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Dielectric spectroscopy of biological materials and field interactions: the connection with Gerhard Schwarz ☆

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When I came to the USA in 1947, I decided to investigate electrical and acoustic properties of biological materials over the broad frequency range which had become available after World War II. There was a need to collect data, to explore mechanisms responsible for such data and to better understand the interaction of electromagnetic and acoustic fields with biological matter.

Little was known in the acoustic case. The mechanism responsible for the strong absorption of ultrasound by tissues was a mystery. One of my co-workers, Ed Carstensen, dedicated most of his energy to this topic. I had obtained the necessary funds and we decided to study the absorption in cell suspensions first. In short order we noted that the sound absorption was largely due to haemoglobin and that cellular structure made only a small contribution. Later I extended this work with Pauly to tissues and found that in this case also the absorption occurs at the macromolecular level. However, in both cases the strongly frequency-dependent absorption is characterized by a broad spectrum of relaxation times. At this time, about 1953, Kurtze, Tamm and Eigen published their absorption data on binary electrolytes. Here the processes were sharply defined, i.e. each process was characterized by a single time constant. Encouraged by these results of the Goettingen team, Carstensen further increased the resolution and was able to measure velocity exact to 1:100 000. This enabled us to detect the small amplitude velocity dispersion which we anticipated from the absorption data. I believe that this led to our first contact with Manfred Eigen, a contact which proved to be so productive a few years later.

Most of our other efforts were dedicated to the electrical case. There had been already considerable progress due to Fricke, Cole, Oncley and others over a restricted frequency range. Our initial efforts concentrated on the same frequency range previously explored, i.e. on the dispersion which I later termed β-dispersion and which occurs at RF-frequencies. We obtained modern instrumentation which was available after World War II. I refined the relevant mathematical formulation, adding simple closed form expressions which permit the easy extraction of cellular

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parameters from experimental data. We carried out dielectric spectroscopy of all sorts of cellular systems including analyses of *E. coli* with Fricke and mitochondria with Pauly and Lester Packer, several vesicle systems with Thompson and Stoeckenius and pleuropneumonia-like organisms with Morowitz. An analysis of erythrocytes with Pauly led to better values of their internal conductivity and its response to osmotic shock. An important result was achieving an understanding of the factors which determine cytoplasmic cell conductivity values.

Data at low and very high frequencies were virtually non-existent and we concentrated our efforts here. Our data above 100 MHz together with data from the Mayo clinic extended the range to 10 GHz. They clearly demonstrated the role of water, with biological water being identical with normal water except for a small fraction of protein-bound water whose relaxational spectrum we found to occur between 0.1 GHz and a few GHz.

Another major interest focused on the low frequency range (LF). One motivation was a concept which to this day is important in dielectric theories. It is the concept of a polarization element which is characterized by a frequency-independent phase angle. It is used in dielectric theories, electrode polarization theories and provides a model for the Cole–Cole distribution function of relaxation times. It is a mathematical tool used in models describing dielectric responses, but its physical basis remains obscure. Its great popularity is due to the fact that the Cole–Cole function yields circular behaviour in complex plane plots of dielectric data. However, I noted in 1956 that circular plots are approximated by almost any logarithmic symmetrical distribution function. Only at frequencies well above or below the relaxation frequency do differences become apparent. The applicability of the Cole–Cole model to tissues and cell suspensions can be tested at low frequencies. However, to do so requires extraordinarily sensitive equipment. I had developed such equipment. Instead of the predicted logarithmic response demanded by the Cole–Cole model I found an entirely new relaxation process for muscle tissue, centered at 80 Hz. The initial response to our 1950 lecture at the annual physiological society meeting and our 1954 paper was not encouraging.

