

## PALL CONFERENCE

It's on, it's on (microphone)

Denay and zeem (doctors?) suggested that three of the CFS Fibromyalgia multiple chemical sensitivity (mcs) may simply reflect different aspects of a common underlying mechanism and there are other people have made similar statements. In CFS and Fibromyalgia one has a literature that suggests that some people do recover completely from those, roughly 10%. With the msc group such recoveries rarely if ever occur. So, in general these are huge problems because almost all people who become ill stay ill through their life although they may see improvements over time. And...so this talk will focus on mcs, however a comparison with these other multisystem illnesses is useful and at times during my talk I will refer to some of the others because often the mechanisms that one sees in the others also show up with mcs. So I will refer to all three of them.

Now, one of the great puzzles about mcs is how can diverse chemicals, that are implicated in initiating cases of mcs, how can they produce these exquisite sensitivity responses. And basically the same set of chemicals that seem to be involved in the initiation also produce sensitivity responses in those who are already sensitive. So we have that great puzzle about how we get initiation and how that then leads to that high level sensitivity. Some of the mcs skeptics, including Ronald Gots(?), have argued that there cannot be a common response to such a broad group of chemicals, and I will show here that they are wrong about that.

So among the chemicals that are implicated in initiating cases of mcs one has a very broad group of organic solvents and related compounds, and then there are three classes of pesticides. There are organophosphorous and comperment (?) pesticides organic chlorine pesticides and pyrethroid (?) pesticides, and then occasionally these other three also have roles in initiating cases, OK? So the question is how can these broad groups of chemicals produce a common response in the body. And what I have proposed is basically that they act along known pathways, and that they all end up leading to increases in NMBA (?) activity.

So we have here the organic solvents, we think that they work primarily but not solely via a receptor called a vanilloid TRPV1 (?) receptor and that can increase NMBA (?) activity. There are the organophosphorous and compermen (?) pesticides which act as cholinesterase inhibitors and they in turn produce increases in NMBA activity, the organic chlorines, including lindane and chloridane (?) and several others act as Gab A (?) antagonists and that, in turn increases NMBA activity, and the pyrethroids (?) act to open sodium channels and that in turn leads to NMBA increases.

So we have all of these things acting along different pathways but producing a common response. So immediately we have an interesting model here, of how these various agents can act to produce a common response in the body.

OK, now let me just say one other thing about this and it's actually quite important, and that is there are members of each of these classes of chemicals that have been tested in animal models. And it's been shown that if you use an NMBA antagonist – that is use a drug that lowers the NMBA activity – you can greatly lower the toxicity of these compounds in the body. So we not only know that you get an increase in NMBA activity, but that that increase is very important for the toxic responses in the body.

There are similar properties for the other three compounds that I talked about before – hydrogen sulphide, carbon monoxide and mercury. Mercury acts via its product, methyl mercury. And each of these can

produce increased NMDA(?) activity and you can use an NMDA antagonist and you will lower the toxic responses to all three of these. OK?

So we have really quite a stunning convergence of mechanism of all of these classes of chemicals that are implicated in mcs. So this very strongly suggests that this increase in NMDA activity is involved.

There are six other types of evidence which implicate increased NMDA activity in mcs, and I am not going to go through these in detail because we have limited time but basically, what I want to say here is that some of these implicate increased NMDA activity in the initiation of cases of mcs, including, say, number two, where a genetic change that produces increased NMDA activity is associated with increased prevalence of mcs, but also that chemicals that act to produce sensitivity responses in people who are already sensitive, such as in number three for example, that you can lower the responses in people who are already chemically sensitive by using an NMDA antagonist. And what does that do? That suggests that the increased NMDA activity has a crucial role in responding to the chemicals in people who are already sensitive. So that is important.

So we have six additional types of evidence for a role of increased NMDA activity, and I think this is really compelling evidence for a common toxicological response to all of these chemicals that are implicated here.

There is one other very important type of evidence which shows that the chemical exposure is causal. That it is involved in initiating cases of mcs, and that is this genetic evidence. And there are three important studies that have been done on the genetics here. One was done by Haley (?) and co-workers with the Gulf War Syndrome people and another one was done by McKay and Ison (?) on Canadian mcs patients, and the third was done by Schnakenburg (?) and co-workers on German mcs patients. And what they have shown is that a whole series of genes which have crucial roles in the metabolism of these same compounds, particularly the organic solvents and the organophosphorous pesticides, have roles in determining whether people are especially susceptible to coming down with mcs. And so we have five of these genes where there is really quite strong evidence for that and then a sixth, the bottom one, where there is weaker evidence.

