TO THE NOBEL COMMITTEE & ROYAL SWEDISH ACADEMY OF SCIENCES STOCKHOLM, SWEDEN

TO THE SCIENTIFIC & ACADEMIC COMMUNITY AND MASS-MEDIA WORLDWIDE

TO ALL AUTHORITIES IN ROMANIA

PETITION FOR THE RECOGNITION OF GHEORGHE BENGA, AS A DISCOVERER OF THE FIRST WATER CHANNEL PROTEIN IN THE HUMAN RED BLOOD CELL MEMBRANE, SEVERAL YEARS BEFORE PETER AGRE (2003 NOBEL PRIZE FOR CHEMISTRY)

In 1986, Benga and coworkers (1) clearly demonstrated for the first time the presence and location of a water channel protein in the human red blood cell (RBC) membrane among polypeptides migrating in the region of 35-60 kDa on the electrophoretogram of RBC membranes, labeled with ^{203}Hg-*p*-chloromercuribenzene sulfonate (PCMBS) under conditions for the specific inhibition of water diffusion. I suggested that a minor membrane component that binds PCMBS is involved in water transport and also indicated the way in which the specific protein could be further characterized: by purification and reconstitution in liposomes. In the same year the labeling experiments were confirmed and extended (2) and in the following 2-3 years I described the novelty of our work in several reviews (3-8).

In 1988, Agre and coworkers purified a new protein from the RBC membrane (9), nick-named CHIP28 (channel-forming integral membrane protein of 28 kDa) (10). However, in addition to the 28 kDa component, the protein had a 35-60 kDa glycosylated component, i.e, the one we detected as the binding site of PCMBS under conditions for the inhibition of water transport across the RBC membrane (1,2). They suggested that CHIP28 may play a role in the linkage of the membrane skeleton to the lipid bilayer (9).

In 1990, Parker first suggested in personal discussion to Agre that the novel protein might be the water channel, and in 1992 1Agre and coworkers (11), based on Windager's suggestion to use oocyte expression as a mechanism to study water transporters, found that oocytes from *Xenopus laevis* microinjected with in vitro-transcribed CHIP28 RNA exhibited increased osmotic water permeability. The water permeability was inhibited by mercuric chloride, therefore, it was suggested that CHIP28 is a functional unit of membrane water channels. By reconstitution in liposomes it was shown that CHIP28 is a water channel itself rather than a water channel regulator. In 1993 CHIP28 was renamed aquaporin 1.

It is obvious and overwhelmingly documented from the facts presented above that the first water channel protein (aquaporin 1) was first discovered in 1986 by Benga et al. (1,2). He described one of its essential components (a molecular weight of 35-60 kDa for the glycosylated component) and the way to distinguish it from other proteins (reconstitution in liposomes and measurement of water permeability). Aquaporin 1 was first purified in 1988 and its water transport property was identified in 1992 by Agre and coworkers (9, 11). It is also obvious that what we identified by labeling experiments is the same protein that Agre and coworkers later purified, since they mentioned (11) that "the characteristics of CHIP28 are consistent with other known features of water channels, e.g. CHIP28 proteins in intact RBCs are impervious to proteolytic digestion (9, 10), as are water channels (12)".

As Agre and coworkers cited our 1983 paper (12) it is very surprising that they never cited our landmark 1986 papers (1,2); in contrast they referred only to work by other American scientists who pointed to a non-specific "pore" that allowed for permeation of anions, cations, nonelectrolytes and water (13). In contrast, we strongly argued all the time that there were indeed water channels in the RBC membrane and indicated the way in which specific water channel proteins could be further characterized by purification and reconstitution in liposomes.

I continued to be very active in the field, by achieving the purification of aquaporin 1 and developing a new procedure for its quantification by densitometry of silver stained gel (14). Over the

last decade, we have characterized the water permeability of RBCs from over 30 species (reviewed in ref. 15, 16); we reported a positive correlation between the water permeability values of RBCs from maternal venous blood and fetal RBCs isolated from cord blood taken at delivery. This points to a genetic basis for the determination of RBC water permeability (17).

Our landmark papers in 1986 can be compared with the first detection of a child "in utero" by ultrasonography, since we discovered one of the essential components of the "aquaporin child" (a molecular weight of 35-60 kDa for the glycosylated component); we have also indicated the way to recognize him after birth (among other children of his group!): placing the isolated children in a certain environment and asking them to perform the same task (one should read: reconstitution studies in liposomes and measurement of water permeability), like aligning athletes for a running test. This was the only certain way to know that the child is really the fastest runner and not just one that is helping (by various means) another child to be the fastest runner. A "new child" was observed in 1988 by Agre and coworkers, however only in 1992 the child we first detected was recognized as having the predicted qualities.

