretically, it would be one of the greatest triumphs of humanity if the act responsible for procreation could be raised to the level of a voluntary and intentional behavior in order to separate it from the imperative to satisfy a natural urge.' Was Freud a naive dreamer? This was certainly the case in 1898, but no longer in 1959. This was the year in which the Food and Drug Administration (FDA) in the USA approved the first oral contraceptive. Enovid, followed 2 years later by the approval of Anovlar by the Bundesgesundheitsamt in Berlin and a year later by the approval of Ortho-Novum by the FDA[4]. The contraceptive revolution had arrived.

Global population had reached the three billion mark by about I 960, and there was a lot of anxiety worldwide. Prophets of doom and gloom were talking about a final population size of 15 billion oreven 25 billion, with standing room only on the planet. There were projections that by the end of the century the world population might reach eight or nine billion. The United Nations (UN), in .1 much more sober projection, projected that by the war 2000 the world population would surpass six billion people[2]. Table 2 shows that this is exactly what happened.

Enormous pressure from the more-developed countries was put on the governments of the less-developed world to introduce national family planning programs. India started in 1951, and by the mid-1990s more than

90% of all developing countries had adopted family planning programs. However, they did this mainly for reasons of human rights, gender equity and reproductive health rather than for demographic considerations. By 1993, more than 550 million couples worldwide were using contraceptive methods, the most important being female sterilization, intrauterine devices, oral contraceptives, condoms and male sterilization[5] (Table 3).

Tabic 2 Estimated and projected world population millions). Data from reference 2

Regions	***Year***	
	2000	***2050***
All regions	6056	9322
More developed	1191	1181
Less developed	4865	8141
Of which less developed	658	1829

Table **3** Estimated number of couples (millions) in the world using specific contraceptive methods in 1993. Data from reference 5

Method	*n*	*%*
All methods	552	100
Female sterilization	180	33
Intrauterine devices	120	**22**
Oral contraceptives	78	14
Condom	43	8
Male sterilization	41	7
Withdrawal	37	6
Rhythm	27	5
Injectable and implant	16	3
Vaginal barrier or other	10	2

The key question is where do we go from here? During the next 50 years human kind will grow rapidly and will age rapidly. It is projected by the UN that the global population will increase by more than 50% fromsix billion to

more than nine billion, but that the population of the more developed countries will remain stable (Table 2). There will be a major increase in North America (USA and Canada) and also in Australia and New Zealand, but this will be counterbalanced by a major decline in the populations of Europe (by some 120 million) and also of Japan (Table 4).

The most important change in the more- developed regions will be an aging process. In 1975, the percentage of elderly people (i.e. people aged 60 years and over) was about 9% worldwide and 12—16% in the more-developed regions. T he projection is that by the year 20.5(1 it will exceed 21% worldwide, 27% in North America, more than 36% in Europe and more than 42% in Japan (Table 5).

The fraction of the aging population growing most rapidly is the population aged SO and beyond. The UN terminology refers to them as 'oldest- old'. For the time being, the biggest oldest-old population is in Europe (about 22 million), followed by China (11 million) and North America (10 million). The UN projections are thatBy the year 2050 there will be some 100 million oldest-old in China, 60 million in Europe, 34 million in North America and 17 million in Japan (Table 6).

Tabic 4 Population of the more-developed regions (millions). Data from reference 2

Regions	***Year***	
	2000	***2050***
All more developed regions	1191	1181
Europe	727	603
North America	314	438
Japan	127	109
Australia/New Zealand	23	31

Tabic 5 Percentage of elderly persons (aged 60 sears and over). Data from reference 2

Year	World	North America	Europe	Japan
1975	8.6	14.6	16.4	11.7
2000	10.0	16.2	20.3	23.2
2025	15.0	25.1	28.8	35.1
2050	21.1	27.2	36.6	42.3

In percentage terms, the oldest-old are increasing in all parts of the world, particularly the developed regions. As recently as 1975, the percentage of the oldest-old population was less than 1% worldwide and 1-2% in the more- developed regions. The UN projection is that by the year 2050 it will exceed 4% of the world population, 7% of the North American population, 10% in Europe and 15% in Japan (Table 7). In Europe, although it is projected that the total population will decline between 2000 and 2050 from 727 million to 603 million, the number of octogenarians will more than double (almost triple), the number of nonagenarians will quadruple, and the number of centenarians increase by a factor of 13 from 50 000 to 670 000 (Table 8).

What about the aging male who, after all, is the subject of this paper? There are approximately 100 million men aged 60 and over in the more developed regions, of whom some 60 million are in Europe, 22 million in North America and 12 million in Japan. These numbers are projected to increase almost two-fold, so that by 2050 about 100 million aging males can be expected in Europe, 50 million or more in North America and 20 million in Japan (Table 9).

Table 6 The oldest-old (aged 80 years and over) populations (millions) in 2000 and 2050. Data from reference 2

	Year 2000		*Year 2050*
Europe	21.8	China	99
China	11.5	Europe	60
North America	10.0	India	48
India	6.1	North America	34
Japan	4.8	Latin America	33
Latin America	4.7	Japan	17

Table 7 Percentage of the oldest-old (aged 80 years and over). Data from reference 2

Year	*World*	*North America*	*Europe*	*Japan*
1975	0.8	2.1	1.8	1.1
2000	1.1	3.2	3.0	3.8
2025	1.9	4.2	5.2	10.0
2050	4.1	7.3	10.0	15.4

Again, the number of oldest-old men is growing the fastest. The projection is that between 2000 and 2050 their number will more than treble in all regions, except Australia and New Zealand where it will quadruple (Table 10).

While the elderly population will increase very rapidly, there will be an equally impressive decline in the number of children. This has to be brought into the equation. As late as 1975, almost 37% of the global population was aged 14 and below. It is projected that by the year 2050 the proportion will be 21% worldwide, with 18% in North America and only 14% in Europe (Table 11).

A situation in which there is a major increase in the elderly population associated with an equally impressive decline in children will have a major impact on population structure. In speaking about aging, if we take a 100-year perspective over all the developed countries, the projection is that between 1950 and 2050 the percentage of children will decline by almost 50%, the percentage of the working-age population will be reduced by 50%, the percentage of the elderly will increase three times and the percentage of the oldest-old almost ten times (Table 12). In the most extreme example,that of the population structure of Japan, the percentage of children was the same as in the rest of the developing world (35%) as late as 1950.

Table 8 The oldest-old population (millions) of Europe. Data from reference 2

Age group (years)	*Year*		
	2000	*2025*	*2050*
All ages 80-89 90-99	727	683 29.1 6.4603	
> 100	18.3	0.23	46.6
	3.1		12.8
	0.05		0.67

The aging male' (aged And over). Data from 60 years developed regions (millions)

Region	*Year*	
	2000	2050
All regions	95.8	173.4
Europe	59.3	96.1
North America	22.0	53.5
Japan	12.8	19.9
Australia/NewZealand	1.7	3.9

It is projected to decline to 12% by 2050, the working-age population to below 30%, the elderly population to increase to close to 60%, and the oldest-old to increase to 15% (Table 13). In a society with so few children, so many elderly and so few peopleof working age, the question is who will provide for whom and how?

A number which illustrates the problem is the potential support ratio - i.e. the number of persons in the working-age groups divided by those who are expected to be in retirement. In 1975, there were more than eight people in the active workforce in Japan for every retired person. This is expected to decrease to 1.4 by 2050. In Europe it is projected to be less than two and in North America about three (Table 14).

If the elderly are increasing in number and the children are decreasing, obviously the median age will also increase. In 1975, worldwide, the

median age was 22 years. It is projected to increase to 36 years worldwide, to 41 years in North America, to almost 50 years in Europe and to 53 years in Japan (Table 15).
Try to imagine a world in which there are two, three or perhaps even four elderly people for every child.

Table 10 Oldest-old (aged 80 years and over) men (millions) in the developed regions. Data from reference 2

Year / *Region*	*2000*	*2050*
All regions	11.3	40.4
Europe	6.2	21.1
North America	3.4	12.7
Japan	1.5	5.7
Australia/New Zealand	0.2	0.9

Table 11 Percentage of children (persons aged 14 years or less). Data from reference 2

Year	*World*	*North America*	*Europe*
1975	36.7	25.2	23.7
2000	29.9	21.5	17.5
2025	24.3	18.3	13.6
2050	21.0	18.3	14.0

Shakespeare says, 'Oh brave new world that has such people in it'. If I may paraphrase, I would say 'This is a brave new world with brave old humankind in it'.
What is behind this? There are obviously many factors; it is a complex situation. However, one of the important contributing factors appears to be a decline in total fertility rate. In the space of 30 years, between 1965 and 1995, the number of children per woman worldwide declined from almost 5 to 2.8, in North America to 2.0, while in Europe it fell to 1.4 — below the replacement level, which is supposed to be 2.1 children per woman (Table

16). All the projections seem to suggest that this will remain the case in Europe for the next 50 years — unless some unforeseen events occur.
If the number of children born remains below the replacement level, a major decline in the population can be expected. Indeed, the UN projects that Europe will diminish by some 124 million people to 603 million during the next 50 years. The greatest reduction is expected to be in Eastern Europe and the smallest in northern Europe (Table 17). There are countries like Ukraine in which it is projected that there will be a reductionof 40% of the total population from the number it is today.

Table 12 Population structure of the developed world (%). Data calculated from reference 2

	Year		
Age group (years)	*1950*	*2000*	*2050*
0-14	27.3	18.3	15.6
15-59	60.0	59.2	41.3
60-79	11.7	19.4	33.5
80+	1.0	3.1	9.6
Population (millions)	814	1191	1 181

structure of Japan (%). Data

Table 13 Population
calculated from reference 2

	Year		
Age group (years)	*1950*	*2000*	*2050*
0-14	35.5	14.7	12.5
15-59	56.3	58.3	29.8
60-79	7.7	23.2	42.3
80+	0.5	3.8	15.4
Population (millions)	84	127	109

Is this a sign of what the malicious sometimes call 'Eurosclerosis'? I do not think so. In fact, we are in good company because we will be followed by

the rest of the world. In 1955, less than 0.1% of the world population was living below the replacement level of fertility, whereas today that figure is 44%. It is projected that in 2015 two- thirds of humankind will live in countries with fertility below replacement level". I think it is therefore justifiable to say that never before in history have birth rates fallen so far, so fast, so low and for so long all around the world, and also that the implications for good and for ill are as yet unclear. It is, however, clear that our institutions, which were designed by our forefathers in a masterly way in the 19th century, such as social security, health care, education, housing, marketing and even the nation state, do not meet the needs of today because theyare catering for a population structure that no longer exists.

The problem with our cherished and sacrosanct nation state today is that it has become too small to do the big things and too big to do the small things. Thus, the fundamental challenge confronting humankind today, as formulated by Professor Soedjatmoko from Indonesia, the first President of the United Nations University, Tokyo, is how to deal with problems for which we cannot findanalogies in older, often petrified systems of wisdom.

Table 14 Potential support ratio (number of persons aged 15-64 years for each person aged 65 years or over). Data calculated from reference 2

Year	*Europe*	*Japan*	*North America*
1975	5.7	8.6	6.3
2000	4.6	4.0	5.4
2025	3.0	2.0	3.4
2050	1.9	1.4	2.8

Table 15 Median age (years). Data from reference 2

Year	*World North America*	*Europe*	*Japan*
1975	22.0 28.7	32.1	30.4
2000	26.5 35.6	37.7	41.2
2025	32.0 39.7	45.4	50.0
2050	36.2 41.0	49.5	53.1

What is responsible for this drop in fertility rate? A fairly simplistic discussion is going on which says that it can all be attributed to

contraception, that in a world in which 550 million couples are practicing contraception, it must have an impact — which indeed it has. There is a highly significant correlation between total fertility rate and contraceptive prevalence, the latter being more strongly associated with fertility rate than other proximate (direct) determinants, such as the pattern of marriage and sexual behaviour outside marriage, the duration of breastfeeding and the practice of induced abortion'.

However, contraception is the means, not the cause of these demographic changes. The cause is something much deeper and much more complex. It has both classical ingredients and new ingredients, excellently summarized by Jean- Claude Chesnais7:

(1) Classical ingredients:

(a) The rise in life expectancy;

(b) New contraceptive methods;

(c) Urbanization and the densification process;

Table 16 Total fertility rate per woman (replacement level: 2.1 children per woman). Data from reference 2

Year	*World*	*North America*	*Europe*
1965	4.9	2.5	2.7
1975	3.9	1.8	2.0
1985	3.3	1.9	1.8
1995	2.8	2.0	1.4

Table 17 Estimated and projected population of Europe (millions). Data from reference 2

Year

Region	*2000*	*2050*	*Difference*
Northern Europe	95.1	92.8	-2.3
Western Europe	183.3	170.9	-12.4
Southern Europe	144.9	116.9	-28.0
Eastern Europe	304.2	222.7	-81.5
Total	727.5	603.3	-124.2

(d) The elimination of illiteracy;

(e) Instability replacing tradition in many societies;

(2) New ingredients:
(a) Social atomization and related feminism;
(b) Collectivized pension benefits;
(c) Globalized nomadism;
(d) The youth loss of majority (they are becoming a minority);
(e) The 'end of work' syndrome (where there is no longer any use for our hands because everything is done by machinery).

The term 'social atomization' perhaps needs some explanation. Two examples will serve to illustrate its meaning. First, the proportion of Japanese women who never married increased significantly in all age groups between 1975 and 1995*. Second, the percentage of Italian women aged 25 and men aged 30 born in the late 1960s who were not living in any kind of partnership was double that of the cohort born in the late 1940s'\

There are also other key determinants of low fertility, particularly those reviewed by Lesthaeghe and Willems10:
(1) An increasing female autonomy;
(2) Gains in female education;
(3) Increased female labor force participation;
(4) A major change in the pattern of union formation;
(5) An increasing instability of unions;
(6) Some rather diffuse and foggy, but nevertheless strong, ideological changes about the future role of women in society and in the family.

