Biol Metals (1990) 3: 61-63

BIOLOGY OF METALS



# A quarter-century Biochemistry of copper with Bill Blumberg

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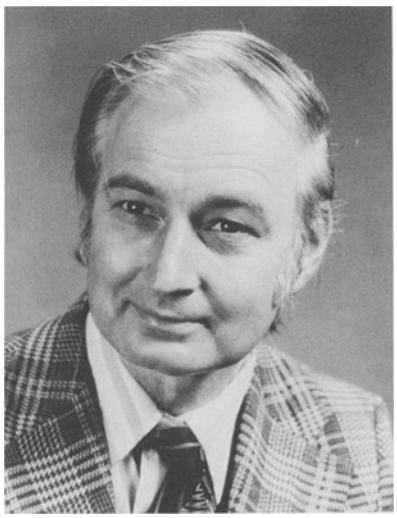

I consider it an honor to be given the opportunity to open this symposium in memory of a colleague to whom I have looked up ever since I knew him, who has been the confessor and last resort for help and advice to all of us who ventured out on the thin ice in the border field of biochemistry and solid-state physics. Bill had a flair for explaining complicated experimental findings and concepts to us in simple terms and his expositions at meetings excel in this respect. Bill was always patient to listen to us and to step down in the hierarchy of the sciences to the lowly level of biochemistry. I remember an occasion, in this context, when Albert Szent Györgyi introduced the physicist Michael Kasha (to a mostly biological audience), he said: "You know, Michael Kasha is a very dangerous man; when you listen to him, everything is so easy and clear, but then, when you find yourself again left to your own devices struggling with the subject, you are lost".

When I now, after having gathered a little more experience and seen more action, go back to Bill's papers and published lectures, I always find things in his writings that I either previously did not understand or that I did not try to understand, because I was not aware that they might ever be of concern to me. I find now that Bill has said many things years ago in which we are still very much involved in our current work. We are particularly lucky if the discussion remarks were recorded and published, because there we find much wisdom laid down, very pertinent to particular problems which are still in focus today. Thus, for instance, in a discussion remark after Peter Hemmerich's paper at the Arden House meeting in 1965 [1], Bill spelled out the conditions and consequences of copper–copper interaction (cupric–cupric in this case), a topic which is particularly timely today. With evidence rapidly accumulating that in those blue copper proteins which are oxidases there is a trinuclear metal cluster, I expect that there will be discussion on this subject later in this meeting on interactions in trinuclear copper or metal centers and also in mixed-valence dinuclear and trinuclear centers.

Since I have been mentioning Bill's incisive and precious discussion remarks, I would like to bring to your attention a more covert role of a related nature that Bill has played at meetings through the years. Bill has often, or even mostly, not been visible. He habitually populated the last row of seats or even stood at the rear wall. So the speaker in front was never entirely sure whether Bill may not be somewhere back there. I am sure that the mere thought that Bill might indeed be somewhere in the back of the room has prevented much loose talk or nonsense from being said.

As far as I am aware, Bill made his debut before larger biochemical-type audiences in 1965 at the Kettering meeting [2] on Fe-S proteins at Antioch and the Symposium on *The biochemistry of copper* (cf. title) at Arden House [3] in the fall, which is more to the point for the present meeting here. He spoke at this meeting about models for copper coordination and explained lucidly in some detail the symmetry considerations that are pertinent here, ending with this remark: "The purpose of these fictitious models is to encourage new lines of thought on the mechanism of action of cupric enzymes and the configuration of the cupric ion in such enzymes". I am quoting him in full, because it says ex-

actly what Bill has always done, namely encouraged new lines of thought and of approach to biochemical problems. Before I go on and remind you of some of the highlights of Bill's contributions, I would like to anticipate that I see three main threads of thought and endeavor going through his development of concepts, approaches, and interpretations. The first, clearly spelled out at Arden House and later recurring in his systematic analysis of the EPR spectra of low-spin and high-spin heme compounds and proteins at Airlie House 1968, and at the Johnson Foundation [4] in 1971: To systematize the measurable EPR parameters of metals in proteins, determine in this way the ligand environment of the metals and relate this information to or predict features of the electronic and MCD spectra. The second, foretold above in the discussion remark to Hemmerich's paper and beautifully documented in the paper on turacin, the dimer Cu-porphyrin, in 1965 [5]: To determine the types and predict the effect of spin-spin interactions. And the third is essentially the thread we are picking up here today: The development of new physical methods or new interpretative approaches to existing methods for the study of metal proteins or metal complexes.