Looking in retrospect, asking the crucial question, when was the first water channel protein, aquaporin 1, discovered, a fair and clear cut answer would be: the first water channel protein, now called aquaporin 1, was identified or "seen" in situ in the human RBC membrane by Benga and coworkers in 1986, It was again "seen" when it was by chance purified by Agre and coworkers in 1988 and was again identified when its main feature, the water transport property, was found by Agre and coworkers in 1992.

If a comparison with the discovery of The New World of America is made, the first man who has "seen" a part, very small indeed, of The New Land was Columbus; later, others, including Amerigo Vespucci (from whom the name derived), have better "seen" a larger part of the new Continent and in the subsequent years many explorers discovered the complexity of the Americas!

I presented the complete history of the discovery of water channel proteins in an invited review (18) that was published one month before the Nobel Prize for Chemistry was awarded to Peter Agre for "the discovery of water channel proteins". It appears that our seminal contribution in 1986 was grossly overlooked by Peter Agre and also by the Nobel Committee. It is another example of mistakes in awarding Nobel Prizes, when a scientist who made the very first landmark contribution to a discovery is left aside. This is my case in regard with the discovery of the first water channel protein in the human RBC membrane.

For any scientist in the world dedicated to the truth and justice there is only one conclusion: Dr Benga's initial discovery must be properly credited by the Nobel Prize Committee.

The daily newspaper "Adevarul de Cluj" (19), this should be emphasized, has mentioned and put together for the first time the two names Benga and Agre and the possibility of awarding to both scientists the Nobel Prize for the discovery of aquaporin 1..

References:

1. Benga Gh, Popescu O, Pop VI, Holmes RP, 1986c. *p*-(Chloromercuri)benzenesulfonate binding by membranes proteins and the inhibiton of water transport in human erythrocytes. *Biochemistry* **25**: 1535-1538
2. Benga Gh, Popescu O, Borza Victoria, Pop VI, Mureşan A, Mocsy I, Brain A, Wrigglesworth JM, 1986b. Water permeability of human erythrocytes. Identification of membrane proteins involved in water transport. *Eur J Cell Biol* **41**: 252-262.
3. Benga Gh, 1988. Water transport in human red blood cells, *Prog in Biophys Mol Biol* **51**: 193-245.
4. Benga Gh, 1989a. Water exchange through the erythrocyte membrane. *Int Rev Cytol* **114**: 273-316.
5. Benga Gh, 1989b. Permeability through pores and holes. *Current Opinion in Cell Biol* **1**: 771-774.
6. Benga Gh, (Ed) 1989c. *Water transport in biological membranes*. CRC Press, Boca Raton.
7. Benga Gh, 1989d. Membrane proteins involved in the water permeability of human erythrocytes:

binding of *p*-chloromercuribenzene sulfonate to membrane proteins correlated with nuclear magnetic resonance measurements. In Benga Gh, (Ed). *Water transport in biological membranes*. CRC Press, Boca Raton, Vol. 2, pp. 41-61.
8. Benga Gh, 1994. Water channels in membranes. *Cell Biol Int* **18**: 829-833.
9. Denker BM, Smith BL, Kuhaida FP, Agre P. Identification, purification and partial characterization of a novel Mv 28,000 integral membrane protein from erythrocytes and renal tubules. *J. Biol. Chem.* 1988, **263**:15634-15642.
10. Smith BL, Agre P. Erythrocyte Mv 28,000 transmembrane protein exists. as a multisubunit oligomer similar to channel proteins. *J. Biol. Chem*. 1991, **266**:6407-6415.
11. Preston G.M, Carroll TP, Guggino WB, Agre P. Appearance of water channels in *Xenopus* oocytes expressing red blood cell CHIP28 protein. *Science*. 1992, **256**:385-387.
12. Benga Gh, Popescu O, Pop VI. 1983b. Water exchange through erythrocyte membranes. V. Incubation with papain prevents the p-chlormercuribenzene-sulfonate inhibition of water diffusion. *Cell Biol Int Rep*, **7**: 807-818.
13. Solomon AK, Chasan B, Dix JA, Lukacovic MF, Toon MR, Verkman AS, 1983. The aqueous pore in the red cell membrane: band 3 as a channel for anions, cations, nonelectrolytes and water. In: *Biomembranes and cell function*. FA Kumerow, Gh Benga, RP Holmes, Eds., *Ann New York Acad Sci*, **414**: 97-124.
14. Benga Gh, Banner M, Wrigglesworth JM, 1996. Quantitation of the water channel protein aquaporin (CHIP28) from red blood cell membranes by densitometry of silver stained polyacrylamide gels. *Electrophoresis* **17**: 715-719.
15. Benga Gh, Borza T. 1995. Diffusional water permeability of mammalian red blood cells. *Comp. Biochem. Physiol*., 112B, 653-659.
16. Benga Gh, 2001. Diffusional water permeability of red blood cells from various vertebrate species. *Bull. Mol Med* **7-8**: 27-42.
17. Benga Gh, Frenţescu L, Matei H, Ţigan Ş, 2001. Comparative nuclear magnetic resonance studies of water permeability of red blood cells from maternal venous and newborn umbilical cord blood. *Clin Chem Lab Med* **39**: 606-611.
18. Benga Gh. Birth of water channel proteins-the aquaporins. *Cell Biol. Int.* 2003, 27:701-709.
19. Sofron D.Aquaporina 1 – prioritate mondiala pentru cercetatorii clujeni. *Adevarul de Cluj*, *24 iulie 2003, p.14*