All of these have roles, then, in the metabolism of these same compounds, and the only interpretation for this set of data, is that these chemicals are acting as toxicants, in their role of initiating cases of mcs, and therefore the genes that influence the rate of metabolism of those chemicals will influence people's susceptibility to them, and to coming down with mcs.

So this is, I think, very important evidence and it really produces a compelling argument that these chemicals are acting as toxicants in initiating cases of mcs and we also have evidence, as I mentioned a while ago that the chemicals are also acting as toxicants when they produce sensitivity responses in those who are already sensitive.

Now, one thing that you may have noted already, is that the receptors for those chemicals that I discussed are not the olfactory receptors. They are not the receptors that are involved in detecting odours. And yet people have over and over again talked about sensitivity to odours. And I think the important point that needs to be emphasized here, is that mcs people are sensitive to chemicals. Many of those chemicals have odours but that does not mean that the primary sensitivity is to odours.

Now, that is not to say that people with mcs do not have changes in their olfactory mechanisms - some of them, I think, do - but that is not the primary mechanism of mcs.

So, what happens then when you stimulate the NMDA receptors is that there are channels that are part of the receptor mechanism that allow calcium to flow into the cell. Calcium stimulates these two enzymes Nnos and Enos (?) which are both nitric oxide synthases. These are both enzymes that produce nitric oxide and both of them are calcium dependant, that is, they have to have calcium there in order to be active. So when

the calcium flows into the cell you get an increase in nitric oxide as a consequence of that, and some of that reacts with another compound called superoxide to form a compound called peroxynitrite, which is a potent oxidant. And so all of these things are known to occur in response to the increased NMDA activity, and, as I think you will see, it appears that all of these responses are important in the mcs mechanism. So we have NMDA activity, we have calcium, we have these forms of nitric oxide synthases, we have nitric oxide and we have peroxynitrite, all of those things are important and I will show you, I think, how they all come in to this mechanism that produces this exquisite chemical sensitivity.

So the central chemistry here, if you will, it is the only chemistry I am going to talk about, is this reaction of nitric oxide, which is this structure  $\text{NO}(\cdot)$  and this is a free radical. And superoxide, which is another free radical, and those two react extremely rapidly with each other to form this compound, peroxynitrite. Peroxynitrite is not a free radical but it is a potent oxidant and it can break down to form other free radicals. And so it can produce a lot of damage in the body.....and we will talk about some of that but probably not in much detail, given the amount of time that we have here.

So, each of these illnesses, here is mcs and here are the compounds that we talked about that can initiate cases of mcs. There are also a number of stressors that can initiate cases of chronic fatigue syndrome, fibromyalgia and also post traumatic stress disorder – which I have argued is actually a member of this group. So these are all, or almost all, short term stressors, and yet they can initiate these chronic illnesses.

And let me just say, there are a total of seventeen different short term stressors that are shown in this table here, and there may be others in addition. But these are the ones that are best documented in the literature as stressors that can initiate these illnesses. And so one of the great puzzles is how can all these things produce a similar response in the body and produce a chronic illness, and what I have argued is that all of these can act to increase nitric oxide levels in the body. Some of them, as you have already seen, do that through the NMDA receptors but some of them do that through other mechanisms, like the infectious agents, viral and bacterial infection, this protozoan infection – toxoplasmosis -, ionizing radiation exposure, all those act, not via NMDA receptors but via the induction of the inducible nitric oxide synthase that is often abbreviated iNOS (?).

So we have different mechanisms but they all produce a nitric oxide response. That seems to be the common response for all of them. Now, as I said before, all of the ones that are involved in mcs work via NMDA, and I think there is an important reason why that is true. And I will show you that in a little while.

But in general, the ability to initiate these chronic illnesses is not dependent on the NMDA receptors. It seems to be dependent on nitric oxide and, probably more importantly, dependent on peroxynitrite – its oxidant product.

So what I propose is that the way in which nitric oxide works is through this peroxynitrite compound over here. And that it works to initiate a complex vicious cycle here, which is then the cause of illness.

