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Interview

In pursuit of communication

An interview with Bob Ruben

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There was a time when all science was carried out with brain and hands. Far from huge investments and large organizations, people would build up equipment, manipulate animals and do experiments. The process took place on a single-individual scale. This prompted people to think that they were able to emulate scientists and to develop the drive to become a scientist. Science is still an individual issue, but it is also true that today, it is more heavily dependent on complex technology, organization and managing structures than it was 50 years ago. *No regret, this is merely descriptive.* Indeed, modern science becomes more and more a labor of teams, in which the individual becomes an integral part. There is also a tradition in life sciences in which basic biology attracts physicians to research. They want to know in order to cure, of course, but many get more involved due to their interest in knowledge and many even become basic scientists. So they walk far away from the immediacy of so-called "applied research" and similar euphemisms and dedicate their professional lives to scientific research. Not forgetting that they want to cure disease and alleviate suffering, they become firm supporters of the benefits of basic science. These two ideas are perhaps incarnated in Robert J. Ruben, a born New Yorker who won a scientific prize while at high school for breeding at home a mutant mouse with audiogenic seizures. He obtained his undergraduate degree from Princeton University in 1955 and went on to study medicine at the Johns Hopkins School of Medicine. He then did his post graduate medical training at the Johns Hopkins Hospital, spent two years at NIH and then went to New York City where since 1968 he has been at the Albert Einstein College of Medicine. Ruben then in the eighties was a critical force for the creation of a new NIH institute to fund hearing related and other communication diseases, the National Institute of Deafness and Communicative Disorders (NIDCD).

Bernd Fritzsch and Fernando Giraldez went to his appartment on the 5th avenue in Manhattan to talk to him and know more about his scientific life and his thinking (Fig. 1). They found a charming academician with a refined intellectual wit and a love for science and art history. Bob also amused the improvised interviewers by showing them only a tip of his amazing library of ancient books on hearing and communication. What follows is the result of that day in Manhattan which ended with us wondering through Retzius' 1884 edition of *"Das Gehörorgan der Wirbelthiere"* [The Auditory Organ of Vertebrates] and other fascinating books on the earliest steps of our understanding of the sense of hearing, including the original work of Corti, whose name is so intimately connected to the hearing organ.

Tell us about your personalized view of your detour through science.

I would say that the detour was into clinical medicine - I went to Hopkins Medical School to study biology -, since Science in general and human biology in particular, has always been and

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continues to be a major part of my life. I won a Westinghouse honorable mention for isolating from the wild, from the woods at the back of our home, a mutant stain of mouse with audiogenic seizures for the Jackson Lab; the Bar Harbor lab burnt down and they asked high school students to trap and in breed wild mice in the late 1940s.