However, all this can be changed. Uncertainty must be accepted as the most important ingredient of everything surrounding us and our present situation. Chesnais points out that there are some reversible factors7:
(1) There is still a strong latent demand for a family policy of two children;
(2) Some interesting decapitalization mechanisms are emerging;
(3) Reforms are taking place in the welfare systems;
(4) There is a shift to post-materialistic values in several societies;
(5) All the above may result in an end of pessimism and a revival of hope.

I would submit that the future is not yet written, and cannot be written because it is created in the mind of people, the new generation who will have a new vision.

When Dostoevsky was in Paris lie wrote a letter home in which he said that it was strange but he thought that most of humankind is suffering from the Don Quixote disease, which is a passionate search for reality. This may have been true in his time. Today, we do not have to search for it. It is surrounding us; it is all over the place. Cur problem is how to cope with the new realities.

In a world in which the amount of new scientific information doubles every 7th year, the classical value systems of our different societies, based on historical tradition, parochial identities and religious dogma, can no longer accommodate the new realities. As I see it, the wind of the new realities is blowing with increasing strength. Some people will try to construct protective wind shields, others new types of windmills. There will always be pessimists and there will always be optimists. In our world today, it is rather easy to become a pessimist when reflecting on human behavior, and rather easy to become an optimist when reflecting on the incredible progress achieved by humankind.

I am a pessimist several times every day, but I am also an optimist several times every day, so you may call me an optimo-pessimist. I am much more an optimist than a pessimist, but I am not a naive optimist and do not deny that our contemporary history, as depicted in the newspapers, gives the impression of a stormy sea in which the fragile vessel of rationalism is constantly threatened by the high waves of passion, fanatic faith and emotion. Nor do I deny that we have failed so far to convince people that fundamentalism and obscurantism cannot improve the human condition. Only science has the proven ability to do this.

I do not deny either that we are living in a world of contradictions and paradoxes in which scientific progress goes hand-in-hand with political confusion, when so-called globalization is associated with increasing fragmentation, when there is plenty of fluctuation, uncertainty and unpredictability. This is all true, but at the same time it should not be forgotten that there are incredible possibilities for the human genius to do things to improve the human condition. We have never had this before. Also, 1 do not think that human nature is fixed. It can change, and it can be changed by time and circumstances.

1 am also an optimist because I have learnt at my age to look back; this can sometimes be rewarding. In an address to the Royal College of Physicians in 1944, Winston Churchill said that the longer you can look back, the further you can look forward. Therefore, in my talks I frequently repeat the remarks of two American atomic physicists, Lesher and Howick" , who looked back 50 000 years and said that 800 life spans can bridge more than 50 000 years, but 650 of these 800 people spent their lives in caves or worse. Only the last 70 had any truly effective means of communicating with one another, and only the last six ever saw a printed word or had any real means of measuring heat or cold. Only the last four could measure time with any precision, only the last two used an electric motor, and the vast majority of the items that make up our material world were developed within the lifespan of the 800th person.

If I may add a personal note, in my own lifetime I have seen more progress in science than all the scientists of all preceding periods taken together since the dawn of history. This is because science is progressing in a geometric fashion. When I graduated from medical school in 1944, I assume that what we called the 'crude size' of medical knowledge was probably less than 5% of that known today. Shakespeare was right: what is past is prologue[12].

At the beginning of Wittgenstein's *Tractatns Logico-Philosophicus*[ji] there is a motto which comes from Kiirnberger. It says that whatever a man knows, whatever is not mere rumbling and roaring that he has heard, can be said in three words. During these past years I have tried to make three-word summaries of what others say and what I try to say. Fairly recently, the Secretary General of the UN, Kofi Annan gave a beautiful talk about the role of the UN in the 21st century, from which I could extract the following three words: *conflict, poverty and democracy.* The major role of the UN in this century should be to prevent conflict, to diminish poverty and to increase democracy. In a world in which more than half of mankind is living in absolute poverty and in which a fraction of mankind isliving in what we call 'democracy', these are worthwhile goals.

What should be the three-word summary for the International Society for the Study of the Aging Male (ISSAM)? I would suggest *faith, science and collaboration.* As a young medical student, I learnt from Claude Bernard that scientists should have a strong faith, but should not believe; also

members of this society should have faith in the uniqueness of their mission and that they can solve many of the problems by research, especially if carried out in collaboration.

Dostoevsky said that the formula 2 plus 2 makes 5 is not without its attractions. 1 submit that in human relations 2 and 2 are very rarely 4; it can be 6 or more. If you collaborate, it will be more.

Four years ago, on 5 February, 1998, I was privileged to give the opening talk at the first meeting of ISSAM[14]. The first slide I showed said, 'Congratulations to Professor Bruno Lunenfeld for your courage, determination, foresight and vision'. There was a major reason for showing this slide because there were a lot of misgivings about it at the time. There is no need for such misgivings today. Jean Monnet wrote in his memoires (1978) that when an idea meets the necessity of the epoch it ceases to belong to those who invented it and it becomes stronger than those who are in charge of it. ISSAM is no longer yours [Professor Lunenfeld's]; it belongs to humankind and the scientific community at large.

Let me finish with a last quotation. A week before the *First International Congress on the Aging Male,* in 1998, 1 was in Bangkok and heard the King of Thailand address a group ot foreign scientists. He said, 'May you be blessed with good health, physical and mental, as well as intellectual capability, so as to be able to contribute further to the well-being of people the world over'.

I cannot finish with a better quote than that and wish you [Professor Lunenfeld] all success in your professional work.

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Reprinted from: The Aging Male, no 3/ 2002, 139-146

The Story of the Limoncello Society

By now, the authentic medieval history of the Society is fairly well known, thanks to the meticulously careful research work of this historians of the Society. Different aspects of this history have already been presented at the Annual Meetings of the Society during the past 5-6 years. Therefore, this section will mainly deal with a single aspect of paramount importance, namely the historical relationship between the Society and the Autonomous Republic of San-Limone. Wiliam Faulkner masterfully characterizes such a relationship by saying that "*The past is never dead; it is not even past*"…

The medieval School of Salerno – the first medical school in human history – was founded sometime in the last decades of the 11th century; it reached the peak of its blossom in the 12th century with some outstanding teachers, like Dr. Limoncello, the professor of pharmacognosi and the inventor of the wonderful elixir and miracle medicine that carries his name. Professor Limoncello is shown in Figure 37 (Photo taken around 1190 AD). He was one of the first champions of *evidence-based medicine (EBM*) as indicated by the recurrent statement in medieval textbooks: "Limoncello is good for you". Of course, he had no way of knowing, that in 900 years' time a new epoch would come, perhaps around the end of the 20th century, in which EBM would no longer stand of *evidence-based medicine*, but rather for *efficiency –based medicine*, with a health care delivery process that minimizes treatment variation while targeting superior financial outcomes. As inscription of every new Sanlimonese coin says, *tempora mutantur*…

At any rate, the philosophy of Professor Limoncello went much, much further… He perceived the quintessence of the human condition, that was verbalized by T.S. Eliot some 750 years later: *"Human kind cannot bear very much reality"*.In fact, he felt that one must go far beyond *evidence – based medicine* in order to really meet the major exigencies associated with the human condition; this was the reason why Professor Limoncello decided to establish the basic principles of *prejudice – based medicine (PBM),* one of the most powerful and popular philosophical ideas even of our contemporary medicine…

It may sound incredible to some, but it was Professor Limoncello, who established the first Limoncello Society of human history as early as in 1186! Moreover, only the most erudite contemporary professors of ophthalmology are aware of the historical fact that the classical pathological entity called *dyslexia muralis* (*characterized by the inability to read the writing on the wall*), was described originally by Professor Limoncello, who also called attention to the very high prevalence of the syndrome among the political leaders of the time.

Professor Limoncello, who was the first Research Professor in the Department of Pharmacognosi at the Medical School of Salerno, was justly noted for two percepts that have continued to light the way for scientists of today: *faith in science and imagination in research.*One example of his imaginative research into excessive skin folds that appear to cause aging in humans. He explored various mechanisms by which wrinkles could induce premature aging in several species. He was lucky enough to be able to focus mainly on the Char-Pei dog, and in so doing discovered that Char-Pei breeders in China were less likely to show wrinkles than a control Sicilian population. This stimulated further studies in which the hair of the dog's ears was boiled in a gallon of the dog's urine, with calamint, catnip, fenel, pellitory, savoury, artemisia, rue, wormwood and anise to make up a medicine delaying aging.

A further interest was Professor Limoncello's exploration of the composition, specifically the mineral content, of the hole in the doughnut. Continuing experiments in his laboratory have been cited by nutrition researchers even today as the first description of a balanced diet – the calorie content of the doughnut hole neatly balanced the calorie content of the remainder of the doughnut. **(Figure 38).**

It was professor Limoncello who carried out the very first subhuman primate studies in human history, following up an accidental observation that no caries was present in animals treated by gavage with palm sorbet containing 30% (v/v) limoncello (Figure 39). A real pioneer in improving *oral health*! He considered marketing a parented formula under the trade name *dentricello*. Unfortunately, this project didn't meet with success, due to the fact, that a suitable *delivery system* (called "toothbrush") was not yet invented.

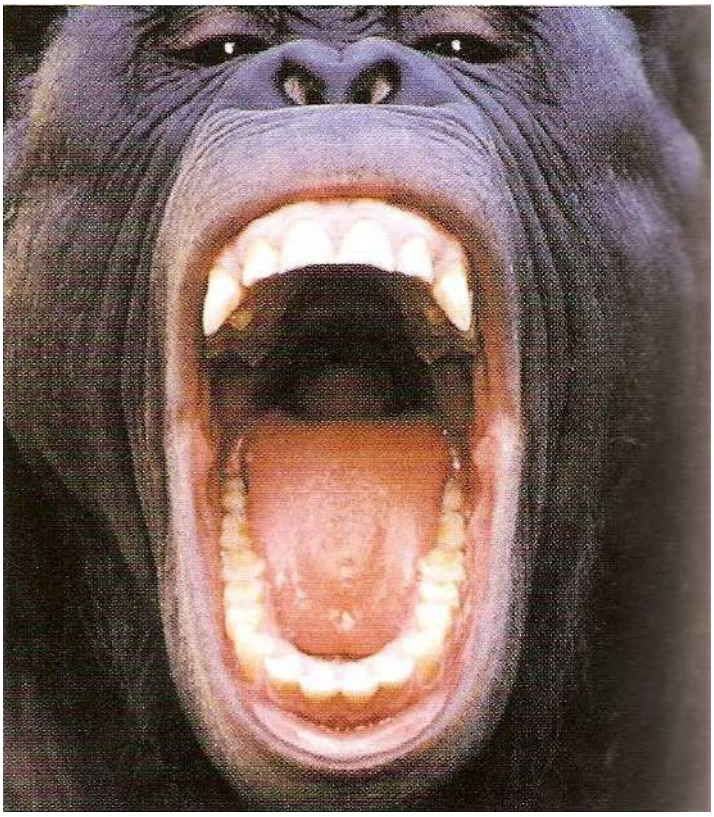

Figure 38 A LATE DESCENDENT OF PROFESSOR LIMONCELLO'S ORIGINAL DOUGHNOUT. EVEN THE HOLE WAS BIGGER AND BETTER IN THE GOOD OLD DAYS.

Figure 39.THE FABULOUS OUTCOME OF THE FIRST EVER EXPERIMENT ON ORAL HEALTH IN SUBHUMAN PRIMATES WITH DENTRICELLO (AROUND 1194 AD).

The premature discontinuation of this line of research was received with lot of disappointment not only among the prospective beneficiaries, but also

among the prospective volunteers for continued investigations some of them became depressed, others almost aggressive as shown in Figure 40.

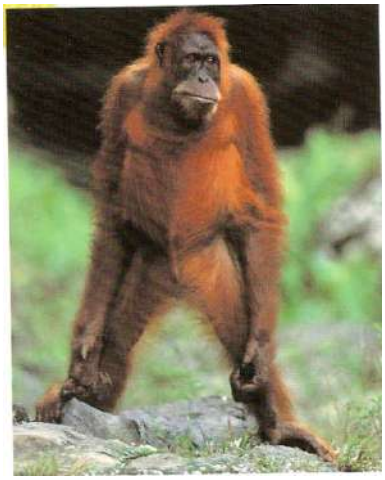

Figure 40. A COMMON YEAR CONSISTS OF 365 DISAPPOINTMENTS.

There is still some controversy as to the proper interpretation of the famous statement made by Professor Limoncello in one of those fascinating after-dinner speaches: "*testis unus, testis nullus*". Many historians believe that it simply related to a classical sentence in the Procedural Rules of the Roman Law. Others, however, sense that there is in it also a subtle hint to the normal and abnormal manifestations of male reproductive physiology.

Be it as it may, there can hardly be any doubt that his other famous maxim: "it is better to have an empty purse than an empty scrotum" expresses a deep socio-endocrinological involvement.

Those scientists, the true scholars, who have read the original papers of Professor Limoncello are profoundly convinced that he was not only the Founding Father of Limoncello Society, but also of modern reproductive endocrinology. His classical definition of puberty was widely quoted in every renowned medieval textbook of reproductive endocrinology with sufficient self-respect. Unfortunately, the two Jesuit fathers who translated it from Sanlimonese into Latin and then into English, were not medically qualified and hence not entirely familiar with the conventional terminology of reproductive endocrinology. In fact, the widespread vulgar definition "Puberty is the period in life between infantry and adultery" has never been accepted by the Academy of San Limone.

The few examples described above convincingly indicate that Professor Limnoncello was a most remarkable individual, far ahead of his (and also our) time. He has never used his first name after a terrible episode when he was kidnapped by the Neapolitan mafia on the request of the *Acqua Santa Corporation,* the sole distributors of authentic holy water, who feared that their business would be damaged by the enormous popularity of Limoncello as a veritable health-promoting beverage. The pious Professor Limoncello was forced to kill three of the gangsters during a successful attempt to escape.