Basically each of these arrows here represents one or more mechanisms by which one of these things increases the second. And I would like to go through some of these with you to give you some idea as to how they fit together. But I think the point that you can see already from this figure, from this diagram here, is that there are a number of cycles in here that are interacting ... you've got this one and you've got that one, and this one and this one, and then you've got some others going up around here. So basically what we have here is a set of interacting vicious cycles and the idea is that the whole cycle, which I call the NO/ONO cycle, based on the structure of nitric oxide, and the structure of peroxynitrite, but obviously involves a whole bunch of other things as well, that once this cycle gets going it is the cause of illness, and that the initial cause is to get this thing started.

So you have these initial short term stressors that get the cycle going and then you have the cycle itself which is the cause of the chronic illness. And you can kind of see why it would be chronic, because these things are going to keep triggering responses and so you'll have these things.

And so what are they? We have nitric oxide here, we have superoxide, we have...they react with each other to form peroxynitrite, peroxynitrite being a potent oxidant produces oxidative stress, which is an imbalance between oxidants and antioxidants (antioxidants?), ummm these two can increase intercellular calcium levels which in turn can activate these two nitric oxide synthases, and make more nitric oxide. This oxidative stress, and to a lesser extent the calcium, can increase the activity of this. This is NFkB (?). This is a transcription factor. This is a protein which turns on certain genes in the nucleus of the cell. So when it is activated, NFkB, it turns on these inflammatory cytokine genes and it also activates this inducible nitric oxide synthase gene – and the cytokines in turn activate INOS (?).

So you get an INOS induction both direct from NFkB, and indirect through the cytokines. And what does that do? Well, it produces more nitric oxide. And so it can kind of come round and act here. There are a number of mechanisms that are also expected and, in fact, have been shown to increase superoxide levels, involving peroxynitrite, involving nitric oxide, and I'm not going to talk about what those are but all of these are discussed in great detail in my book, Explaining 'Unexplained Illnesses'.

And, and, there's an increase in, here is our NMDA activity, and I will show you how NMDA activity can be increased in a little while.

## **Pall Conference Part 2**

And there is another receptor here called the vanilloid receptor which we also think has a role, and in fact there are probably some other members of this group of receptors, which I will refer to very briefly, that also have a role here.

So this is what we call the NO/ONO cycle, and actually even though this is rather complex, it turns out it is even more complex than this, so I apologize for that but that's the way science is sometimes.

So there are two aspects of this cycle which are not obvious from the discussion that I just gave you. One is that there are a number of mechanisms by which mitochondria, and consequently energy metabolism, is dysfunctional. As a consequence, the attack of peroxynitrite on a number of the components of the mitochondria as a function of the role of superoxide also in the mitochondria and also nitric oxide, all three of these can inhibit or inactivate important components in the mitochondria that will then lead to mitochondrial disfunction and energy metabolism disfunction and a depletion of ATP (?) which, of course, is the energy currency in the cell.

So these mechanisms turn out to be important not only because they have roles in producing some of the symptoms and signs, but also because they have actual roles in the cycle itself. And I will talk to you a little bit about a couple of the roles in the cycle, where we think that they have important roles.

There is another set of mechanisms here (and I apologize for giving you all of this biochemistry but you kind of have to .....and I have to say I have learned a lot of biochemistry myself when researching this)....um The nitric oxide synthases have a co factor that is essential for their production of nitric oxide called tetrahydrobiopterin (?) and it is usually abbreviated BH4, ok so we have this BH4, and when you have a deficiency in tetrahydrobiopterin then some of the nitric oxide synthases become what has been called uncoupled. That is, they no longer produce nitric oxide, and they start producing in place of that – superoxide. And so they produce superoxide in place of the nitric oxide. And so what you can have when you have a ...some cells or tissues that have particularly high levels of nitric oxide synthase and where they become partially uncoupled you can have adjacent enzymes, right next to each other, one of them producing nitric oxide, one of them producing superoxide, and these will then react very quickly to form more peroxynitrite .. and what will that do?

Well, what peroxynitrite does is it oxidizes tetrahydrobiopterin (?) and so it can keep this partial uncoupling going. And what I think is true, and I have come to this fairly recently, is that this mechanism here is likely to be the core of the NO/ONO cycle. And one of the things that is kind of interesting about it is that if you improve this part of it, that is, if you increase the availability of tetrahydrobiopterin, BH4, what you will do is increase nitric oxide levels, but you will also get a clinical improvement.