Princeton was no different; my junior paper in the Psychology department dealt with the differences in results between a culturally based IQ test (the Stanford Benet and non-culture based test using Raven's Progressive matrixes) on a socially deprived population - a prison population of juvenile delinquents. Yes, the results were as expected – some were retarded but many were very bright – we just needed to know how to measure this. My faculty advisor was Ernest Glenn Wever (I gave one of his eulogies at his memorial service as we had become very good friends). His first assignment was physiological optics. This led to me working in Kiefer Hartline's lab at Hopkins during summer of 1954 (I learned about omatidia and how to eat hard shell crabs) and I found out who E.G. Wever was. Then for my senior year, I worked as his lab technician and stayed the summer of 1955 catching local reptiles and doing their physiology. My thesis was to build a modification of Ted MacNichol's amplifier and then complete recording of a single active frog cutaneous nerve to extend Adrian's work [neurophysiologist Edgar D. Adrian, Nobel prizewinner for Medicine in 1932]. They liked the thesis and I received 0.7. That would be equal today to an A++. At Hopkins, in 1955 there was no ear research (Stacy Guild and the temporal bone collection was in a janitors closet!), so I became involved with temporal lobe epilepsy with A.E. Walker (Poblete *et al.*, 1959). John Bordley, who held the Chair of the division of Otology and Laryngology at Johns Hopkins Hospital summoned me to his office at the end of my third year at Hopkins Medical School and offered me a job as director of a neurophysiology lab; the person who was supposed to do this decided to stay in Europe and there was an NIH grant which needed a PI [personal investigator] (unthinkable today – I was 24 years old). The acceptance was not an easy decision as I was already thinking of a career in neurology/ neurophysiology and/or neurosurgery and ENT [ear, nose & throat] did not appeal. The attraction of being able to do my own science was the tipping point – I would make the Faustian bargain – do the science and worry about the nose bleed and the tonsil. My decision was greeted with overt hostility by an influential segment of the Baltimore Jewish community – the social invitations stopped! But who had time or cared for that anyhow – I was doing what I wanted to do. I began with studies of the CNS and my interest in the inner ear and deafness grew out of a casual conversation with Stacy Guild (see my adenoid poster or the Association for Research in Otolaryngology [ARO] abstract) as to why no one was recording human cochlear potentials. He said that it could not be done, since Wever, Békésy, Meltzer and Lempert, amongst others, had tried and failed. I found out that the Russians probably did it in the 1930's and it seemed illogical that it could not be done (I had recorded this from snake, lizards, turtles etc., so why not a great ape?). So, I built another Mac Nichol preamplifier, borrowed that developed by Kouwenhoven and Knickerbocker (deifibulation fame) and off to the OR [operating room, surgery]. Dr. Alfred Lieberman exposed the middle ear, showed me where the round window was and what do you know! – we recorded both the microphonic and the eighth nerve action potential. We did it again on a second patient and obtained the same results (Ruben *et al.*, 1959). I then visited Lempert's hospital (that is another story) and immediately saw their problem – signal to noise – their low level amplifier was about 30 feet from the patient! This human electrocochleography led to a number of publications, one of which has turned out to be prophetic (Ruben, 1963).

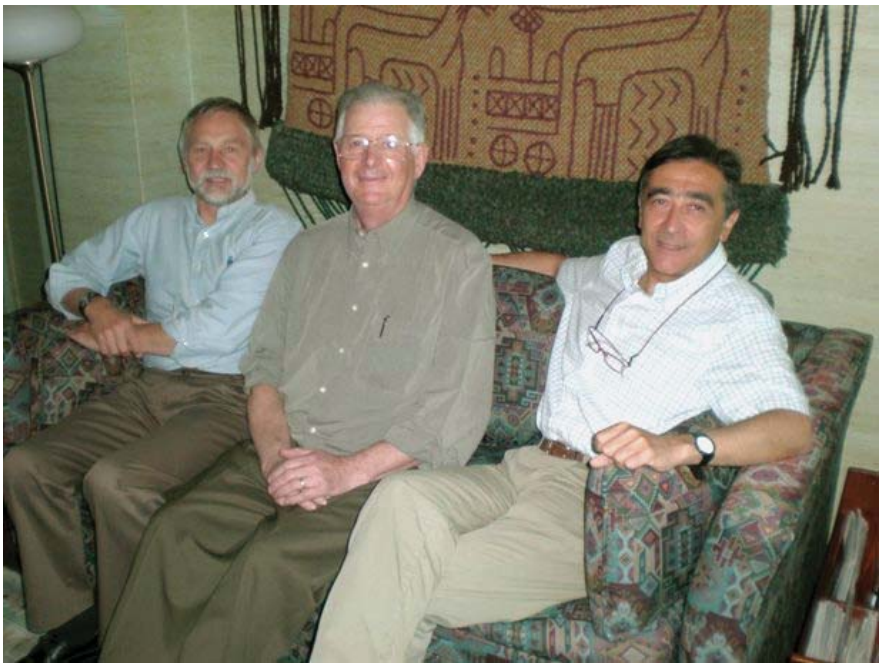

Fig. 1. Bob Ruben interviewed. *Bob Ruben (center) with Fernando Giraldez (right) and Bernd Fritzsch (left) during the interview in Manhattan, May 2007.*

How did you get involved in the field of ear development?