Perhaps, topics of a biophysical character were at that time not readily appreciated. Kacy Cole brought my work to the attention of Fatt and Falk. They confirmed the α-dispersion in 1964 and proposed that the tubular system of muscle cells was the cause of the effect. Their papers were published in the Proceedings of the Royal Society. By this time interest increased in this low frequency effect. In the meantime I had become interested in colloidal particles suspensions as a model for tissues. Fricke had published a paper about the dielectric behaviour of suspensions of small glass spheres. He found that the permittivity increases as the frequency is lowered. However, Fricke did not reach the low frequency limit necessary to recognize the α-relaxation effect. Furthermore, it was uncertain to what extent electrode polarization could have contributed to the observed data. We found that milk also displayed the low frequency dispersion behaviour which I had first noted with muscle. Shortly thereafter monodisperse particles became available and Mazcuk and myself carried out extensive measurements on these suspensions, concentrating on the pronounced size dependence of the relaxation time constant. I reported these findings in 1957 in my review article on dielectric properties of cell suspensions [1] and at the first Biophysical Society Conference in the United States. I considered all sorts of possible mechanisms, including the existence of a surface conductance as suggested by O'Konski. He had suggested the existence of a frequency-independent surface conductance while discussing the radio-frequency dispersion of protein solutions. He was interested in my measurements since I was the only one at the time able to obtain relevant properties at low frequencies. But I concluded that a frequency-independent surface conductance could not explain my data. Instead, if surface effects were considered responsible, the surface conductance had to be frequency-dependent, i.e. possess dispersive characteristics. This was a formal exercise providing little physical insight. However, it strongly suggested that counter ions must be somehow involved. I dis-

cussed this with several colleagues, including Chester O'Konski. He did not believe my data since they contradicted his belief in a frequency independent surface conductance. I also talked with Larry Oncley, who had done outstanding work about the dielectric properties of proteins. He believed that counter ions might cause an effect only at much higher frequencies, if at all, arguing that ion mobilities would place it there.

Let me turn now to the next decade, and the significant contributions of Gerhard Schwarz in my laboratory. I had met Cerlinski, originally from Eigen's Institute and at the time working at the Johnson Foundation. He directed my attention to the work of Gerhard Schwarz then working in Eigen's Institute. Schwarz was studying the anisotropy of the conductivity caused in polyelectrolytes when exposed to fields strong enough to evoke the effect. I read the Schwarz and Eigen papers and the role of counter ion displacement suggested as the cause of the observed effect. This added to the idea that counterion displacement might be the cause of the LF-dispersion. At about that time Manfred Eigen visited Philadelphia and my laboratory. We agreed that Gerhard might be interested to join us for some time and do further work on the α-dispersion of monodisperse systems. Gerhard accepted my invitation and joined us for a period of 3 years. He looked at the data which we had collected, noticed the R^2 radius (R)-dependence of the time constant and began to work out his relevant theory.

We published two papers in 1962. One contained most of our experimental data and a detailed discussion of non-applicable concepts [2]. Gerhard's paper contained the theoretical treatment [3]. This paper by Gerhard Schwarz was subsequently cited more than 1000 times. Robert Cole wrote me that the discovery of the effect and its explanation was perhaps the most significant dielectric contribution during the past decades. Since then many more papers have been published on the dielectric properties of colloidal particles and the theory has been refined. The basic approach chosen by Gerhard stimulated the emergence of an entirely new field of dielectric research.

Has everything been done? In the case of tissues and cell suspensions more work needs to be done. There is but little doubt that counter ion relaxation is at least in part responsible for the LF-dispersion which I had termed α-dispersion. But Fatt and Falk in Katz's laboratory had convincingly demonstrated that the tubular system in muscle tissue is a strong contributor. Gersing recently demonstrated that gap junctions in liver tissue cause α-dispersion effects. Furthermore, linear membrane conductance properties are dispersive at low frequencies, at approximately 100 Hz. This is predicted from the Hodgkin and Huxley equations. Harvey Fishman et al. presented experimental data to support this. Thus, if tissue cell membranes behave similar to the axon membranes studied by Hodgkin and Huxley, then tissue properties will reflect this by corresponding α-dispersion effects. To sort out all these mechanisms remains a task for the future.