After this tragic event he felt he could no longer use his first name Pius and Society at large showed sympathetic understanding for this during the rest of his life.

Unfortunately, the Limoncello Society, just like the Medical School of Salerno couldn't survive the turbulent and powerful; waves of medieval history too long; it rapidly disintegrated around the time when humankindentered the 13th century. However – and most fortunately for us – the Limoncello Family kept the secrete recipe of the magic elixir also after moving from Salerno to Capri in order to seek protection among its cliffs from the wild attacks of the saracenic buccaneers. I t is easy to understand that – under these conditions – the distribution of Limoncello remained extremely restricted during many centuries to come, until – very recently – in 1986 some visionary scientists decided to re-establish the Limoncello Society and to make its benefits (including the wonderful potion) available to the entire human race. As far as the commercial distribution of the Limoncello elixir is concerned, this was left to the official representatives of the Autonomous Republic of SanLimone.

The year 1986 signals not only the re-establishment of the Limoncello Society, but also the premature death of a great artist of the second half of the 20th century, Joseph Beuys; his painting entitled "*Capri-Batterie, nach 1000 Stunden Batterie auswechseln*"("Capri-battery, replace it after 1000 hours"), which was originally prepared for Edizione Amelio Napoli, is reproduced below (Figure 41), not only because of its particular relevance to Capri, but also because of its philosophical use of the Limoncello Society's two major symbols: *lemon and light.*

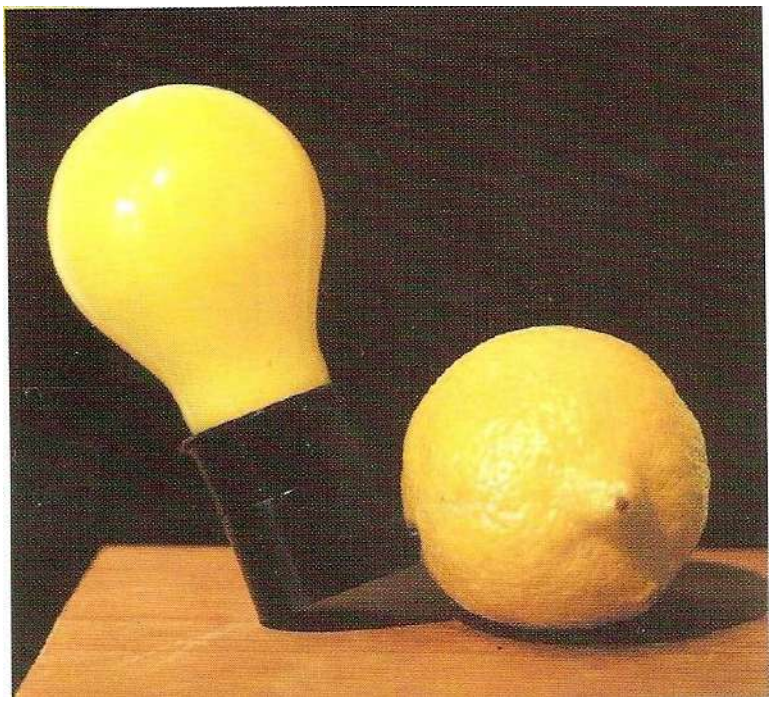

Figure 41 PAINTING BY JOSEPH BEUYS ENTITLED “CAPRI-BATTERIE, NACH 1000 STUNDEN BATTERIE AUSWECHSELN” (“CAPRI-BATTERY, REPLACE IT AFTER 1000 HOURS”).

Reprinted from: Diczfalusy E., “In San Limone we trust!”, 73-80

A BRAVE NEW WORLD FOR AGEING POPULATIONS; *QUO VADIMUS ?*

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As it was presented in Arad, December 2, 2009, with the ocasion of receiving the title Doctor Honoris Causa from the West University "Vasile Goldis" Arad

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Sors bona nihil aliud

Miklòs Zrinyi
(1600 –1664)

There is false modesty,

but there is no false pride.

Jules Renard,
Journal, 1909.

PRAISE shames me,
for I secretly beg for it.

Rabindranath Tagore
"Stray Birds"

Knowledge is proud that he has learned so much; Wisdom is humble that he knows no more.

William Cowper (1785)

... The only wisdom we can hope to acquire is the wisdom of humility: humility is endless.

T.S. Eliot
East Coker

When the amount of our knowledge increases, with it also increases the perimeter between the known and the unknown so that we get a much wider horizon of all the things we still have to learn.

E. Diczfalusy 2009

It's all that the young can do for the old, to shock them and keep them up to date.

George Bernard Shaw
Fanny's First Play (1911)

L´homme arrive novice à chaque âge de la vie.

(One is a beginner at each age.)

S.R.N.Chamfort
(Maximes et pensées)
(1805)

At fifty you begin to be tired of the world and at sixty the world is tired of you.

Axel Oxenstierna
(1583 - 1654)

The day has only 24 hours...
Yes, for other people.

For me it has only 2; the rest is stolen from me by others.

E. Diczfalusy 2009

Behold,
I do not give lectures or a little charity,
When I give
I give myself.

Walt Whitman (1819-1892)
Song of Myself

A concise and modern curriculum vitae

At forty I lost my illusions,
At fifty I lost my hair,
At sixty my hope and teeth were gone,
At eighty life has clipped my claws,
I'm bent and bowed and cracked;
But I can't give up the ghost because
My follies are intact.

E.Y. Harbourg
Gerontology or Springtime for Senility, 1965

...omnem crede diem
tibi dilexisse supremum...

(Hold for yourself the belief
that each day that dawns is your last)

Quintus Horatius Flaccus (65 – 8 BC)
Epistles, bk.1.II (13 BC)

Albert Einstein says that he has learned more from Fyodor Dostoevski than from all great physicists of the world...

...and I have learned more philosophy from my beloved quotations than from all textbooks of philosophy.

E. Diczfalusy 2009

There is no proverb which is not true.

Cervantes, Don Quixote

Fundamental truths are characterized by the fact that the opposite proposition is also a fundamental truth.

Thomas Mann

There are no whole truths;
All truths are half-truths.
It is trying to treat them as whole truths that plays the devil.

A. N. Whitehead,
Dialogues, 1954, Prologue

Pure truth hath no man seen nor e'er shall know.

Xenophanes of Colophon (570 - 480 BC)

Ego mundi civis esse cupio.
(I wish to be a citizen of the world)
Erasmus, 1522.

However you can't become a citizen of the world without having friends all over the world, simply because without friends you will remain an eternal alien.

E. Diczfalusy, 2009

Once we dreamt that we are strangers,
We wake up to find that we are dear
to each other.

Rabindranath Tagore
(Stray Birds, 1916)

Why is Medical Science the most important one ?

Because - as Albert Einstein says -
"The creations of our mind
shall be a blessing
and not a curse
to mankind"

Friendship
is the only cement
that will ever
hold the world together.

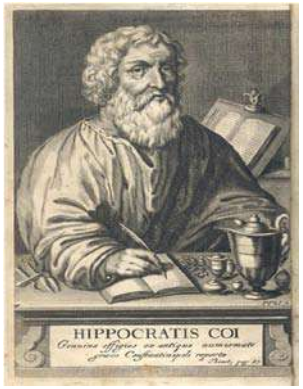
Woodrow Wilson

Science is a dialogue between mankind and nature, the results of which have been unpredictable.

Ilya Prigogine
The end of certainty.
(1997)

**When an idea meets the exigencies of an epoch,
it becomes stronger than political power; it becomes
the common property of humankind and it may resist
the historical forces of destruction for a long time**

**Monnet, J.
Mémoires (1978)**



Hippocrates

**Perhaps the greatest intellectual achievement
of *Homo Sapiens;* the Medical School**

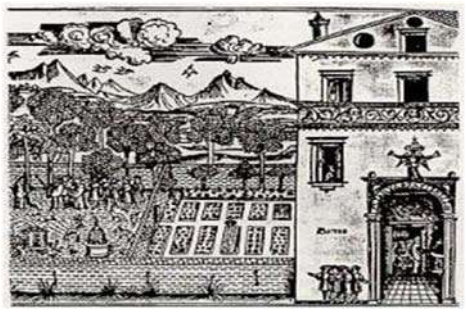
School	Established
Kos	30 BC
Salerno	830 AD
Bologna	1088
Paris	1150
Oxford	1167
Cambridge	1209
Salamanca	1218
Montpellier	1222

E.Diczfalusy 2009

TEXTO ORIGINAL EM GREGO

The

Hippocratic Oath



The supposed building of the Scuola Medica Salernitana (also known as Centro Studi Hippocratica Civitas)

It has been said that although God cannot alter the past, historians can.

Samuel Butler: Erewhon

Trotula de Ruggiero (Trotula of Salerno, 1050 - 1097), author of the first textbook of Obstetrics and Gynaecology, entitled *"De passionibus mulierum ante in et post partum".*

(from a manuscipt from the late 12th or early 13th century)

Illustration from the manuscript of Trotula de Ruggiero; *"De passionibus mulierum"* (12th - 13th century), or "Hip-bath is good for you !"

Illustration from the manuscript of Trotula de Ruggiero;
***"De passionibus mulierum"* (12th - 13th century),**
or "Hip-bath is good for you !"

Diploma rilasciato nel 1778 dal Priore Nicola Giro a Casimiro Greco di Salerno. Salerno, Archivio di Stato

Diploma rilasciato nel 1687 dal priore Antonio Mazza a Giuliano Guarino da Solofra.
Salerno, Archivio di Stato

Fonte:
La Scuola Medica Salernitana. Storia, immagini, manoscritti dall'XI al XIII secolo, a cura di M. Pasca, Electa Napoli, 1988

Progress is the way of life…

I have graduated from the Medical Faculty of the University of Szeged in 1944, more than 60 years ago. Compared to our colleagues from Salerno nine hundred years before, how much more medicine did we know than they; and how very little, compared to the medical knowledge of today !

E. Diczfalusy 2006

Examples of the oldest and the more recent Medical Schools in Central Europe;

School	Established
Praha	1348
Crakow	1364
Wien	1367
Pécs	1367
Novi Sad	1960
Arad	1990

E.Diczfalusy 2009

Mankind
cannot get on without a
certain amount of absurdity.

Arthur Schopenhauer
(The Christian System)

Science is not
the opposite of mysticism;
it is just a very special subgroup of it.
Scientists are no supermen,
only the worshippers
of an other kind of religion.

E. Diczfalusy 2009

There are seven billion human beings
and as many interpretations of each word.

What Schopenhauer calls *Absurdity*
I call *Mysticism*.

E Diczfalusy 2009

Science must begin with myths,
and with the criticism of myths.

Sir Karl Popper
in
British Philosophy in the Mid-Century (1957)
(ed. C.A.Mace)

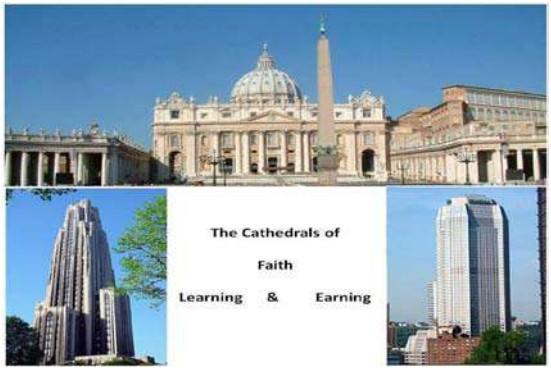
Science without religion is lame,
Religion without science is blind.

Albert Einstein
Science, Philosophy and Religion,
A Symposium 1941, Ch. 13

“We have just religion enough
to make us hate,
but not enough
to make us love one another”.

Jonathan Swift ,1720

Three Cathedrals, or perhaps even four ?



The wonderful Cathedral of *Egodulia*

Egodulia = a pathologically exaggerated form of egomania or self-adulation.

This cathedral is still under construction and continuous perfection. When it is finished it will be the biggest among all cathedrals.

It will have three essential pillars:

I, Me and Myself

E. Diczfalusy 2009

Si possis recte,
si non,
quocomque modo rem.

(Quintus Horatius Flaccus)
Alexander Pope's translation:

Get place and wealth, if possible with grace;
if not, by any means get wealth and place.

Thy money perish with thee.

Bible: Acts of the Apostles
ch.8, v.20 (to Simon Magnus)

"Whoever prefers wealth, or might to the possession of good friends , thinks amiss."

Euripides : Heracles (420 BC.)

In my lifetime, I have seen more progress in *science,* than *all* scientists of *all* preceding periods together since the dawn of history. I profoundly believe that *without medical science life would be a mistake…*

E. Diczfalusy

Most drugs used today by millions and millions of fellow-men and women have been developed in our lifetime and many more are in the coming…

"What is past is prologue"

(The Tempest, II.i.261)

The nature of science is self-correcting:

"DIES DIEM DOCET"

(one day teaches the other)

Publilius Syrus (c.a 100 A.D.)

and

each generation continues and improves the achievements of the preceding one.

E. Diczfalusy

To every new epoch in history belongs a new spiritual and philosophical content and I think that the philosophical content of the 21st century will be anthropocentric; it will place focus on human creativity and on ethical issues emanating from it.
It will also deconstruct the old deterministic worldview.

E. Diczfalusy 1994

What is *Anthropocene* ?

It is a neologism coined in the year 2000 by Nobel laureate Paul Crutzen to indicate the most recent period in the life of the Earth, when – for the first time - the impact of human activities on the environment reached the same dimensions as natural forces.

OUR EARTH

The most "recent" stages in its history of 4 600 millions of years or more.