The ability to make an accurate, although qualitative, assessment of hearing/cochlear and statoacoustic nerve function, in infants and children who could not be assessed by psychophysical methods, brought me, early in my professional life, into contact with may deaf/non-speaking/severely language impaired patients. As the metric I was using was neurophysiological, I began to carry out a series of studies on the neurophysiology of deafness, either genetic, acquired - sound trauma and neural lesioning - using mice (Mikaelian and Ruben, 1964; Alford and Ruben, 1963; Fisch and Ruben, 1962), dogs (Hudson and Ruben, 1962) and cats (Fisch and Ruben, 1962) as models. I also reviewed and generated a library on the temporal bone histopathology of deafness and the development of deafness in humans and other animals. I soon came to the conclusion that much of deafness was due to early cell death and not malformation. I then began to look at the developmental physiology of hearing which

also served as a 'control' for the few studies of the progressive loss of hearing (op. cit.).The physiological data was phenomenological but did not offer insight into the cellular mechanisms underlying the cell death. When the cells got 'sick' and then died they did less to no physiology - no surprise.

At this time, we are in the middle of the Vietnam war and I had expected to serve – but was found to be medically unfit because of colitis (which later turned out to be an infection picked up in Qintana Roo when playing as a Pre-Columbian archeologist - another story). However, I felt obliged to serve and was given the opportunity to be in the Public Health Core at NIH on the condition that I could not claim any medical disability or care for my colitis. Also, that I be solely dedicated to the lab (I was a research associate - not a clinical associate). Needles to say, I was not saddened by this. I knew that I wanted to begin to find out how the inner rear was put together embryologically and to find out how the cells that made up the inner ear went about their life cycles, for I thought that this should begin to give some insight into the biology of the inner ear's cell cycle. I went to the library and soon realized that I could best, at that time (1964), study this by initially determining the birth dates of the cells of the inner ear and finding out which, if any, may continue to reproduce after the inner ear had matured. The technique would be to use tritiated thymidine (this was all that was available in the early 1960's). I then identified a lab at NIH, in the eye institute, which was doing embryology of a sensory structure, the eye. Dr. A.J. 'Chris' Columbre headed this research and he accepted me. He was a teacher, mentor and very good friend. These were two wonderful years. I had to develop the histological techniques and was helped by Richard Sidman from Harvard (Ruben and Sidman, 1967; Ruben, 1967).

I repeated the studies three times, for I was surprised at the findings but they came out the same all three times so I published them (Ruben, 1967). I had a lot of data to process and to handle all this, I wrote a computer program. This maybe was one of the earliest three dimensional anatomical programs written. It was in Fortran and filled boxes of IBM cars, but it enabled me to see the development in time and space.

Having done this, having studied and been exposed by Columbre to, at that time, classical experimental embryology, it was obvious to me that there must be a technique developed that would allow for the manipulation of the mammalian ear (the bird, reptile and amphibian did not have human genetic diseases and/or their inner ears would grow back and a basil papilla was not a really a cochlea - this was before Cotanche, Corwin and Rubel). So, in order to further understand the cell biology of the developing inner ear, my orientation was much more the biologist than the ototologist, but the subject of deafness gave a rationale for NIH funding. I decided that an organ culture of the mammalian inner ear was needed which could be further developed into cell culture. Again, I was told, that this could not be done, since others had tried and failed; that it might be possible to do this with the chick, but not with the mouse. I thought that this was not correct and we should go and try just as we did with human electrocochleography. As I did not have any skills, real knowledge or experience in cell culture, I needed to recruit a knowledgeable person. Tom van de Water was a tissue culture technician with experience and skills in monkey kidney cell culture. He was looking for a position and was going to peruse his doctorate. I showed him where the otocyst was and how to remove it with little damage so that it was viable (I learned this from Columbre who let me operate on chick – another story). He then developed the organ culture technique which is now used worldwide (Van de Water and Ruben, 1971) There followed a series of studies which documented the development of the inner ear at a cellular, molecular and now beginning