There are also some unresolved problems about the dielectric properties of colloidal particle suspensions at somewhat higher frequencies. The late Fritz Sauer worked on this in Reinhard Schloegl's physical chemistry division at the Max Planck Institute of Biophysics in Frankfurt Main. He became motivated by our discussions. Schloegl invited me frequently for extended visits and I came almost every year after my retirement from the University of Pennsylvania. I worked on manuscripts, gave lectures and was with Sauer and some students to set up instrumentation and study colloidal properties.

Sauer believed that additional dispersive effects should become apparent at higher frequencies. He suspected that frequency-dependent viscosity effects should be considered. He had discovered a relevant theoretical thesis carried out at the University of Upsala. There were only a few measurements available at higher frequencies, including those carried out by Gerhard Schwarz in our lab and extending up to 10 MHz. They did not indicate any unusual dispersion. Blum, one of Sauer's students worked with me on this and obtained many additional data. An additional and predictable Maxwell–Wagner effect was observed. It is caused by the surface conductance which appears to be frequency independent in this frequency-range. The effect occurs above 1 MHz. It

is of small magnitude and not biologically significant and we discussed this in our recent paper in the *Journal of Physical Chemistry*. The effect has nothing to do with Sauer's suspected viscosity effect. It did not contradict Gerhard's earlier result since its magnitude was shown by us to strongly depend on the suspending medium's conductivity and the magnitude of this Maxwell–Wagner dispersion had to be very small in the case of the earlier result.

Sauer continued his theoretical work. His first attempts to treat the complex problem was limited to a first order treatment. Its failure to explain our results motivated him to start inclusion of the next term of a series development. He died while working on this.

Let me next turn to another significant contribution of Gerhard Schwarz while visiting us the second time in Philadelphia. It pertains to field interactions at the cellular level. A number of curious phenomena had been reported which appeared to suggest that weak electrical fields might adversely affect biological function and, hence, be dangerous. It was obvious that fields strong enough to cause undue heating can cause harm. This happens when fields are fairly high, typically comparable or higher than fields which cause membrane excitation responses at low frequencies, say below some kilohertz. Another school of thought claimed that even very weak electric fields can directly interact and cause significant bioeffects. They believed in effects caused by fields weaker than those needed to cause cellular excitation responses or undue heating. Most of the reported relevant results could not be reproduced. But two withstood criticism: They were the so called pearl chain effect and the alignment and turn over phenomena of cells exposed to modest electrical fields. We called the alignment of cells and particles in chains in the direction of the applied field the pearl chain formation effect. The second effect was first observed in the 1960s. It reported their orientation with long or short axis in the field direction and the existence of 'turn over' frequencies where cells rotate from the one to the other direction as the frequency is changed. These effects were cited as examples of not understood responses in terms of existing physics.

Sher and I published in 1969 the first quantitative results on the pearl chain phenomena, demonstrating that there is a threshold, i.e. the field strength must be higher than this threshold in order to cause the effect. This threshold appeared to satisfy the Langevin criterion that relevant potential energy change compares with the kT. The same was true for the preferential orientation of non-spherical cells exposed to an alternating electrical field. However, our argument had been semiquantative at best and a more thorough analysis was done by Schwarz for the orientation case.

Schwarz derived the equations which specify conditions necessary for preferential orientation of ellipsoidal particles and the turn-over effect from one to another direction as the frequency is changed [4]. In a second paper with Saito the relevant analysis is applied to biological cells, modelled as conducting particles surrounded by a membrane, resulting in considerably more complex responses [5]. The papers demonstrated that classical physical theory could explain the observed phenomena and that there exist threshold values which exclude the potential for bioeffects at weaker field strength values. From thereon these phenomena were never again to my knowledge cited as proof of the existence of mysterious and dangerous non-thermal effects. Furthermore, this work anticipated interest in the effects of alternating fields on cells, reawakened a decade or two later. Cell rotation, alignment, separation, fusion, electroporation and manipulation for all sorts of purposes establish now areas of interest for many scientists [6]. Schwarz was an early pioneer also in this field. A decade later membrane electroporation was demonstrated by Eberhard Neumann (1972) and rotation of cells exposed to AC fields noted by Zimmermann: it was only present if there were other cells nearby. I suggested to Zimmerman that the superpositioning of their dipole field to that of the rotating cell must induce a rotating field component which was probably responsible for the effect.