So nitric oxide is not always the bad guy in this thing. In this context actually increasing nitric oxide via this mechanism, via lowering, uncoupling, should be helpful rather than hurtful. OK?

So this is the new NO/ONO cycle which includes all those things that I just talked about. And....actually sometimes I think I am not very quick on some of these things....it took me about two years to figure out how to diagram this.

Here we have peroxynitrite, it is abbreviated PRN, and here we have BH4, and so this is depleted, and so here we have this reciprocal relationship between PRN and BH4 ...

(requests for more microphone.....problems hearing....from the audience)

So here we have this reciprocal relationship between these two, which as I said before, I think this may be actually the core of the cycle. And then we have ATP (?) depletion, energy metabolism depletion, over here, and these all have important roles then, in the cycle. And I have diagrammed these, and I am not going to go into them in detail here.

And here are some of the other things that we have talked about before – nitric oxide, superoxide, oxidative stress, NFkB (?), INOS (?), the other nitric oxide synthases here, intracellular calcium, NMDA receptors, and there is these receptors here. This is the vanilloid receptor that I mentioned before as a member of this receptor family and I think actually now there are some other members of this family that have a role here and I will talk about perhaps one or two of those later on.

OK, can you hear me better now?

So we have here five principles which underlie this NO/ONO cycle mechanism. And these are important for at least three reasons that I will share with you shortly. Let's look at these principles. The first one, the first two, we have already talked about – that short term stressors act to initiate these illnesses by increasing either nitric oxide or other cycle elements and so they have to start out, they have to be able to interact with the cycle in it. These increases then, particularly in peroxynitrite, initiate this NO/ONO cycle which is then the cause of these chronic illnesses. And one of the consequences and predictions of this is that each of the elements of the cycle will be elevated in the chronic phase of illness. And of course, that is testable. And we have substantial data on that, particularly with the CFS Fibromyalgia group, but also some with the mcs group.

The symptoms and signs of illnesses must be caused by elevated elements of the NO/ONO cycle. So, in other words, all the symptoms and signs should be caused by some of these things – nitric oxide, superoxide, peroxynitrite and NFkB, oxidative stress etc. So those have to be involved in the production of the symptoms and signs in order for this to be a NO/ONO cycle disease.

The fourth principle turns out to be a very important one. And that is that the basic biochemistry of this cycle is local. What do I mean by that? What I mean by that is that it will be present in certain tissues in particular individuals and in other tissues in other individuals. That is, it is basically a local mechanism. And the reason that it is local is because the three compounds involved, nitric oxide, superoxide and peroxynitrite, have short half lives in biological tissues. So they don't go very far from where they are initially made to where they are destroyed, typically. And the mechanisms of the cycle...all those arrows that we talked about before ... act at the level of individual cells.

So we are talking about a mechanism which will be present in certain tissues in one individual and a different set of tissues in another individual. And because of that, you can first of all have multiple diseases caused by the same mechanism, depending on where the tissue impact is, and secondly you see a tremendous amount of variation in symptoms and signs from one individual to another. Even those that have been diagnosed as having a particular disease. So, for instance, with the CFS group you see a lot of variations, with the mcs group you see a lot of variations. This has been a great puzzle, because people said how can they be real diseases if they are so variable – and the answer I think is really very simple. The answer is that the tissue impact in the different individuals is different. And that will then provide a lot of variation in symptoms and signs.

### **Pall Conference Part 3**

The fifth principle is that therapy should focus on downregulating the NO/ONO cycle. That is we should focus on lowering the cause rather than relieving the symptoms, and so that's obviously the most important principle from the standpoint both of sufferers and also from the standpoint of physicians that are trying to effectively treat them.

So, basically what I have argued is that these NO/ONO cycle diseases represent what I argue is the tenth paradigm of human disease, that is, there are nine well accepted paradigms that I have listed here, of human diseases, and I am arguing this is the tenth, and in fact it may be one of the more important ones of those ten as well. So this is, I think, quite exciting. And there are a number of other diseases in addition to the ones that I have discussed, where one can make a case that they may be NO/ONO cycle diseases. I think I will talk about some of those later if we have time.