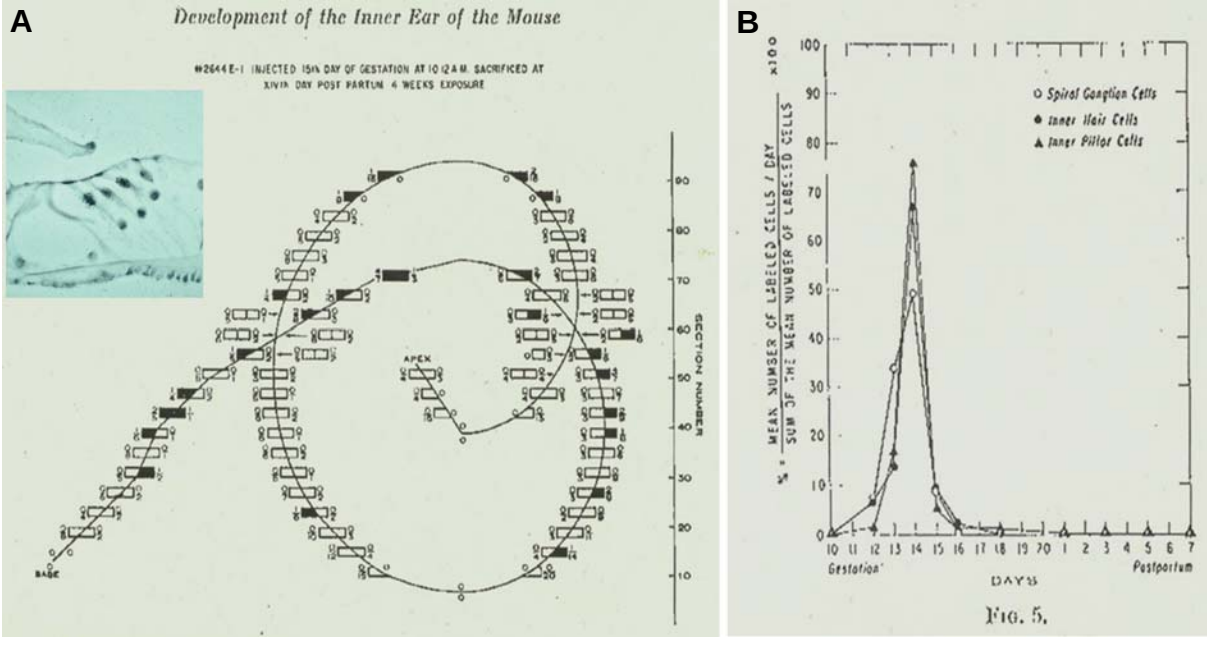

Fig. 2. Terminal mitoses in the ear. *Reconstruction of the cochlea and birth-date of outer hair cells.* **(A)** *Reconstruction of ear no. 2644E-1. Outer and inner hair cells are tabulated respectively on the inside and outside of the spiral. The denominator is the number of cells present and the numerator the number of labelled cells. Filled boxes indicate that labelled cells were present. Inset: Hair cells labelled with 3H-thymidine.* **(B)** *Labelling activity of spiral ganglion. Inner hair and inner pillar cells throughout development. Days, days of injection of tritiated thymidine. From (Ruben, 1967).*

at the protein level. A long way from the physiology of the young postdoctoral student.