EPOCH	**Million years ago (mya)**	**Human relevance**
Pliocene	**5.0 - 1.8**	**1st primitive hominids (appr. 4.4 mya)**
		Homo habilis **(appr. 2.4 - 1.5 mya)**
Pleistocene	**1.8 - 0.012**	***Homo erectus*** **(appr. 1.8 – 0.3 mya)**
		Homo sapiens neanderthalensis **(appr. 0.2 – 0.03 mya)**
		Homo sapiens sapiens **(appr. 0.1 mya)**
Holocene	**0,012 - →**	**Humans aquire agriculture and technology**
Anthropocene	**< 0,0001**	**Humans change the global environment**

***Homo sapiens in the anthropocene epoch*.**

For the first time in history, *homo sapiens* is significantly changing his Earth, his environment, and his own development. Will he be able to do it in a responsible way ? In fact, how sapiens is *homo sapiens* today ?

E. Diczfalusy

THE SEED OF CIVILISATION

(Aquisition of agriculture)
or
Chance is a nickname for Providence (Chamfort)

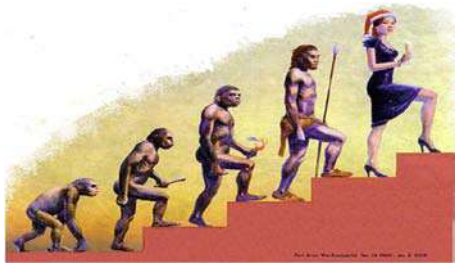
WHEN **Years ago (approx.)**	**WHAT**	**WHERE**
10 000	**Barley, Wheat**	**Middle East**
10 000	**Maize, Squash**	**Mesoamerica**
10 000	**Manioc, Squash,Yams**	**Neotropics in South America**
8 000	**Rice, Millet**	**China**
7 000	**Banana, Sugar cane**	**Papua New Guinea**

800 lifespans can bridge more than 50 000 years.

But of these 800 people, 650 spent their lives in caves or worse;
only the last 70 had any truly effective means of communicating with one another,
only the last 6 ever saw a printed word or had any real means of measuring heat or cold,
only the last 4 could measure time with any precision;
only the last 2 used an electric motor;
and the vast majority of the items that make up our material world were developed within the lifespan of the 800th person.

R.L.Lesher and G.J. Howick , 1966

WHERE ARE WE HEADING ?



THE HUMAN CONDITION

” This is a long road knows no turning.”

Sophokles: Ajax (appr. 430 BC)

D Y S L E X I A M U R A L I S ?

or

Our inability to read the writing on the wall

“The least debated of all phenomena of our day, the surest in its progress, the easiest to foresee ahead and perhaps the most pregnant with consequences is the aging of the population.”

Alfred Sauvy 1963

Old age is the most unexpected of all the things that happen to a man.

L. Trotsky
Diary in Exile, 1935

Aging is one of the major achievements of this century. Yet it is so often approached not as an achievement, but as a problem, the "tidal wave that is going to swamp us all"

Alex Kalache,
Paris 2000

For Age is Opportunity no less
Than youth itself, though in another dress,
And as the evening twilight fades away,
The sky is filled with stars, invisible by day.

H.W.Longfellow
Morituri salutamus (1875)
Stanza 24.

No man loves life like him that´s growing old.

Sophokles (496?-406 BC)
(*Acrisius*, Fragment 64)

François de la Rochefoucauld in 1664 tells us, that when we grow old we become more stupid and more wise at the same time.
He carefully avoids mentioning the comparative aspects.
One can always hope that the relationship between these two properties will be 50/50 and not 99 to 1.

E. Diczfalusy, 2009

DEVELOPMENT OF THE GLOBAL POPULATION

YEAR	MILLIONS
14	256
1000	280
1500	500
1920	1 860
1958	2 919
2000	6 115
2005	6 512

United Nations, 2008.

In my lifetime
I have seen the birth of
ANOTHER TWO WORLDS,
equal in numbers, needs,
aspirations, hopes and dreams.

E. Diczfalusy

GROWTH OF POPULATIONS (millions)

REGION	YEAR		
	1950	2005	2050
WORLD	2 529	6 512	9 150
AFRICA	227	921	1 998
ASIA	1 403	3 937	5 231
EUROPE	547	729	691
LATIN AMERICA and THE CARIBBEAN	167	556	729
NORTHERN AMERICA	172	335	449
OCEANIA	13	34	52

United Nations 2008

All projections are uncertain, but some of them are less uncertain than others.

E.Diczfalusy

POPULATION AGED 65 YEARS AND OVER

(Percentage of total.)

REGION	YEAR		
	1950	2005	2050
WORLD	5.2	7.3	16.2
AFRICA	3.3	3.4	7.1
ASIA	4.1	6.2	17.3
EUROPE	8.2	15.9	27.4
LATIN AMERICA and THE CARIBBEAN	3.5	6.3	19.5
NORTHERN AMERICA	8.2	12.5	22.0
OCEANIA	7.3	10.2	18.7

United Nations 2008

POPULATION AGED 65 YEARS AND OVER

(Percentage of total. Selected countries)

COUNTRY	YEAR		
	1950	2005	2050
Italy	8.1	19.6	33.3
Spain	7.3	16.8	31.8
Romania	5.3	14.8	28.5
Serbia	7.6	14.7	23.7
Albania	7.0	8.7	21.5
India	3.1	4.6	13.7
Europe	8.2	15.9	27.4
Africa	3.3	3.4	7.1

United Nations 2008

POPULATION AGED 80 YEARS AND OVER

(Percentage of total.)

REGION	YEAR		
	1950	2005	2050
WORLD	0.6	1.3	4.3
AFRICA	0.3	0.4	1.1
ASIA	0.4	1.0	4.4
EUROPE	1.1	3.5	9.6
LATIN AMERICA and THE CARIBBEAN	0.4	1.2	5.5
NORTHERN AMERICA	1.1	3.6	8.0
OCEANIA	1.0	2.6	6.5

United Nations 2008

POPULATION AGED 80 YEARS AND OVER IN SELECTED COUNTRIES

Estimates and projections

(Percentage of total.)

COUNTRY	YEAR		
	1950	2005	2050
Japan	0.4	4.9	15.6
Germany	1.0	4.3	14.1
Italy	1.0	5.1	13.4
Austria	1.2	4.3	12.1
France	1.6	4.6	11.3
Spain	1.0	4.3	11.3
Romania	0.6	2.4	7.6
Hungary	0.8	3.3	6.9
Serbia	1.2	2.4	6.3

United Nations 2008

SEX RATIO AT 80 YEARS AND OVER

(men per 100 women. Selected countries)

COUNTRY	YEAR	
	2005	2050
India	87	73
China	67	68
Greece	75	65
Serbia	66	60
Romania	55	48
Hungary	44	43
Ukraine	29	35
Russian Federation	24	35
Europe	43	58
Africa	69	64

United Nations 2008

INCREASES IN LIFE EXPECTANCY AT BIRTH

(by sex, years)

REGION	YEAR			
	1950 - 2005		2005 - 2050	
	Male	Female	Male	Female
WORLD	20.2	21.8	7.9	8.1
AFRICA	15.4	15.3	12.8	13.9
ASIA	26.5	28.9	7.6	8.1
EUROPE	8.1	11.1	7.4	5.4
LATIN AMERICA and THE CARIBBEAN	20.5	23.6	6.5	6.2
NORTHERN AMERICA	10.9	9.6	4.1	4.5
OCEANIA	16.0	16.0	5.9	5.3

United Nations 2008

MEDIAN AGE

(years, in different regions)

REGION	YEAR		
	1950	2005	2050
WORLD	24.0	27.9	38.4
AFRICA	19.2	19.1	28.5
ASIA	22.3	27.4	40.2
EUROPE	29.7	38.9	46.6
LATIN AMERICA and THE CARIBBEAN	20.0	26.0	41.7
NORTHERN AMERICA	29.8	36.2	42.1
OCEANIA	28.0	32.2	39.1

United Nations 2008

MEDIAN AGE (years)

(in selected countries)

COUNTRY	YEAR		
	1950	2005	2050
Japan	22.3	43.1	55.1
Bosnia and Herzegovina	20.0	37.3	52.2
Poland	25.8	36.8	51.0
Portugal	26.2	39.3	50.4
Bulgaria	27.3	40.8	49.5
Romania	26.1	36.7	49.5
Slovenia	27.7	40.1	48.7
Hungary	29.9	39.1	46.6
Czech Republic	32.7	38.8	46.2
Serbia	25.8	36.6	44.7
Albania	20.6	28.5	44.3

United Nations 2008

POPULATION OF CHILDREN

(0-14 years; percentage of total)

REGION	YEAR		
	1950	2005	2050
WORLD	34.1	28.4	19.6
AFRICA	41.7	41.2	27.3
ASIA	36.1	28.2	17.9
EUROPE	*26.2*	*15.9*	*15.0*
LATIN AMERICA and THE CARIBBEAN	40.2	29.8	17.0
NORTHERN AMERICA	27.2	20.5	16.9
OCEANIA	29.9	25.0	19.1

United Nations 2008

POPULATION OF CHILDREN

(0-14 years; percentage of total)

(Selected countries)

COUNTRY	YEAR		
	1950	2005	2050
India	37.5	33.1	18.2
Albania	38.9	26.5	16.2
Serbia	28.1	18.4	15.8
Hungary	25.1	15.5	14.8
Romania	29.5	15.6	13.3
Bosnia and Herzegovina	37.8	16.6	11.8
Europe	26.2	15.9	15.0
Africa	41.7	41.2	27.3

United Nations 2008

TOTAL FERTILITY RATE PER WOMAN

(replacement level: 2.1 children per woman)

YEAR	WORLD	EUROPE
1965	4.8	2.4
1975	3.8	2.0
1985	3.4	1.8
1995	2.8	1.4
2005	2.6	1.5

United Nations 2008

TOTAL FERTILITY RATE

(Replacement level: 2.1 children per woman)

In 1965 total fertility rate in Europe was 2.4 and- in the 26 countries studied - it varied between 2.0 and 5.1.
In 2005 it was below replacement level (<1.9) in all countries.

United Nations, 2008.

Quo vadimus ?

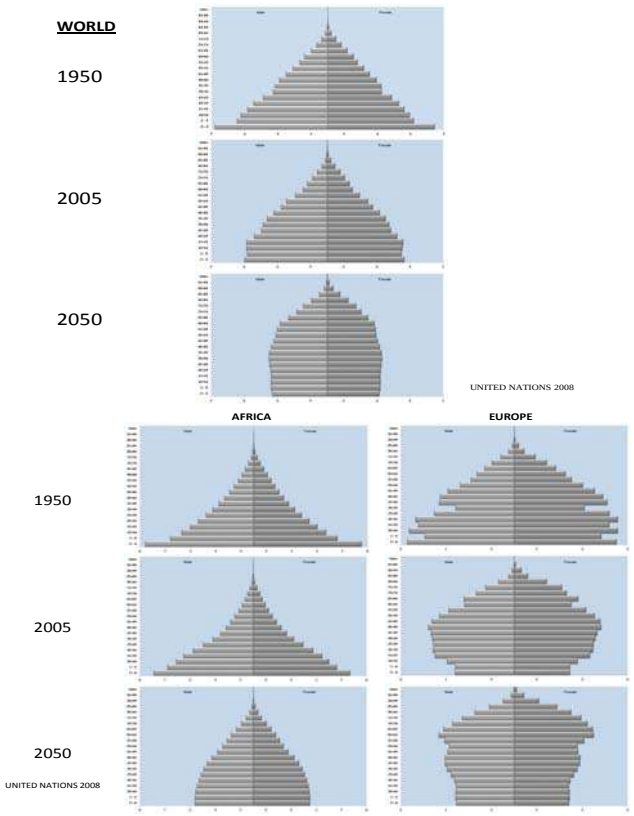
Never before have birth-rates fallen so far, so fast, so low, for so long around the world. The potential implications – environmental, economic, geopolitical and personal – are both unclear and clearly monumental, for good and for ill.

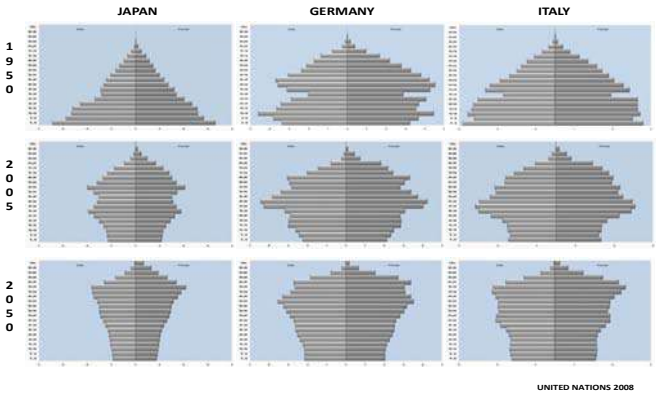
Ben J. Wattenberg
The Population Explotion is Over
in New York Times Magazine
November 23, 1997 Sunday Late Edition

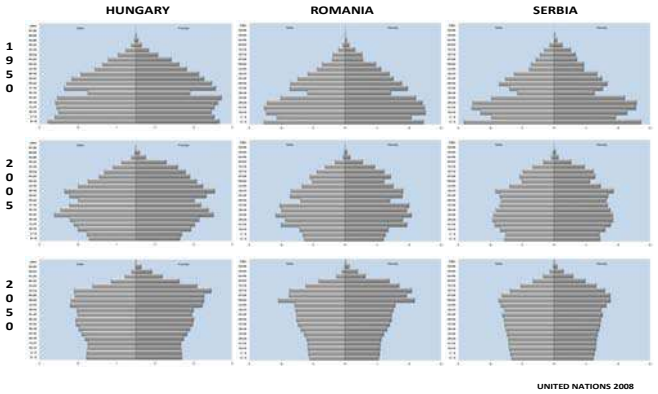
PERCENTAGE CHANGES IN THE PROJECTED POPULATION OF SELECTED EUROPEAN COUNTRIES BETWEEN 2005 AND 2050

COUNTRY	PERCENT
Bulgaria	**-30.3**
Ukraine	**-25.4**
Romania	**-20.1**
Russian Federation	**-18.9**
Hungary	**-11.4**
Serbia	**-6.7**
Sweden	**+16.6**
Spain	**+19.0**
United Kingdom	**+20.1**

United Nations 2008







Need for Institutional Reform

Many of our Institutions are in crisis, because they are catering for for the needs of yesteryear; for a population structure that doesn't exist any more.