Presented at The 8th World Congress on Advances in Oncology and The 6th International Symposium on Molecular Medicine (October 16-18, 2003).

October 18, Hersonissos, Crete, Grece

Gheorghe Benga, MD, BSc. (Chemistry), Ph.D. (Biological Chemistry)
Professor and Chairman, Department of Cell and Molecular Biology
"Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca
6 Pasteur St, 3400 Cluj-Napoca, Romania, St.
Tel: 40-264-594373
Fax: 40-264-596889
E-mail: gbgbenga@gmail.com

Member of The Romanian Academy, Member of The Academy of Medical Sciences,
President of the Cluj Section of The Romanian Society for Cell Biology, Vice-president of The Romanian Society of Medical Genetics, Vice-president of The Romanian -American Association of Laboratory Medicine, Life Member, American Association for the Promotion of Science

I am required by law to write myself the petition. Any support from scientists from all over the world would be gratefully acknowledged.

In support of the PETITION one may sign on this site, as physical persons, while for the Public/Collective Recognition a message should be sent (signed by the representative of the institution or group) by mail, E-mail, or fax to the above addresses.

P.S. After the PETITION and the other relevant information have been placed on the site of The Ad Astra Association, in october – december 2003, several hundred scientists signed in support of the PETITION, aproving my action, some of them adding comments which underline my priority and of the group lead by myself (Benga group) in the discovery of the first water channel protein. Among those who signed in support I mention the majority of members of the Romanian Academy, among whom: Acad. Ionel Haiduc (President of the Cluj branch of The Romanian Academy, later to become President of The Romanian Academy), all members of the Section of Chemical Sciences, all members of the Section of Science and Technology of Information (including the President of the Section, Acad. Mihai Drăgănescu, past President of The Romanian Academy), all full members of the Section of Medical Sciences, including the President of the Section, Acad. Nicolae Cajal (by then President of the Academy of Medical Sciences of Romania), Acad. Victor Voicu (by then Secretary General of The Romanian Academy), distinguished Academicians Ion Baciu and Ion Hăulică, Acad. Laurenţiu M. Popescu (by then First Vice-President of the Academy of Medical Sciences of Romania, later to become President of the Academy of Medical Sciences of Romania), Prof. General (r) Vasile Cândea (President of the Academy of Romanian Scientists), some of the most famous biochemists from Romania, including Acad. Mihai Şerban (President of the Comission of Biochemistry of The Romanian Academy, Dr. Ştefana Petrescu (Director of the Institute of Biochemistry of The Romanian Academy and President of the Romanian Society of Biochemistry), chemists, physicists, matematicians, information scientistis and other specialists of Romania, great personalities of the Academy of Medical Sciences of Romania, other distinguished scientists.

From abroad I received the support and recognition of Professor George Emil Palade (1974 Nobel Laureate in Physiology or Medicine), of some of the greatest world specialists in the field of glycoproteins (Prof. Naoyuki Taniguchi from Japan, Prof. Jean Montreuil from France), of the membrane transport (Prof. Ramadan Sha'afi from the SUA, Prof. Richard Naftalin from England, Prof. Philip Kuchel from Australia), as well as from the foreign co-authors of the two landmark papers published in 1986 in *Biochemistry* (co-author Prof. Ross Holmes from the USA) and *Eur. J. Cell Biol.* (co-authors Prof. John Wrigglesworth and Dr. Anthony Brain from England). Their comments as well as of many other scientsts of world repute may be seen on this site.

In addition, there are dozens of public/collective recognition, among which those of the Academy of Romanian Scientists, Academy of Medical Sciences, National Council for Higher Education Scientific Research, of some branches of The Romanian Academy, of the Romanian Society of Cell Biology, of universities, faculties, departments, research institutes, associations from Romania or from abroad.

The priority of Benga group in the discovery of the first water channel protein is recognized in several comments regarding the 3003 Nobel Prize in Chemistry, as well as in scientific articles mentioned on this site.