The Ruben 1967 paper on terminal mitoses

In 1967, Ruben published a seminal paper on the developmental biology of the ear, which is still used today (we mean "used", not only quoted). This is a forty-page paper that was published in *Acta Oto-Laryngologica*, Upsala, with the title *"Development of the inner ear of the mouse: a radioautographic study of treminal mitoses"* by R.J. Ruben (Ruben, 1967). This is a remarkable piece of work which is still fundamental today to describe the birth-date of cells in the ear. The paper starts with a brief historical introduction where the *aficionado* can trace back ear development and innervation to the very first studies of the late XIX and early XX centuries. Then, it concentrates on the question addressed in the paper, i.e. the search for the *"times of establishment of a permanent cell population"*, considering the terminal mitoses as *"the last divisions which a cell undergoes"*. The principle was as follows (Hughes *et al.*, 1958; Miale and Sidman, 1961): a pulse of ^{3}H-thymidine is given at a particular stage of development and ^{3}H-thymidine incorporation is observed after birth (Fig. 2). The cells that retain ^{3}H-thymidine are those that made their terminal mitoses within two hours after the time of injection, whilst those that are not labelled must be those that either kept dividing and diluted the label, or those that were quiescent and did not incorporate thymidine at all. Now the important thing was to be able to carry out this analysis with precision in time and space and this is exactly what Ruben did. He reconstructed the cochlea and vestibular system from every fourth 10 μm section of treated ears, from animals that were injected at different stages and examined all the different cell types that were histologically recognizable in the cochlea (without any markers whatsoever), vestibular apparatus and ganglion (Fig. 2). Ruben developed a computer program for the reconstructions and by transferring data from individual sections to punch cards, they were processed to give for each cell type the number of cells present, the number of labelled cells and the percent labelling. He produced precise and beautiful spatial and temporal plots that showed when and where cells were born in the inner ear (Fig. 2A). This was how the opposing apex-base gradients of birth-date of hair cells and neurons were discovered.

This work has stood the test of time and two more recent papers have confirmed the basic and astonishing finding using much simpler techniques employing BrdU and whole mounted cochleae (Matei *et al.*, 2005) (Lee *et al.*, 2006). To our knowledge this is one of the few cases in ear development where the early insight was so profound and deep that it virtually shut down any additional attempts to study that process until novel techniques had emerged almost 40 years later. Ruben then