E. Diczfalusy 2009

Some institutions catering for the needs of yesteryear

Social security
Health care
Housing
Education
Nation - state

E. Diczfalusy

Given the rising proportions of the elderly in the population and the increasingly effective and expensive technologies that will be applicable to them, we are at the edge of a new and endless frontier of ethical inquiry.

D.Callahan (1988)

So what is the problem?
Money, money, money
and poverty, poverty and poverty…

E. Diczfalusy 2009

Question: BUT WHAT CAN I DO?

Answer : NOBODY KNOWS
WHAT HE CAN DO
TILL HE TRIES.

Publilius Syrus
(Maxims786)

Never doubt that a small group of thoughtful, committed citizens can change the world; indeed, it is the only thing that ever has.

Margaret Mead
Quoted by The Goldman Environmental Foundation
The Herald Tribune April 20, 1998

SEVEN OBSTACLES ON HUMANKIND'S "GOLDEN ROAD TO SAMARKAND"

Population growth
Ageing of population
Environmental degradation
Global unemployment
Poor health
Persistent poverty
Intra-species aggression

E. Diczfalusy, 1997

A world of contrasts and hope.

Scientific progress
Political confusion
Globalisation
Fragmentation
Fluctuation
Unpredictibility
New realities
New opportunities
Need for Humility
and
Infinite HOPE

E. Diczfalusy

Empathy, Science, Hope

Happiness is the only thing
we can give others
without having it ourselves.

Queen Elisabeth II.

Money doesn't make you happy,
but giving it away for a good purpose
helps a great deal

E. Diczfalusy 2009

Whenever you do good for others,
perhaps only 2% of them will remember
and be grateful.
However, the greatfulness of that 2%
may change your life.

E. Diczfalusy 2009

Fate il bene
e
fatelo bene

(Do well and do good !)

It is not a disaster to be unable to capture your ideal,
but it is a disaster to have no ideal to capture.
It is not a disgrace not to reach the stars,
but it is a disgrace to have no stars to reach for.
Not failure, but low aim is sin.

Dr. Benjamin Elijah Mays (1894 - 1984)
(president of Morehouse College 1940-1967)

Fatelo bene per viverlo bene!

(Do good and live well !)

E. Diczfalusy 2009

The Future of Universitatea De Vest:”Vasile Goldis ”ARAD

- **I am profoundly convinced that the university will not only endure but it will prevail because its dedicated staff does not ask what the university can do for them but what they can do for the university**

THOUGHTS OF FORMER STUDENTS AND FRIENDS

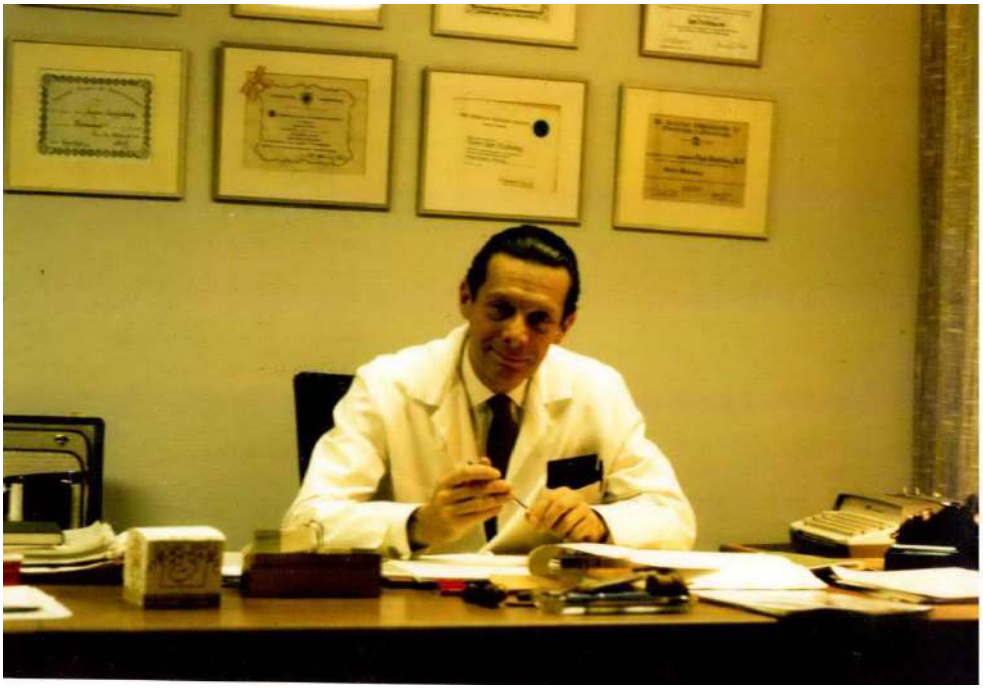
Biran Affandi,

MD, PhD
Professor of Obstetrics-Gynecology at University of Indonesia, Jakarta

"Professor Egon Diczfalusy represents a father of a scientific community which has been growing and developing by itself since he graduated as a doctor. Today the tree of scientific community- consists of (mainly) his students – and has been matured enough to dedicate its fruits to the world.

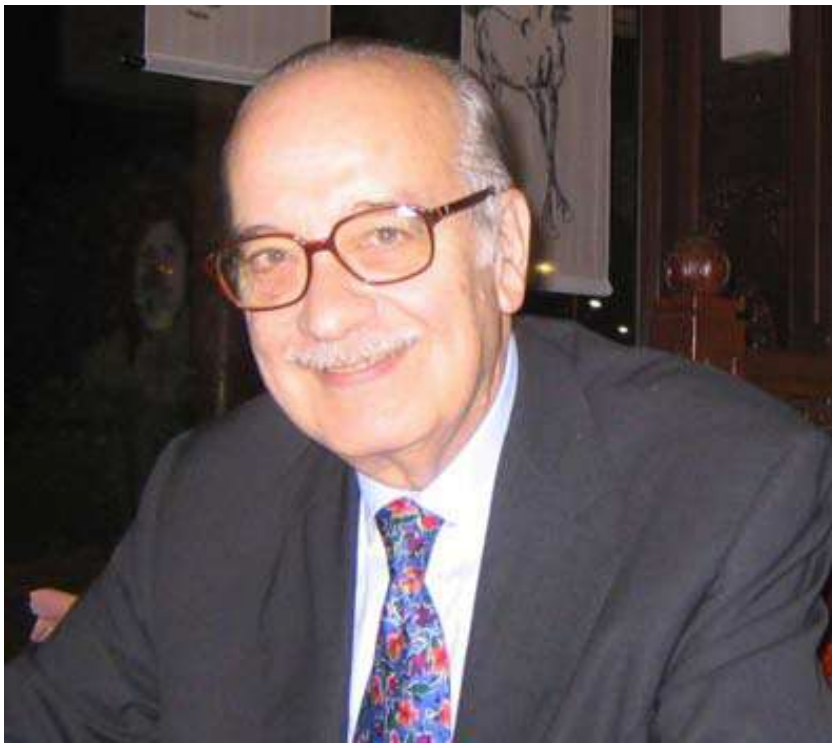
Professor Egon Diczfalusy is a father, a teacher, a pioneer and a friend of medical scientists."

Jakarta, Indonesia, on the 16th of July 2010



Giuseppe Benagiano,

MD, PhD
FACOG(hon), FRCOG(ad eundem) Hon Sen(Szeged)
Professor Emeritus, Sapienza, University of Rome



"*Egon Diczfsalusy is an eminent scientist, a humanitarian, a visionary and a man who - throughout his long life cared for the underprivileged.*

His life has been dedicated as much to science and research as to improving the reproductive health of women and men of the world.

For me, Egon Diczfalusy has been a figure so important as to be second only to my father.

I owe to him my introduction to research, to the world of the United Nations and to the intricacies of international public health."

Geneva, Switzerland, on the 16th of July 2010

Ian D. Cooke,

MD, PhD
Professor Emeritus at University of Sheffield, UK
Director of Education
International Federation of Fertiliy Sciences

"It is difficult in 10 words, so here are more.

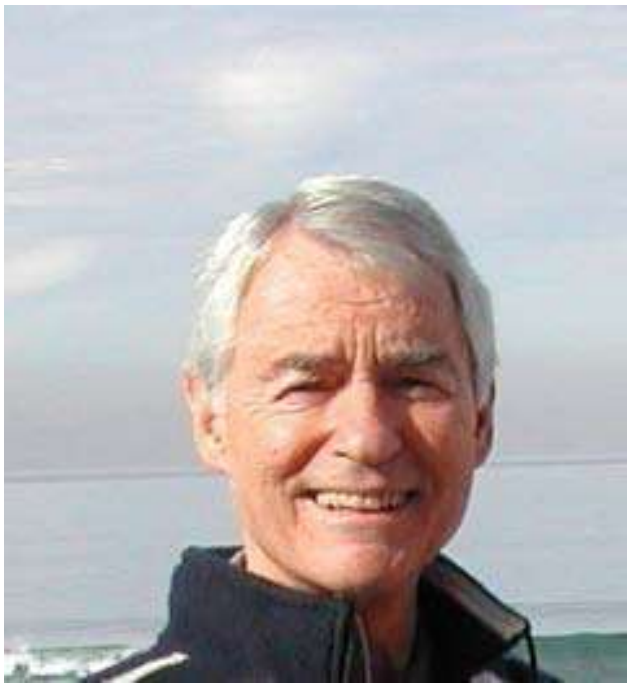
An innovative scientist and a cultured man who first described the functioning of the human feto-placental unit"

It is quickly done, perhaps the best way to highlight the essence of the man as one remembers him and his impact.

Best wishes,

Ian"

Sheffield, UK, on the 16th of July 2010



Ronald J Pion,

Md, PhD
Clinical Professor of
Obstetrics-Gynecology
UCLA School of
Medicine

"*With fond memories of the Hormone Laboriet @ Karolinska Sjukhuset:*

In my second year of a USPHS fellowship (July 1963) under the auspices of the department of OB-GYN at the UCLA School of Medicine I was offered an opportunity to study under the direction and mentorship of Dr. Egon Diczfalusy. I had completed a year's study of androgenic steroids with Dr. Hans Zimmer at UCLA. To further prepare me as a research scientist in academics it was suggested that I pursue additional research in estrogens. Egon's lab was renowned for the work that he guided in evaluating the steroid milieu and relationship between the maternal/placenta/fetal units.
My early work in association with Dr. Bob Jaffe and laboratory staff –Berit and Ulla - involved analyzing blood and urine samples that traced the relationship of injected radioactive isotopes into the identified umbilical

cord vessels at the time of therapeutic abortion. Results pointed us in the direction of analyzing pregnenolone/progesterone pathways – an opportunity that we had not anticipated pursuing. Dr. Diczfalusy's intellectual curiosity permitted our exploration of these precursors and their relationships. The work environment among the other research scientists gathered from around the world was stimulating and Egon's passion for seeking new knowledge was 'contagious'. My year's experience remains a highlight of my career.

Egon is the 'titular' G-dfather to our second child.....Dana Laura..... Born December 15 1963 at the Karolinska Sjukhuset

Egon is my 'heroic' mentor-learning model for me to emulate."

Los Angeles, USA, on the 18th of July, 2010

Paul Brenner,

MD, PhD
Professor and Vice Chairman
Department of Obstetrics and Gynecology
Keck School of Medicine the University of Southern California

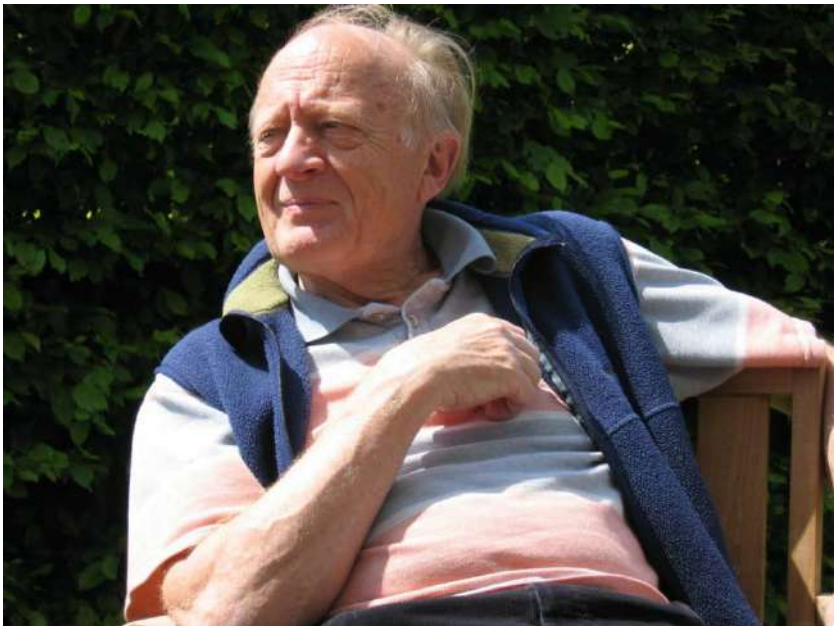
"Professor Diczfalusy was very important in directing my research efforts early in my career. Even more than this, as a mentor he taught me the moral and ethical standards to be a success in life. He was much more than a remarkable scientist, he was a role model and in many ways a "father" figure.