There is one more aspect to Bill's contributions which I must mention here. In these days, few major and sustained advances such as those that we are speaking about here, are made by a single scientist. I think it would be Bill Blumberg's will, and all of those here who have followed the developments which I have touched on would agree with me, that I should emphasize Jack Peisach's contributions to Bill's accomplishments. I have no doubt that Bill could have mastered the physical problems, for whose solutions he is known, all by himself, but I have serious doubt that he would have undertaken to do so. It took the broad knowledge of biological phenomena and problems and the enthusiasm, and intellectual stimulus of a man like Jack Peisach to convince Bill that the problems he was putting his mind to were in fact worth the effort.

Now let me briefly go through just some highlights that I remember vividly from a much larger body of contributions. I mentioned the Kettering and Arden House meetings. Then followed the second conference on *Magnetic resonance in biological systems* at Stockholm, where Bill gave a brilliant and much cited exposition of the EPR features of Fe(III) in a rhombic field [6]. To illustrate Bill's skills in explaining concepts to non-physicists, I would like to mention an example that Bill gave in explaining why there could not be an ever increasing rhombicity as had been suggested by others. In a properly chosen axis system $E/D$ can never exceed a value of 1/3. He likened the experience with the phenomenon of rhombicity to the experience that a hiker has when he goes into a dense forest. At some point, the hiker will no longer get deeper into the forest but will eventually walk out of it on the other side. In effect he has switched his axis system by walking beyond the point of maximal density. In the mid-sixties, Bill published work on blue-copper proteins and peroxidases [7] and in 1970 [8] he explained for us the odd signals we observed with cytochrome *P*-450 on addition of substrate, where a dramatic change of spin state occurs from low spin to high spin with formation of a strongly rhombic high-spin signal ($E/D = 0.087$), to my knowledge never observed before with heme proteins. After a preliminary announcement of his approach to determining the ligand environment of low-spin heme proteins at the magnetic resonance conference at Arlie House in 1968, Bill explained in detail his, by now classical, analysis of the EPR spectra of these proteins in various ligand fields at the Johnson Foundation meeting in 1971 [4]; he presented the 'truth diagrams' which have played a great role in the development of EPR spectroscopy of heme proteins. This was soon followed by an analysis of the spectra of high-spin heme proteins [9–11] where, as alluded to above, the rhombicity is the major variable. Rubredoxin, a mononuclear high-spin iron-sulfur protein, was also studied. The work on this protein, presented at a meeting of the New York Academy of Sciences, contains an exemplary demonstration of how account must be taken of the zero field splitting of a high-spin heme protein and how its value can be determined [12, 13]. In 1974 we find in the Archives a treatment of the EPR of [2Fe-2S] proteins [14] and soon after of copper proteins and complexes [15], basically similar to that used previously for heme proteins, a guide to extracting structural information from EPR parameters. In 1976 at Honolulu, at the American-Japanese joint meeting on copper and iron proteins [16], Bill introduced a novel approach, little if at all heard of in biochemical circles: X-ray absorption spectroscopy. This brings us closer to the present, because Bill's contributions to the metal-protein field since then have mainly been in this area. In 1978 we find his name on a paper on X-ray absorption spectroscopy of Cu and Zn in superoxide dismutase [17], 1978/79 on papers concerned with cytochrome oxidase [18–21], 1982 with stellacyanin [22] and as recently as 1989 he is the first author on a publication describing results on X-ray spectroscopy of dopamine $\beta$-hydroxylase [23]. As with all newly introduced techniques, enthusiasm is great and experience nil at the beginning and so some over-interpretation and other slips can be expected. I was always glad to know that a critical and uncompromising mind like Bill Blumberg's was somewhere in the background in this game, just as he had been in the back of the room in the early EPR days, as I alluded to above. Those of us who had the privilege to know Bill and to enjoy his advice and collaboration, will always sense Bill's presense, somewhere in the background, reminding us to maintain the scientific standards that he has set.

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