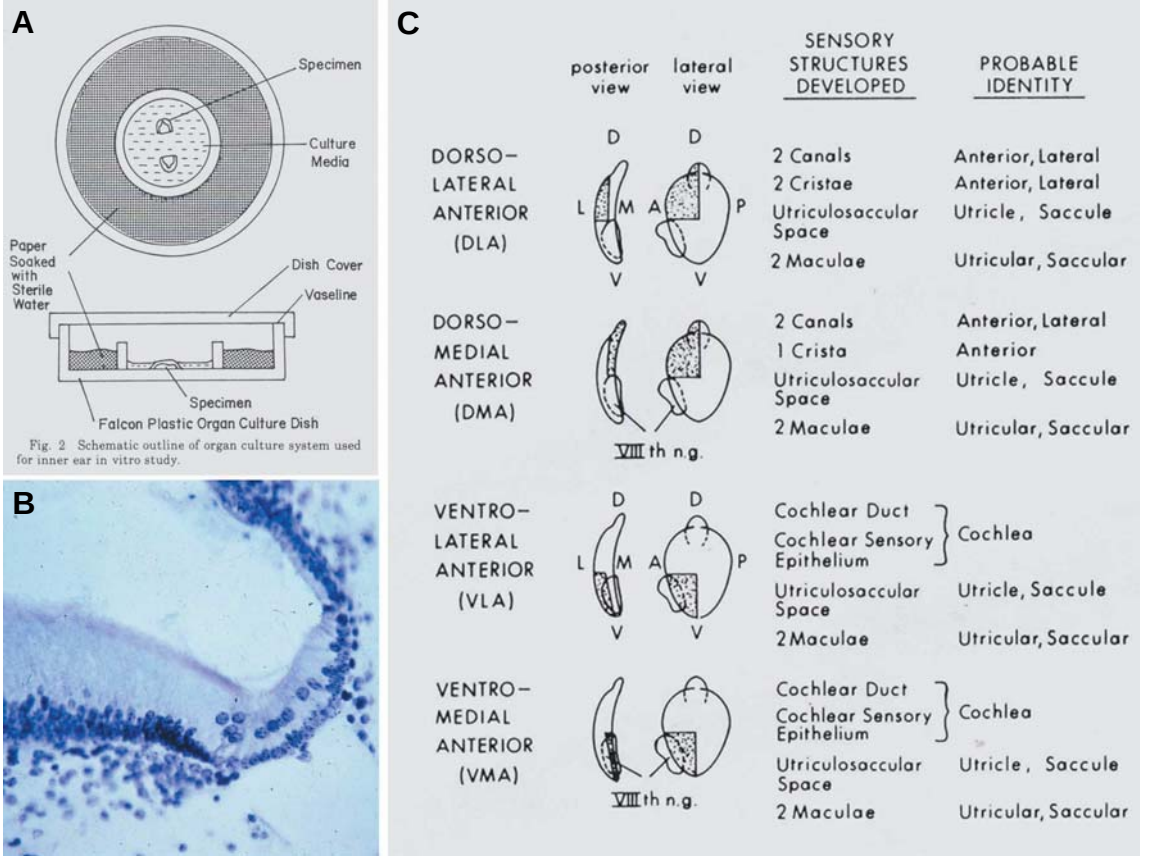

Fig. 3. The extracorporal growth of the inner ear. In vitro *studies of the otic vesicle, the first fate map.* **(A)** *Diagram of the culture system.* **(B)** *Example of organ culture of a mouse cochlea.* **(C)** *Diagram of the fate map of the mouse otocyst from pieces of epithelium that were separated by surgical dissection and grown in culture. From (Van de Water and Ruben, 1971).*

Fig. 4. Bob Ruben and the origin of the NIDCD. *Bob Ruben testifying at the House of Representants for NIDCDF in 1990. Geraldine Fox can be seen in the NIDCD part, sitting behind Bob Ruben (the last face on the right) during the hearing.*

explored further the behavior of terminal mitoses in one of the first discovered mouse mutant strains with a neural tube malformation and an otic phenotype, the *kreisler* mouse (Deol, 1964), a mouse that is still used today to investigate the role of neural signals in otic patterning (see Schneider-Maunoury & Pujades, 2007). Ruben published this work in 1973, showing that the 11-day *kreisler* otocyst has a shortened cell cycle (Ruben, 1973).

Looking back, what do you think about your own contribution to the field; how much scientific return has your insight brought to our area of research?

Looking back, I feel that my studies on the development of the inner ear served as a catalyst and as a basis for the investigation of the developmental biology of the inner ear. The discovery of the regeneration of hair cells in the avian basilar papilla gave a quantal increase to investigation of the development of the inner ear and how one could find the holy grail of mammalian organ of Corti regeneration. This 'practical' endeavor has focused attention quite narrowly and there has been a reduction in fundamental, basic research - that is NOT where the NIH money is. The monograph on terminal mitoses (op. cit.) has and appears to still serve, some 40 year later, as the basic observation of the natural history of mammalian inner ear cell proliferation. This work was and still is a long standing contribution to knowledge – that for me is more than satisfying.

The concept of early cell death has not received the same attention. As I look at the domain of inner ear diseases which result in hearing impairments, I see a very different scenario from what is being pursued now by most. As the predominant problem is early cell death (prenatal, aka congenital; first few years of life, aka early onset; old age, aka presbycusis), the object of the effort should first and foremost be prevention. If that is not working, then there is the possibility to repair the malfunctioning - sick - injured - cells, for they stay around for quite a time (e.g. the Sh1 or congenital rubella or sound trauma or ototoxicity etc.) and, then if all that does not work, you go to regeneration. Simply stated it would do more good to work on prevention and repair than on regeneration, but for this I only get the Cassandra citation[1].

The "extracorporal growth of the inner ear" and the fate map of the mouse otocyst, the Van De Water and Ruben paper of 1973 and the Li et al., paper of 1978.