In ten words: scholarly, brilliant, kind, professional, dedicated, unselfish, modest, supportive, leader and charismatic"

USA, on the 18th of July 2010

Ivo Brosens

FRCOG
Emeritus Professor of Obstetrics and Gynaecology at the Catholic University Leuven, Belgium



"*In the first place it is a great honor for me to be part of the close friends of Professor Egon Diczfalusy.*
My admiration for Egon started in the late 1960s when he and his collaborators published in Acta Endocrinologica the classical studies leading to the concept of the foeto-placental unit. At that time I was a resident at the Catholic University of Leuven in Belgium and involved in the investigation of the role of the placental bed spiral arteries in obstetrical syndromes like preeclampsia and fetal growth retardation. The work of Egon has been the basis of our current understanding of what can be called the maternal-placental- foetal unit.

In the late 1990s when I was involved in ISGE Training program in gynecological endoscop, I had the opportunity of meeting many of his friends in Hungary and Romania and learned how important it is to go and meet other colleagues in their country.
For me is Egon the outstanding person who never stopped learning and teaching."

Leuven, Belgium, on the 19th of July 2010

Philip Troen,

MD, PhD
Professor of Medicine University of Pittsburgh School of Medicine
Assistant Dean for Medical Student Research
Physician-in-Chief Emeritus Montefiore University Hospital.

"Dear Egon,

I am very pleased to have this opportunity formally to acknowledge again the great opportunity you gave me and the important experience I had at the Karolinska with you.

Here are my brief responses to the request by Cristi:

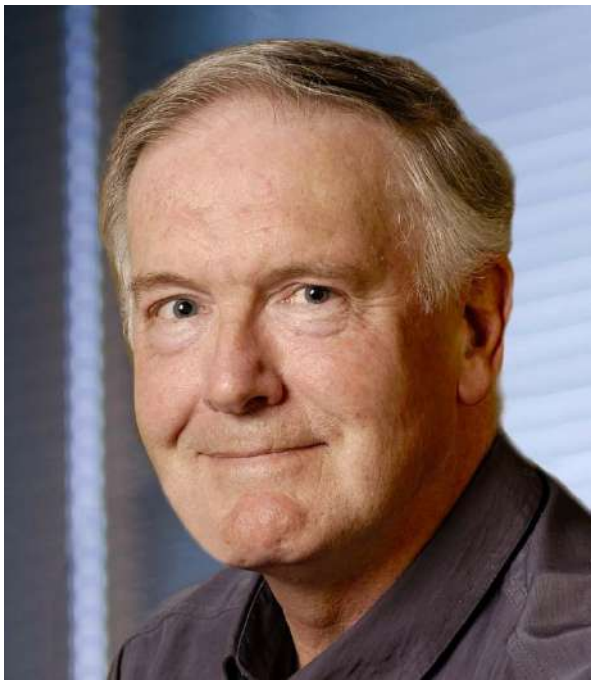
I met Egon at a Laurentian Hormone Conference following the recommendation of Al Albert with whom several years previously I had an endocrine fellowship at the Mayo Clinic including research on the endocrine function of the human placenta. Egon was kind enough to invite

me to join him in Stockholm. This invitation allowed me to receive a Guggenheim Fellowship to make the trip to Stockholm feasible.
The time (1960-1961) spent with Egon in his laboratory represented a major event in the development of my academic career. He provided me with the opportunity, encouragement and support (including space for my 100 tube countercurrent distribution machine) to establish myself further as a productive endocrine researcher. An important aspect of my time in the Hormone Laboratory with Egon was the discipline of analyzing the data and preparing the paper. Particularly rewarding was the writing with him of our major review, "Endocrine Functions of the Human Placenta", for Vitamins and Hormones.
His friendship to my family and me was invaluable in making our stay in Stockholm both pleasant and successful.
I believe the following words capture the essence of this remarkable man:

Dedicated, disciplined, focused
Creative, thorough, organized
Supportive, thoughtful
Literate, intelligent

Egon,
Your picture with its inscription still hangs next to one of Al Albert in my office.
My sincere thanks and appreciation.
Best wishes,
Phil"

Pittsburg, USA, on th 19th of July 2010



David Robertson,

MD, PhD
Principal Research Fellow
NHMRC
Associate Professor of
Obstetrics and Gynecology,
Monash University
Prince Henry's Institute of
Medical Research
Melbourne, Australia

"*I was a research fellow (initially a Ford Foundation Fellow) in Professor Diczfalusy's laboratory from October 1971 until February 1979 when I returned to Australia. This was a very important and by far the most enjoyable time in my research career. I learnt a lot about undertaking research. Professor Diczfalusy emphasized the need for method development as an essential prerequisite in undertaking research as well as the need for project planning with sufficient power to obtain a clear result. In addition, the research should have tangible health relevance even if it is long term. These lessons I have employed throughout my research life. The enjoyable aspects relate to the calm and stable laboratory atmosphere where strong personal relationships between researchers from many countries and staff were fostered. Professor Diczfalusy was my supervisor and mentor during my PhD candidature whose support I highly valued. We published over 20 papers together.*

In ten words:A statesman with a world vision, an excellent scientist, a fair and reasonable man"

Melbourne, Australia, on the 20th of July 2010

Wanda Holmberg

"He is my stepfather and the man who gave my mother a happy life. He also shows that you need not be old because you are aged.

In ten words: Dedicated to science and friends, generous, humorous, caring, multilingual globetrotter, music and art lover."

Norway, on the of 20th July 2010

Petru Chitulea,
MD, PhD
Assistant Professor of Obstetrics-Gynecology at University of Oradea

"The Power of Ideas

Medicine has represented, throughout the history of humankind, a branch of science profoundly anchored in humankind and in the life of the communities, but also of the human culture, in general, of all times.

The social role of the doctor, as a holder of all the information connected with the human being's physiological and psychological needs, as a healer of physical suffering, has represented an important mark in all societies, starting with the dawns of human history until today.

The history of medicine, from the healing shaman to the ultra-specialized doctor nowadays, has been interspersed with a series of technical, pharmacological, anatomo-structural, physiopathological discoveries, but, especially, with the appearance of some new concepts, innovating ideas, which propelled it. If technical and, in general, material discoveries were relatively easily accepted by the members of the medical

corpus, the new concepts were always met with acerb opposition, an opposition which was worth a better cause. Throughout history we find examples of ideas, new in their time, ideas that we nowadays consider self-explanatory, which have become common places of medical thinking, but which in their time caused an uncommon opposition both from the members of the fellowship, and from society, in general.

Thus, the study of anatomy based on dissection, culminating with Andre Vesale's "De humani corporis fabrica", the concept of vaccination against infectious diseases, whose promoter was the English doctor Jenner, Semmelweis' concepts of asepsis and antisepsis, Pasteur's discovery regarding the microbial aetiology of infectious diseases, the concepts of holistic and psychosomatic medicine, Freud and Jung's theories, the concept of family planning and contraception, to Egon Diczfalusy's concept of the feto-placental unit, have met an opposition sometimes active, other times passive, but always consistent.

The discovery of the fact that pregnancy is not represented by the foetus together with its "annexes", that the foetus, the placenta, the umbilical chord and the amnios form a unitary whole, all of them being parts of the fecundated human egg, and that none of them has any sense in the absence of the others, having an equal importance in the accomplishment of the future human being that is going to be born, shocked the community of the obstetricians, despite evidence.

Formulated in 1970, the idea of the feto-placental unit, although it is the expression of an elementary logic, has not been totally adopted even after 40 years, medical students being taught even now, in some universities, about "the foetus and its annexes".

Despite all opposition to new ideas and concepts, medicine has progressed in the last two millennia, exactly because of them. The great medical progresses, which changed the face of medicine, have always been connected to the implementation of the new concepts, despite the initial opposition they were subjected to.

"When an idea corresponds to the necessities of the age, it stops belonging to those who invented it and becomes more powerful than the power-holders and it becomes a common property of humankind." - E. Diczfalusy , 2002.

That is why I consider that accepting the title of Doctor Honoris Causa of the University of Oradea, by PhD Professor Emeritus Egon Diczfalusy, represents a great honour for our university, for all the members of the academic community, and last but not least, for all the members of the medical corpus in our city and county.

Professor Diczfalusy's connection with Oradea started in 1994, when he responded to an invitation made by PhD Professor Emeritus Academician Ioan Munteanu, at the time the President of the Romanian Society of Obstetrics-Gynaecology, by PhD Prof. Eng. Teodor Maghiar, the founder rector of the University of Oradea, and also by me, as the President of the Romanian-Hungarian Congress of Obstetrics-Gynaecology (May 1994), and when he was awarded the title of Honorary Member and the medal of the Romanian Society of Obstetrics-Gynaecology.

Starting then, professor Diczfalusy's connection with the Romanian gynaecologists has been a constant, materialized through multiple meetings at scientific manifestations in our country, at scientific manifestations all over the world, where he always displayed, sincerely and warmly, his friendship and appreciation both for his Romanian fellows, and for the Romanian medical education.

Throughout all these years, I have had the privilege of knowing closely the professor Diczfalusy's exceptional personality, of coming into contact with his exhaustive scientific erudition, with his encyclopaedic culture, and last but not least with his fine and vivacious wit. As a matter of fact, all the great scientific personalities of our specialty, whom I have had the opportunity of meeting, have always expressed a unanimous admiration and sympathy.

That is why Professor Egon Diczfalusy's return to Oradea, on the occasion of being awarded the title of Doctor Honoris Causa of our university, will always represent a glory, a memorable and bright landmark in the history of the higher education in Oradea and also of the entire Romanian scientific community."

Oradea, Romania, on the 21th of July 2010

Balogh Adam,

MD, PhD

Associate Professor, Consultant,
Osteoporosis Clinical Management
and Research UnitDepartment of
Obstetrics and Gynecology
University of Debrecen

"*Thoughts about Professor Egon Diczfalusy*

It is extremely difficult to describe Professor Egon Diczfalusy because of the complexity of his personality. It would be too simple to say, he has been a renaissance man. He is equally knowledgeable of the antiquity, the pre-Columbian art, and the controversial modern age. He has been showing strong sympathy towards Asian people and cultures. I guess, not many of European scientists made more friends, and more devoted ones there. I also recognized his deep affinity to Latin people all over the World, perhaps for their rich history, charming languages, their enjoyment of life and elegance, as I also witnessed him having been working hard for building friendly professional bonds among scientist of Central-Eastern

Europe. Doing so I felt a hint of his hope for better relations of these nations after centuries of troubles.

He has been a real Citizen of World in a good sense proudly, and with perceived duties of this status. He believed in the mission of science in making a better life for mankind. He appreciated the progress of science that has taken place in the world during his life to date. But he warned us watchfully of the evil trends of exploitation, social and gender inequity, continuing enmities, series of wars, their vast expenses and related human suffering throughout the world.

Characterizing Professor Egon Diczfalusy in 10 words / statements. A limited view by one of his past student and present friend. These adjectives are listed from outside to inside, as I imagine him:

1. *Elegant*
2. *Intellectual*
3. *Wit*
4. *Multilingual*
5. *Hard working (still)*
6. *Responsible*
7. *Merciful*
8. *Sentimental*
9. *Alert to problems of individuals and evils of our Merry New World*
10. *Has a sixth sense*

Collected and compiled by heart by

Dr. Adam Balogh"

Debrecen, Hungary, on the 26th of July 2010

Laszlo Kovacs

Professor Emeritus László Kovács MD., PhD., DSc., FRCOG, Szeged University

"I met Professor Diczfalusy and his wife Ann the first time in 1973 during the FIGO Congress in Moscow. In the past almost four decades we have met many times in Hungary and all over the world, and during these years a close friendship developed between us. I look at Egon as my master from whom I have received very much help and advice in the activities of the Szeged WHO Collaborating Centre of Research in Human Reproduction as its Director. In the 1990-ies we have organized several international symposia with his very effective assistance. The "Egon Diczfalusy Lecture Award" that I received in 1999 in Stockholm is one of my most respected medals.

Professor Diczfalusy is highly respected in Hungary as a famous Hungarian-born scientist. He is honoris causa doctor of the Szeged University, invited member of the Hungarian Academy of Sciences, h.c. member of the Hungarian Society of Obstetricians and Gynecologists,

invited member of the Editorial Board of the Hungarian Journal of Obstetrics and Gynecology.

In this year (2010) Professor Diczfalusy will have his 90th birthday. It will be celebrated in his excellent health and activity. I also wish to congratulate him, and wish still many active years and good health!

My short character-painting:

Excellent scientist, fascinating speaker, effective organizer, hearty supporter of young researchers, warm-hearted friend and a real cosmopolitan."

Szeged, Hungary, on the 28th of July 2010

Takeshi Aso

Professor emeritus, Tokyo Medical and Dental University

"Professor Egon Diczfalusy input me the spirit and mission for a medical scientist.

10 words to define Professor Egon Diczfalusy:

*Source of wisdom and origin of energy for activities.***"**

Tokyo, Japan, on the 6th of August 2010

Elisabeth Johannison

MD, PhD; FIAC
Life member of the American Society for reproductive medicine

"In a few words I would like to say that Prof Diczfalusy has been my only and most important mentor in my entire professional life. I started to work in Prof. Diczfalusy's research group already in the early 1960 and my first publication appeared already in 1961 in collaboration with Egon Diczfalusy and Carl Gemzell ("Effect of a single injection of human pituitary follicle stimulating hormone on urinary estrogens and vaginal smear in amenorrheic women" Journal of clinical endocrinology vol;21961) Finally let me say that Prof. Diczfalusy introduced me to the world of the spirit of international research in the field of human reproduction and for me this collaboration has resulted in more than 100 publications (fetal endocrinology the development of the feto-placental unit and the human endometrium). He stimulated a great number of fellows all over the world and he also had a great influence in the development of the WHO programme of research in human reproduction. Last but not least let me mention his creation of the Egon and Ann Diczfalusy foundation for supporting research in reproductive health. The Foundation was introduced in Szeged Hungary in 2008 and it is continually developing. I am convinced that the activity of Prof. Diczfalusy's creative work, his initiatives and his look for the future will continue."