I am going to go through this just very quickly but in chapter three of my book I discuss how a variety of different symptoms and signs of this group of illnesses, things that are shared among all four of them, how they may be generated by mechanisms, initiated by specific elements of the NO/ONO cycle. And what I want to say to you here, basically, and just point out to you is that these mechanisms are pretty well documented in the literature. The question is, are these the mechanisms that are occurring in these illnesses. And there I think in most cases we don't have good evidence for that.

But what is true is that there is a wide variety of different changes that should be produced by these elements of the cycle. Fatigue presumably is caused by energy metabolism dysfunction. There are changes in immune function that can be produced by inflammatory cytokines, oxidative stress, superoxide, including low Natural Killer Cell function which has often been reported with this group of illnesses. Problems with learning and memory dysfunction, problems with circulatory dysfunction, particularly with orthostatic intolerance, pain, there are basically all the elements of the cycle can be involved generating pain in hyperalgesia situations. Depression can be produced by the inflammatory cytokines and by increased nitric oxide. So one can have psychiatric symptoms, one can have pain, one can have other types of brain dysfunction, immune dysfunction, circulatory dysfunction and so forth. And the changes in sleep, anxiety ... this is one I found particularly interesting because there are animal models where one could take a specific NMDA agonist and inject it into the amygdala in the brain of an animal and you can get anxiety and even panic attack responses simply from doing that. So presumably a local increase in the cycle in the amygdala should be able then to produce anxiety responses. Abnormal PET scans SPECT scans and so forth.

So there are quite a number of things that should be able to be produced by these cycle elements. And again, these are well known mechanisms but whether they apply to this group of illnesses is still unclear, OK? So I am not saying that we have proven that these are THE mechanisms with these illnesses.

So we have the four illnesses and presumably the differences among them have to do with which specific tissues are impacted by the NO/ONO cycle, in one illness versus another. So the question is, what is going on with mcs? And how can people become so extraordinarily sensitive to chemicals, as a function of previous chemical exposure? And that is really perhaps the most important set of questions that one can ask about mcs. And I think one of the things about mcs is that one has sensitivities that come both from the central nervous system, and also other sensitivities that come from peripheral regions of the body. I will talk about both of those rather briefly. But the central part is probably the most characteristic thing about mcs and perhaps the most important for us to understand.

So, basically what I am going to discuss here is the fact that there was a previous model of mcs that was developed by Dr Iris Bell (?) who is at the university of Arizona in the US, and she argued that there was a neural sensitization mechanism that was involved, presumably in the hippocampus and other related regions

of the brain. This is the region of the brain that is involved in learning and memory. So her arguments was that the chemical, somehow, and I'll show you how in a little bit, in fact I have already sort of showed you how, could produce great neural sensitization where the synapses between one neuron and a second were much more active than they would be normally, as a consequence of chemical exposure.

So basically, the idea here is that this process of neural sensitization which is known to be activated on a very selective basis in normal learning and memory, may be activated massively in mcs as a consequence of chemical exposure. That was her basic model.

The thing that I want to emphasize here is the following: the main mechanism for neural sensitization is something called long term potentiation, abbreviated LTP. Long term potentiation is known to involve increased NMDA receptor activity, OK? So here are NMDA receptors and I think the reason why all these chemicals have to act through the NMDA receptors is basically this one – that is, this is essential for getting long term potentiation. And this process not only involves increased NMDA activity but it involves increased intracellular calcium, increased nitric oxide, and also increased superoxide. So a number of the elements of the NO/ONO cycle can be seen to have important roles in neural sensitization. So again, what we are arguing is that the chemical exposure that we talked about before, that can increase all of these things, can very plausibly have a major impact on neural sensitization, through a variety of different mechanisms.

OK, so I have got a greatly oversimplified diagram of how some of this plays out in mcs, that I have called the neural sensitization cycle. Basically, here is a presynaptic cell and here is a postsynaptic cell and what happens in neural sensitization is that basically this arrow becomes more active and you get more NMDA stimulation in the post synaptic cell. Now when the post synaptic cell is stimulated you can get an increase in nitric oxide, as we have already said, because when you stimulate NMDA receptors you get an increase in nitric oxide.

One of the things that nitric oxide does in this mechanism is that it acts as what is known as a retrograde messenger. That is, it can go back to the presynaptic cell and make it more active in releasing the neurotransmitter, and the important neurotransmitter here is glutamate, which is the normal NMDA agonist here. You also can then get peroxynitrite as a function of the increased nitric oxide, and you can get energy depletion.