Attempts of growing embryonic organs and tissues *in vitro* date from more than one-hundred years, however, in the 50s cell culture techniques started to develop and new attempts counted with improved means. Mary Faith Orr, in 1968 cultured dissected four-day old chicken otocysts and looked at the histology of the explants at different incubation periods between 3 and 12 days in culture (Orr, 1968). She also dissociated for the first time cells from chicken otocysts into a suspension, to then look at their reorganisation *in vitro*, comparing those tissue arrangements and cell types with the culture grown otocysts. Mary Faith Orr refers in her paper earlier experiments by Fell in 1928 and Friedmannn (Friedman, 1956; Friedmann, 1959) that showed the capacity of explanted otocysts to "self-differentiate" *in vitro*, an idea that later led to the concept of the epithelial autonomy of the otic vesicle, that is, the epithelium of the otic vesicle contains the necessary cues to generate different cell types at their corresponding places (Swanson *et al.*, 1990); see also (Torres and Giraldez, 1998). Bob Ruben recruited Tom Van de Water in the early seventies to develop an *in vitro* system where to analyze the development of the ear in the mouse embryo (Fig. 3, (Van de Water and Ruben, 1971)). They had in mind to tackle two basic problems in ear development, the fate map of the otic vesicle and the trophic interactions, both neurotrophic and epithelial-mesenchymal cross talk, questions that were addressed for the first time in these and following papers. Van De Water continued thereafter an independent carrier contributing important studies on the role of neurotrophic factors in ear development. One need to remember that in 1975 only NGF had been identified by its selective action on spinal sensory and sympathetic neurons, suggesting that there might be a number of comparable trophic factors essential for the long-term survival of other classes of neurones. Van De Water and Ruben undertook a number of studies looking for trophic interactions between the otocysts and the cochleo-vestibular ganglion. But it remained for the technology of knock-out neurotrophin null mutants to show

1. Note: Cassandra (Greek for "she who entangles men") was a Greek princess whose beauty caused Apollo to grant her the gift of prophecy. However, when she did not return his love, Apollo placed a curse on her, so that no one would ever believe her predictions. [Èditor]

that the mammalian ear requires not NGF but two related neurotrophins, BDNF and NT-3 and their associated receptors for maintenance (Ernfors *et al.*, 1995; Schimmang *et al.*, 1995; Fritzsch *et al.*, 1997)

What is your evaluation of the changes taking place in the field of ear development, a field which is currently exploding with novel findings?

There is a huge amount of empiricism with relatively isolated and unconnected findings. These are, to me, in desperate need of organization and creation of a few theoretical hypotheses that would enable more effective and efficient experimental quests. Today's 'novel' findings are tomorrow's standard knowledge. The large amount of empirical data needs to be organized. The data will only increase, probably geometrically, as proteomics develops. This will make it even more important to ask questions which are focused. There is the great danger that the questions may be canonized/ossified and leave little or no room for new thought. This has happened too many times in the history of science.

The involvement of Dr. Ruben in the formation of NIDCD

One important aspect that came up during the interview was the involvement of Dr. Ruben in the formation of the NIDCD in 1988, the NIH institute that funds all ear related research (http://www.nidcd.hih.gov/about/learn/history.asp). Dr. Ruben was disappointed with the way funding of hearing related research was run through several institutes, none of which had the required expertise to handle the specific aspects of ear function, genetics and regeneration. The bill to generate NIDCD was introduced by Senator Tom Harkin (Dem, Iowa) in intense discussion with Dr. Ruben and Geraldine Fox (Fig. 4). Part of this discussion was presented in a meeting report in *Int. J. Ped. Otorhonol* 15 (1988) 1-15. This symposium, entilted *"The biology of sensorineural hearing loss in children"* combined with the book edited by Ruben, van de Water and Rubel (The Biology of Change in Otolaryngology, 1986; Proceedings of the 9th ARO Midwinter Research Meeting) may be considered the start of modern investigations into the molecular biology of inner ear sensorineural development and disease.