Stockholm, on the 7th of August 2010

Salvatore Mancuso

Professor, Head of the Department of Obstetrics & Gynecology Catholic University, Rome

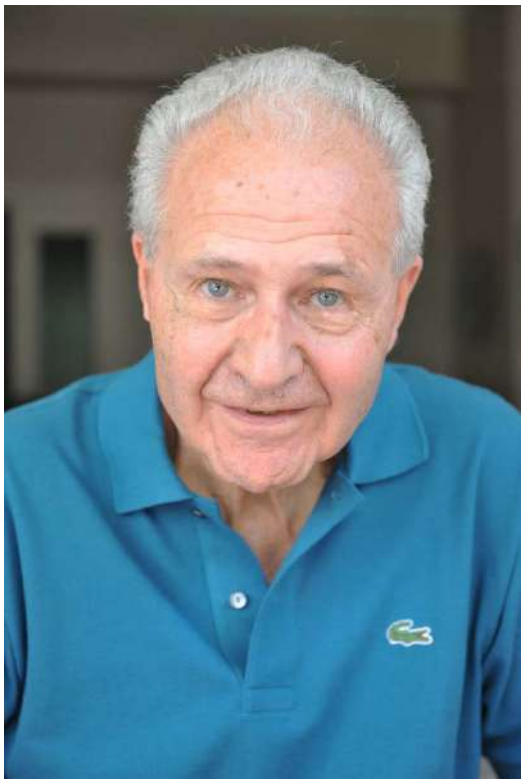
"When I first read his thesis on "Placental Hormones", at the early 60ties, I realized that I could not miss the opportunity to be one of his fellows at the Karolinska group. He accepted my application and once in Stockholm I became during 5 years one of his numerous pupils.

Now, after 50 years, I can say that Professor Diczfalusy provided an essential turning-point to my life, he has greatly influenced my way of thinking, has instilled the philosophy of research and gave the most important incentive to build up my academic career.

Not only he was the perfect mentor in endocrinology and biology, but he greatly contributed to deepen my humanistic culture: yesterday to investigate the early origin of human life and today to search the roots of our western culture, enjoying together the wonderful Greek tragedies.

Scientist able to transfer ideas, culture, knowledge and opinions to a multitude of people."

Rome, Italy, on the 7th of August 2010



Edoardo Menini

Associate Professor of Applied Biochemistry, Chemistry and Biological Chemistry in the Catholic University of Rome
Director of the Hormone Laboratory of the Policlinico Universitario "A. Gemelli". Rome

"A few days ago my close friend Ninny Mancuso told me about a project concerning Prof. Egon Diczfalusy. As you certainly know I was the first "Italian" researcher to work at the Hormonlaboratoriet of the Karolinska Sjukhuset under the direction of Prof. Diczfalusy. In May 1957 I visited Prof. Diczfalusy Laboratory with the intention of spending there a fortnight seeing what was going on in the field of "hormones". To my surprise Prof. Diczfalusy spoke very fluent Italian. As a consequence of an ordinary laboratory incident (in which I was marginally involved) occurred during the last stages of the isolation of 16-oxo-oestradiol from placental tissue by Eva Axelson and Anne Marie von Munsterman I remained in Stockholm over 4 years, until July 1960. At that time the main interest of Prof. Diczfalusy was the development of the new concept of foeto-placental unit

which largely depended on the isolation of oestrogens (and other steroids) in foetal and placental tissues and some body fluids. At that time the identification of the isolated steroids was accomplished, among others, by the technique of countercurrent distribution (CCD) a very tedious and time consuming operation as well as unhealthy (one had to manipulate and breathe consistent amounts of toxic volatile solvents). Being myself a chemistry graduate with some knowledge of analysis, I introduced in the laboratory as a criterion of specificity the techniques of paper partition chromatography and paper electrophoresis. I remember that at the beginning Prof. Diczfalusy was very skeptical about the use of these "new techniques" and that it was very hard for me to convince him that it was worthy to try them: Later on these new techniques became standard and CCD abandoned. I mention this to point out how careful Prof. Diczfalusy was about the validity of the laboratory results and the reliability of the methods employed.

Prof. Diczfalusy represents for me the person that has introduced me to the field of hormone biochemistry a subject that has been the daily bread of my work as a teacher and as a researcher until my retirement in 2001. From Prof. Diczfalusy I have learned the value of good research and how to make research (what I call the "grammar" of research) although I do not remember having never seen him with a test tube or a glassflask in his hands. For this I am indebted to Prof. Diczfalusy.

A wise and highly cultured man as well as a great scientist that considered research a value in itself and a service to mankind".

Rome, Italy, on the 8th of August 2010

Herman Adlercreutz

Professor emeritus Herman Adlercreutz, M.D., Ph.D., born in Helsinki April 10, 1932

"In 1958 I finished my medical studies at the University of Helsinki. My father associate professor Erik Adlercreutz was the first hepatologist in Finland and made me interested in the hyperestrogenism occurring in liver cirrhosis. The only laboratory in Scandinavia measuring estrogens by chemical methods was that of Egon Dicazfalusy in the Department of Obstetrics and Gynecology at Karolinska hospital. Professor Gunnar Birke in the Department of internal medicine introduced me to Dr Egon Dicafalusy, who gave me the opportunity to pursue my ideas by starting to measure estrogens in bile, something that he commented saying that it will be very difficult. Altogether I stayed 34 months in the laboratory (1958-61), finishing the practical part of my doctoral thesis "Oestrogens in human

bile" published as a monograph. At my disputation professor Diczfalusy came to Helsinki that I appreciated very much.

In this way I learned to know a man with many unusual talents. He is a multi linguist and definitely the best lecturer I ever met. From his lectures I learned very much that I have used in my later life. He could see how various aspects of reproduction may influence the whole world. In addition he has a memory that few can surpass. As an example: During writing of a manuscript in his home I wrote and he dictated practically all references directly from his memory. He also read word by word my whole thesis and taught me at the same time much about writing scientific papers which I have used numerous times. There is no doubt that nobody else has had such a major influence on my whole career.

Definition of Egon Diczfalusy: A verbally unusually talented scientist with global perspectives on human life."

Helsinki, Finland, on the 9th of August 2010

Tihomir Vejnovic

Professor of Obstetrics and Gynecology
Medical Faculty, University of Novi Sad, Vojvodina, Serbia.
Head of the Perinatology Department,
Past President of the Ob&Gyn Section of Serbian Medical Association.

"On Himalaya Mountains, there is a spring with streaming water "without gas".

If you take a nip from that spring, a legend says that you would become immortal. Many people have tried to reach the spring that cannot congeal, but using the shortcuts they missed the main lead and never reached the "path of the stars."

A Latin proverb says "per aspera ad astra" and that is why I consider that friendship with professor Egon might be a thorny path, but a path that certainly leads to the stars.

Our friendship for all these years was a motive for me to gain new acknowledgement, but also new friends and new horizons.

*In few words - Professor Egon Diczfalusy is Schiller's "**Ode an die Freude**" for quartetto Vejnovic and me.*

- Definition in 10 phrases:

1. *King of Ronninge*
2. *Crown-prince of 'Libamay'*
3. *Veteran from Stockholm*
4. *The Phantom of the Opera*
5. *Chardonnay Kovacevic*
6. *Formula one fighter-pilot with Corgy*
7. *"No problemo"*
8. *Ziveli!*
9. *Ziveli!*
10. *Ziveli!*

With warm regards,

Prof. Dr Tihomir Vejnovic"

Novi Sad, Serbia, on the 10th of August 2010

Ioan Munteanu

Profesor Emeritus,
Honorary member o Romanian Academy

"In the second half of the 7th decade of the last century, I was a young assistant at the Obstetrics and Gynecology Department of the Faculty of Medicine Timisoara. Back then, my professor, I. Nubert, attentioned me that in our field of research had appeared a new concept. This new concept – the fetal-placentar entity – *replaced the old concepts which were sustaining that each segment (the fetus and the placenta) were something completely different and apart. The promoter of this new concept was a Swedish physician by the name Diczfalussy. My professor was sustaining that this Swedish physician must be a Hungarian physician who has moved to Sweden. The concept was completely new and interesting and had captured the attention of the entire obstetrical world. The book published in relation with the new concept (*Foetus and placenta – *Author Egon Diczfalussy) became the reference book in the obstetrical field in that period. The new concept opened the ways for the perinatal medicine which is today in full development. From the beginning i considered Egon Diczfalussy to be a great scientist, a genius man and I did not allow myself to even think that i could ever meet him and nevertheless become one of his close friends.*

In 1991 I met Professor Laszlo Kovaks from Szeged, in Singapore with who I become, shortly after that, a close friend and together we established to renew the traditional connections between the Romanian and Hungarian practitioners of obstetrics and gynecology. Those connections were disrupted for a period of 12 years by the dictatorial couple who considered the relations of any kind with our neighbors to be forbidden.

In 1993 I organized the first romanian-hungarian meeting of obstetrics and gynecology. At that meeting i invited professor Diczfalussy, who surprised me by accepting the invitation and was the buffer of the meeting. He advised us to continue and to strengthen these meetings. We followed his advice and in the following years we organized alternative meetings of the gynecologists and obstetricians in Romania and Hungary. Even since then, Professor Diczfalussy had the genial vision to advice us to organize meetings grouped on European regions between specialists. In this way we could meet each other and we could become stronger and influence in good the politics in our origin countries. Therefore we could form the organization DKMT (Danube-Cris-Mures-Tisa), an organization of the Romanian, Hungarian and Serbian specialists, which takes place periodically in Timisoara, Szeged, Belgrade and Novi Sad. Professor Diczfalussy accomplished the reunion of specialists in this part of the Europe. Beside this international organism, professor Diczfalussy formed the foundation Egon&Ann Diczfalussy at which reunion are invited professors from all Europe and USA.

For his exceptional career, professor Dicsfalussy was elected in 1994 Honorary Member of the Romanian Society of Obstetrics and Gynecology and received the title Dr. h.c. at the Universities from Timisoara, Cluj, Oradea and Arad.

A memorable event was the summer of the year 2008 when I become Emeritus Professor at the University of Medicine and Pharmacy "Victor Babes" Timisoara. At this event, together with other important professors from Europe and other countries, the main guest was Professor Egon Dicfalussy who sustained a wonderful speech about me. This special

relation that i have with Professor Diczfalussy allows me to consider myself a disciple of the great professor and to carry for him feelings of respect and consideration.

In 10 words:

genious

family man

polyglot

humorous

respectful

intuitive

modest

people oriented

grateful

talented"

Timisoaara, Romania, on the 11th of August 2010

Gheorghe Furău

MD, PhD

Assistant Professor of
Obstetrics- Gynecology
West University
"Vasile Goldiş", Arad,
Romania

"I consider a gift of chance, a twist of fate, the way I met approximately 20 years ago the eminent scientist of whom I read about in almost all my medicine textbooks.

It was an honor for me, the university I represent and the city of Arad, to award the title Doctor Honoris Causa, to the distinguished professor, in the presence of many of his disciples and former students, who wished to be present. For some that meant to cross an entire continent or ocean just to be there a couple of hours for their maestro.

The message he addressed on this occasion: "Take advantage of me as much as possible", proves the altruistic nature of a great MAN. His results show that through determination a man can make a difference in the journey to the stars. Cited from Senece- Hercules Furens- "Non est at astra mollis, e terries via" (From earth to the stars, there is no flat road).

Professor Egon Diczfalusy achieved creating a real school, he himself being a true educator, teacher and mentor for many "students" all

over the globe. This altruistic spirit stood at the base of the creation of the "Egon & Ann Diczfalusy Foundation" whose noble objectives, I am convinced, will remain for many years to come, an investment in and for the future generation.

Behind the scientist, I have to remark the extraordinary psychologist, in the person of the maestro (and friend) Egon is. In my moments of despair caused by the ones I once considered "my brothers", he comforted me telling me that "If 2% of those you helped in some way remember you in a good way, it is an achievement worth the cost".

A man of chosen culture, Egon the artist, in his elegant way, forces you to enter the miraculous world of art, to learn new places, to tie friendships with people from all over the world, who "by chance" were his student. I was impressed by the sheer easiness, the professor communicated with the young generation "in their" and "on their" same language. The qualities of a true pedagogue only prove his devotion to other people, in my opinion, this being the key to the professor's youth.

If I had the possibility to clone only one man for the future generations, this would be no other than the MAN, the scientist, the researcher, the pedagogue, the artist and the forever young, Egon Diczfalusy."

Arad, Romania, the 11th of August 2010

Kerstin Hagenfeldt

MD. PhD. Prof em. Dept of Woman and Child Health, Div of Obst & Gynecol. Karolinska University Hospital and Karolinska institutet, Stockholm, Sweden

"What does Egon represent for me?

He was my tutor in research. I was fortunate enough to spend four years in his lab in an atmosphere dominated by his quest for knowledge, his immense devotion to science, always at hand for discussions on reproductive endocrinology, laboratory techniques, animal experiments or statistics He introduced us fellows to his wide global network of colleagues all over the would- in my case specifically to the WHO Special Programme of Research, Development and Research Training in Human Reproduction: A programme that Egon initiated together with professor Sune Bergström and Ulf Borell from the Karolinska Institutet. Since then I have had the

pleasure to work with this programme during more than35 years. For this I will always be deeply grateful to Egon as this work has been very important in my professional life.

To describe him in ten words are hard:

Let me try:

-in science: devoted, knowledgeable, extremely well- read (in a time before the internet when you actually had to read publications and correspond and discuss by mail with colleagues !!)

-to families and friends (including former fellows): always concerned, generous with advice and support

-in leisure times. a connoisseur on food and wine

-in life: an old-fashioned gentleman"

Stockholm, Sweden, on the 12th of August 2010

Katalin Barabas,

Habil, MD, PhD
Associate professor, head of University of Szege, Faculty of Medicine, Department of Behaviour Sciences

"What does Professor Diczfalusy mean to me?