After my retirement from the University of Pennsylvania, I gave lectures on these phenomena at the Max Planck Institut of Biophysics. Fritz Sauer was always present and began to work

in this field. His and Schoegl's papers on the theory of field interactions with particles were summarized in an Erice Nato Conference volume in 1985 [7]. They establish the basis of the relevant theory. This volume and the papers of Chizmadjev and Postashenko are recognized that way. Interest in direct field interactions with cells has attracted many investigators. Some surprising results were obtained. While working in Wuerzburg with Arnold and Zimmermann, I suggested to compare the results obtained with colloidal particles using two different techniques, namely dielectric spectroscopy and electro-rotation. We obtained different results at low frequencies and a negative dispersion with the rotation technique. The evaluation of the rotational response gave permittivities which decreased when the frequency was lowered, while the dielectric spectroscopy data gave the usual positive response. The dielectric data were obtained on the same suspension by us, using equipment at the Max Planck Institute for polymer research in Mainz, while the rotational data were obtained in Wuerzburg. How can it be that different techniques reveal different properties? Is it possible that the combination of these data tells us something about the movement of the fluids about the particle surface and that the inductive mass term, usually neglected in dipole theories, becomes important in the rotation case? We never got around to publishing these data. A complete theory which covers both the results from electro-rotation and dielectric spectroscopy is still to be achieved.

Since then the field of bioelectromagnetics has been blooming. Much had been done about macrodosimetry, that is specific absorption rates (SAR) resulting from exposure of man to electromagnetic fields. All this work considers the distribution of fields strength in tissues of different properties, such as fat, bone, tissues of high water content, etc., and their relative arrangement in the human body. Here the body's response is calculated by using finite element techniques and compartmental physiological models.

The variation of the SAR at the cellular level is equally significant. I refer here to fields induced in membranes, cytoplasm, cellular organelles, etc. Little has been done about micro-dosimetry even though I published several articles on this [8]. It is clearly a prerequisite to understanding direct weak field interactions, if there are any at that. An example of this is the earlier work of Gerhard Schwarz on the cellular orientation effect. Much later the effect was rediscovered when Blakemoore demonstrated the response of mud bacteria near Woodshole to weak magnetic fields and Adrian Kalmijn, now at the La Jolla Scripps Oceanographic Institute formulated the relevant theory.

In contrast, there has been too much emphasis on experimental data reporting on complex biological responses to exposure and epidemiological observations. Most of this work could not be reproduced. A recent report of the National Research Council and the National Academy of Sciences denies the need for support for this sort of work and calls for more relevant basic research. An NIH report is a bit more cautious, stating that subtle effects cannot be ruled out. Relevant standards in the USA appear to be reasonable, but international standards recognize European acceptance of the principle that subtle effects cannot be ruled out and that 'prudent avoidance' should be recommended, whatever that may mean operationally.

About 1962 I visited Goettingen for the first time again after World War II and gave a lecture in Manfred Eigen's institute on Electrical Relaxation Phenomena and Biological Structure (6 July). In 1968 I spent 1 month there working out the details of our dielectric analysis of vesicles which Stoeckenius had prepared. Since then I have been there several times, talking about all this and benefiting from discussions with Manfred Eigen and his co-workers Porschke, Eberhard Neumann, Eggers and Funk and later Reinhard Schloegl whom I met there first. I cherish these occasions and an environment which brought Gerhard to Philadelphia where he did such outstanding work and later work with Schloegl's group. I have only concentrated on those few fields where Gerhard did outstanding work in my laboratory, work I am familiar with. I did not discuss his dielectric work on the helix-coil transformation, his interest in membrane channel function and many other topics. I admire his

continued creativity, a creativity which enabled him to continue to enter new fields and be productive to this day.

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