Scientific research has changed in many ways since you began inbreeding mice in your back garden. What is your point of view today about the general changes in science over those years, the way it was done when you started and how it is today?

To preface this question I will state one of my favorite mantras: "*The only constancy in life is change and the lack of change in a biological system is death!* " One major change in biological/medical science in the last half century has been organizational. The discipline has evolved from small individual workshops to large science factories and the extensive use of consortia at the national and international levels. The advantages are obvious – more hands and resources to do more work. The disadvantages are less obvious. Science has become institutionalized with fewer and fewer people making decisions as to what and how things are to be studied/considered. There is much less room or tolerance for new ideas and new approaches and this can be extremely deleterious to the effort. A by-product of institutionalization is the extension of the period of indenture /post doctoral fellowships etc. There are very few principal investigators less than 30 years of age and the majority of resources appear to be controlled by those >50. The average age for a new principal investigator at NIDCD is said to be now 42 years. For at least a millennium it has been obvious that the advances have come, in the main, from the younger/youngest investigators. During the Renaissance and to some extent into the 19th century, a young person's observations may not have been made public until he (and the occasional she!) had achieved institutional status -Versalius is an exception and so is , to some extent, Darwin.

Another dangerous consequence of 'big' institutional science is that the science may be done by a committee in which the majority rules. There are too may examples when the majority of a committee determining science has been wrong such as 'ruling' what caused death following blunt injury after World War I.

A second major and very deleterious change is that of the commercialization of science. This resulted is pernicious, destructive and, for science and the commonwealth, somewhat self defeating outcomes. All science that is either conducted at a University, a Foundation or directly at a government institute (NIH etc,) is supported by taxes. The granting systems are obvious but one must also realize that the University, Foundation etc. all are recipients of gifts - donations - which are tax deductible and thus essentially all of the citizens are contributing to science. The same public 'ownership' applies to their tax free status. Based on this, I feel that no person who develops an idea, a mouse, a potion, etc. which in anyway is tax supported - subsidized - should be allowed to claim a patent, copy right and/or an intellectual property right. The effect of the present system is to have much critical research carried out in secret and/or with limited access by the public. This is a waste of human resources and grossly impedes the advance of science, for there is no full disclosure of methods or of data. When such things, which have been tax supported, become commercially useful, the cost to the consumer, the tax payer who supported the work, should be only that associated with the production – the fundamental discovery should be ethically and legally in the public domain. I have seen too many researchers and their ideas lost and subverted by their, to me, illegal and immoral quest for wealth through the processes of a patent, copy right and/or an intellectual property right. I have also seen corporations, by the rights of this or that developed by means of tax support, then suppressing the item, so that another item that they "own" and have developed can continue to monopolize or dominate the field. This maneuver may be carried out, even when the suppressed item is superior to the established one.

Summary

Due to the achievements of the NIDCD, combined with the important influence of the initial work on 'birthdating' by Dr. Ruben and his leading role in the field through 1975-1995, the editors of this Special Issue consider Dr. Ruben to be a landmark in modern research on ear development. It was a great delight to talk to a person who has been of such paramount importance for the development of the entire field, having contributed to the main issues of the science of normal ear development, and to the orientation of the administrative resources towards the application of basic research to the treatment of disease.

At the end of this day in Manhattan, there was as strong feeling that there is a need to continue with this conceptualization now that the molecular foundation of ear development has been laid down. It appeared possible that current efforts may have an immediate relevance for translational research to benefit those with impaired hearing. Conceptualization of the issues as an intellectual 'blueprint' for others, in particular younger researchers with not as broad a perspective, might benefit the entire field. It is the intent of the editors of this Special Issue to provide at least a rough outline of this future direction of research.

KEY WORDS: *interview, mitosis, birth dating, inner ear, otic vesicle, cochlea, NIDCD.*

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