I have known Professor Diczfalusy personally for four years.

During our first discussion that was on the moral basis of scientific research, he convinced me that I had met an exceptional personality. This meeting has developed into a nice relationship that involves discussions and correspondence about the medical science and the history of medicine, providing me experiences in music and the fine arts pervaded by humor and containing several cultural references.

His excellent organizing ability, physical strength and indefatigable social activity elicited my deepest respect and admiration.

If it is possible to characterize somebody in the 21st century as the Renaissance man, he is the very one. He is a real humanist and a polyhistor.

Tradition and progress is mixed in a peculiar proportion in him.

He is experienced both in the field of modern scientific achievements and in the world of the Old Greek dramas. His love for operas is not unintentional as this is the very genre that presents man in the most complex way, and he is mostly interested in the human being.

Professor Diczfalusy has brought me a message from a world that is very much desired by me, the scale of values and the style of which is much over our hurrying, globalized world.

I consider the gift of Fate that I have the privilege of knowing him. Thank you.

If I had to characterize the personality of Professor Diczfalusy, the first word that comes into my mind would be Elegant. He has elegant thoughts, elegant taste, elegant physical appearance and elegant life style; he is an exceptional man.

Katalina the Second"

Szeged, Hungary, on the 12th of August 2010

Britt-Marie Landgren,

MD, PhD,
Professor of Obstetrics- Gynecology Karolinska University Hospital and Karolinska institutet, Stockholm, Sweden

"*In one sentence, I would say that Egon is my teacher, mentor and dear friend."*

Stockholm, Sweden, on the 12th of August 2010

Gyorgy Bartfai
Professor University of Szeged
Department Ob. & Gyn.

"I spent more than 1 year in Professor Diczfalusy's Lab, at the Karolinska Institute, Stockholm. I was lucky enough to be there in the "golden age" when he was one of the leading figures in science on the field of reproductive health, as well as the eminence grise of the WHO.

*Professor Egon Diczfalusy is my father in research. I never forget his frequent citation by Zrínyi "*Good fortune, nothing else*". However, I would like to continue with another citation by Pasteur:* "Chance favours only the prepared mind."

He is my role model of a committed scientist obtaining outstanding professional results yet remaining an uncompromising humanist always with a good sense of humor and staying sensitive toward others. "

Szeged, Hungary, on the 16th of August 2010

Jeff Spieler

Senior Technical Advisor for
Science and Technology
Office of Population and Reproductive Health
Bureau for Global Health

"*What does Professor Egon Diczfalusy represent for me?*

A world class leader in research in human reproduction who has mentored and trained many of the other world leaders in family planning, reproductive health and research from all over the world.

-To define him in 10 words.

A scholar, friend and renaissance man with the best quotes.

One story - Egon and I spent a fabulous week together in Milan in the late 90s as guests of the Rockefeller Foundation editing the Supplement

to the International Journal of Gynecology & Obstetrics on "Contraception 21 - The Promise of Public/Private Sector Collaboration" that resulted from a series of three Bellagio Conferences convened by Mahmoud Fathalla and the Rockefeller Foundation. While we worked hard from 8:30 AM to 12:30 PM and from 2:00 - 6:00 PM, we had great lunches and fabulous dinners with marvelous Italian wines every evening, followed by a nice walk. Egon had the expense account and it was great to be his "guest" for lunch and dinner and to share our work and non-work lives with each other.

Geneva, Switzerland, on the 16th of August 2010

Emmet Lamb

Professor Emeritus
Ob&Gyn Stanford
University

"Your two questions -- 1. What does Professor Egon Diczfalusy mean to you? and 2 How would you define him in 10 words? -- posed quite a challenge and I spent several enjoyable days exploring the proper answers.

Egon Diczfalusy was my most influential mentor and advisor. I officially retired fourteen years ago from a long academic career but am still slightly active in research. Throughout those years I have profited from my time with him even though the minutia of steroid metabolism have long ago left me.

I was in the Hormone Laboratory for a little over a year in 1965-66 during the peak years of Egon's work on the fetoplacental unit and contributed one small piece of the jigsaw puzzle he was solving. In his 1997 book The Contraceptive Revolution: an era of scientific and social

development, Pantheon, page 7, there is a list of 35 scientists who worked in the lab at that time. Fourteen were in Stockholm during the time I was there and it is likely that each had a publication to show for their efforts and some had many. The first two words, therefore, in my litany of adjectives to describe him are ***organized*** *and* ***indefatigable*** *as a research mentor. He found time to meet with each of us to discuss the state of our project on a regular basis and reviewed our data with unflagging enthusiasm and interest.*

He was a ***polyglot****, speaking often to his students in their native language. Occasionally when he would find himself using words from two different languages of the half dozen or more in which he was fluent, he would say "Oops" and twirl an imaginary dial to switch his brain and tongue from one to the next.*

Egon was also a ***polymath****, a renaissance man with an encyclopedic knowledge in many fields. Every lecture and every publication began with an apt quotation. He must have memorized many of these since he also sprinkled his every day conversation with quotations. I have suspected that he used his long late evening commute time to memorize quotes or perhaps to read books by the likes of Blaise Pascal.*

He was ***demanding****, requiring that we meet rigid standards. Recrystalizing to constant specific activity the steroids we isolated from various tissues and fluids obtained from subjects who had received injections of a radioactively labeled precursor condemned us to many hours in the weight room in those days of mechanical, not electronic, balances. This did not make up a large proportion of the time I spent on my project but it was lonely grunt work which I dreaded. Much of the other time was fun with 14 other postdocs with whom to socialize.*

He was ***gracious*** *in occasionally entertaining us for a noon meal of corn flakes and fil mjolk in his office with his chief technologist (was her name Leda?) or for an evening feast of reindeer meat and all the Swedish fixings with his wife Anna at his home in the distant suburbs.*

*He was **polished** and impeccably dressed, a suave, and urbane European **intellectual**, a delightful conversationalist.*

*He was **trusting** once he came to know you. He even sent his son Bo to live for a summer with us when we had returned to California. I was delighted to find forty five years later that the shy teenage pianist who joined our family for a few months had become a very important figure in green energy in Sweden, or so a Google search revealed."*

California, USA, on the 17th of August 2010

Nevena Secen

Prof of Internal medicine, ex-Vice dean for foreign communications and foreign students, Faculty of medicine Novi Sad, Vojvodina-Serbia

"It is a great privilege to know such a huge person as Prof Egon Diczfalusy is with his outstanding personality,energy and fruitful life.It is amazing inspiration for us!

Dear Prof Egon Diczfalusy, we wish you a very happy and healthy birthday and may you have many, many more!

Yours,

Very truly,

Nevena Secen"

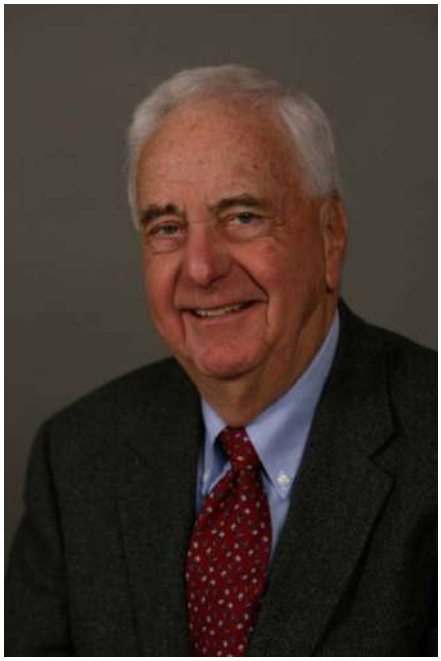
Novi Sad, Serbia, on the 17th of August 2010

Attila Pal

Professor Ob&gyn University of Szeged

"He is not just a pure scientist and an ideologist but a person who has recently shown philosophical characteristics also. "

Szeged, Hungary on the 18th of August 2010

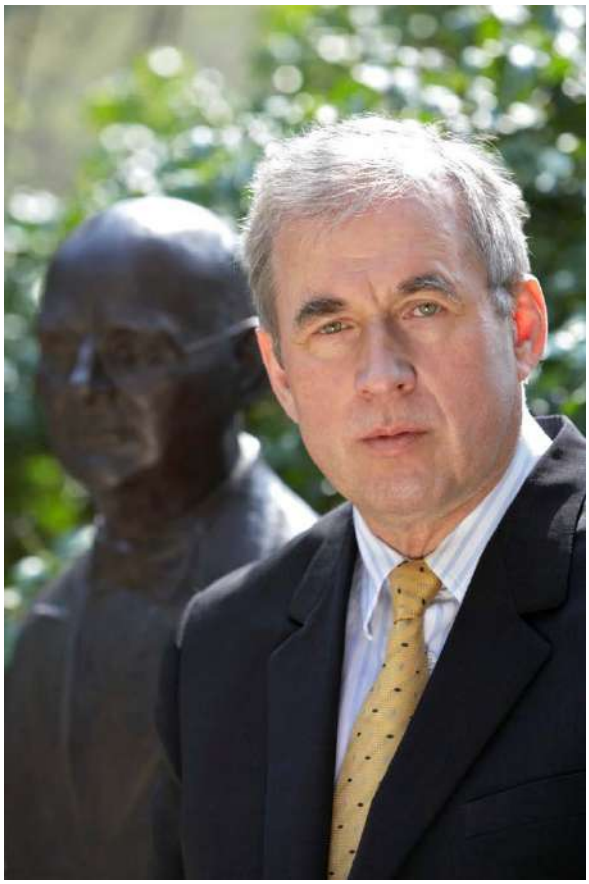


Daniel R. Mishell Jr., M.D.
Professor, Department of Obstetrics and Gynecology
Keck School of Medicine
University of Southern California
Los Angeles, California

"Professor Diczfalusy has been a close friend and colleague of mine for nearly 50 years. I have read his marvelous books on modern contraception and attended many of his outstanding lectures at various international symposia. I have also been present at several meetings of the WHO which were chaired by Professor Diczfalusy. I learned from his actions at these meetings how to develop consensus and conclusions from a diverse group of scientists from many different nations. I utilized his methodology when I needed to chair scientific meetings and am grateful for having the opportunity to learn from this master. In addition to science, Egon Diczfalusy taught me how to enjoy good food and fine wine. I am forever grateful that he taught me that wines from Burgundy were superior to those from Bordeaux. I recall fondly the erudite conversations we had at many outstanding dining events.

The ten words I would use to define Egon Diczfalusy are brilliant, sophisticated, erudite, charming, cosmopolitan, elegant and an outstanding educator, mentor and gourmand. I wish to extend my special congratulations on the occasion of his ninetieth birthday."

Los Angeles, USA, on the 18th of August 2010



Thomas Rabe,

Senior consultant,
Department of
Gynecological
Endocrinology and
Reproductive Medicine,
University Women`s
Hospital Heidelberg,
Germany.

„Outstanding scientist, brilliant analyst of past and future global development with view to need of mankind. Unique global player in gynecological endocrinology and reproductive medicine fighting lifelong for Women`s Health. Grand senior, multilingual gentleman with multicultural behaviour, homo sapiens extraordinarius.“

Heidelberg, Germany, on the 19th of August 2010

Riccardo Marana,

Director of Gynaecological Oncology at the Catholic University of Sacred Heart, Rome, Italy

"My two year stay at Karolinska, from 1978 to1979, represents a milestone in my professional and personal preparation. It greatly influenced my growth and my career in the years to follow.

It was my first time studying abroad for such a long period and my knowledge of English was poor. I immediately felt welcomed in Prof. Diczfalusy's laboratory, as if part of his big family. There I learned, starting from the bench, all that is fundamental to become a knowledgeable researcher. Under his supervision, as well as that of his team, I cultivated a critical eye, an intuition for research and the ability to develop a project and write papers. I still remember fondly the staff reunions during tea, often in his company.

Prof. Diczfalusy taught me to have faith in myself and in my capabilities, and demonstrated appreciation in my work. I greatly admired his humanity and empathy that always accompanied his indisputable leadership.

Even though I was stimulated by pure research and was offered to join his team, I realized that my real interest was in gynaecological surgery. Therefore, I returned to Rome, applying my acquired knowledge in clinical research.

No doubt, Prof. Diczfalusy's Letter of Recommendation led the way to many opportunities abroad, especially in the U.S. Since my years at Karolinska, Prof. Diczfalusy has always been near and genuinely interested in my achievements, both academic and personal. If I am where I am today, it's thanks to his precious teaching, guidance and support. I am grateful having him as a mentor and friend."

Rome, Italy, on the 21th of August 2010

Alessandro Pala,

MD, PhD,
Professor at at the Catholic University of Sacred Heart, Rome, Italy

"Egon represents for me a model of clearness of mind.
Trying to define him, I would say he is able to teach his students how to give
Invaluable and true pieces of scientific information"

Rome, Italy, the 21th of August 2010

Peter Petrusz,

Professor of Cell and Developmental at the School of Medicine, University of North Carolina at Chapel Hill
External Member of Hungarian Academy of Sciences

"Professor Diczfalusy belongs to a breed of scientists that, unfortunately, is becoming nearly extinct. He possesses the rare combination of extraordinary talent, an almost super-human memory, power of reasoning, capacity for work, high culture, unfailing moral compass, and a compassionate, deeply human, and politics-free philosophy that only the greatest, if anyone at all, can match. It has been one of the greatest honors of my life to be able to work under his guidance and supervision for almost five years during the late 1960s and until 1971. His name is forever preserved in the scientific literature but I hope that we, who had the good fortune to be close to him personally, will be able to pass on some of what we learned from him (and what we learned goes way beyond "simple" science) to future generations.
In 5 words only I would say about him: the best mentor I ever had."

North Carolina, USA, on th 2th of August 2010

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