So what does energy depletion do? Well, energy depletion acts in two ways to increase NMDA activity. One is that it actually makes these receptors more sensitive to stimulation. And the other thing is that it actually means that the glutamate that is released from the presynaptic cell is actually more active in the stimulation. So there is a double effect here.

So immediately you can kind of see how chemical exposure coming in and stimulating these could lead through a number of different mechanisms to giving you an increase in neural sensitization and consequently an increase in chemical sensitivity.

Now, I have talked about seven different mechanisms, and again, these are all well known mechanisms but their role in mcs is primarily hypothetical at this point. But still and all, these are all mechanisms which are predicted to be stimulated as a consequence of that, and each of them will lead to an increase in chemical sensitivity at a different level. So the idea is that each of these, here is the retrograde messenger function, here is a function of just NO/ONO cycle elevation, and the two roles of energy metabolism that we discussed a little while ago are here. But also nitric oxide can inhibit the metabolism of these chemicals via this mechanism. Peroxynitrite can lead to a breakdown in the blood/brain barrier, which would lead then to increased chemical access to the brain, that's another mechanism. And let me just say that Doctor Bodokuklinski (?) in Germany has published some studies showing that this does occur in mcs patients -this

particular breakdown of the blood/brain barrier does occur in mcs patients. Abudonia has shown this in animal models as well.

And then there is an effect on the vanilloid receptors that would be specific probably for organic solvents. So we have argued that the organic solvents were primarily through this vanilloid receptor, so if you increase this activity you will get increased stimulation by organic solvents.

So you can kind of see how relatively small changes in each of these could lead to a huge change in chemical sensitivity, because they are going to multiply on each other. So, you know, if you have a threefold increase here and a fivefold increase here and so forth, and you multiply each of these together, you can see how you can get a thousandfold or even greater increase in sensitivity based on all of these different mechanisms. So basically I think that is what is going on with mcs, and why mcs people can be so exquisitely sensitive to chemicals at levels that normal people have no problems with at all that one can see. So this is the basic explanation for the phenomenon that one sees here.

Let me just say I am going to skip through most of this.....

Basically, I think that some of the peripheral sensitivity mechanisms are the same as the ones we talked about before, for the central nervous system. But there may also be other ones that come in here. And specifically Bill Maggs (?) and Gunar Hoyser (?) have published evidence that there is a phenomenon called neurogenic inflammation in the peripheral sensitivities now and also Masso (?) activation have roles. And both of these .....both of these are compatible with the NO/ONO cycles mechanisms, and so these may be part of the NO/ONO cycle in the peripheral tissues.

So in mcs you often see sensitivity in the nasal passages, in the upper respiratory tract, in the bronchii, the ones you basically have asthma type symptoms. You have sensitivity in regions of the skin, you have sensitivity in the GI tract, and there are several other types of tissues that can become sensitive as well. Which tissues are impacted varies from one mcs patient to another. So you don't see all the same patterns. And in fact (?) knew a person in (????) Washington who only had, as far as I could figure out, sensitivity in her upper respiratory tract, she had no sensitivity in the brain, she had had no asthma type symptoms, she had no skin sensitivities and ..she just had sensitivity in the upper respiratory tract.

So you can have, and that at least by some definitions, doesn't qualify as being mcs, but I think that basically the mechanism that she was seeing in her upper respiratory tract was essentially the same as what mcs people have. It is just she didn't have other tissues impacted.

So you can kind of see how the local nature of this can come into it, and can give you a variety of different symptoms from one individual to another. And that is obviously important in terms of understanding what is going on here .

Now, I think what I am going to do here is just to spend some time on this, and then I'm going to skip over to therapy because we have a limited amount of time here, and I want to leave some time for questions.

One of the obvious great needs in mcs, and in fact in this whole group of illnesses, is the need for specific biomarker tests. For tests in which you can take a patient who at least has been tentatively diagnosed as having mcs, in this case, and ask : is there an objective test that can be done that confirms that diagnosis? And one of the things that I found surprising actually, is that there are a number of such tests that in my judgement have been described in the literature, that look to me like they should be very good specific biomarker tests. And yet one of the things that has been an important thing in this area is that none of these have been sort of widely recognized as specific biomarker tests.

#### Pall part 4

I want to speak basically about three of them because I think three of them are particularly attractive as potential tests that could be used in a clinical setting. One of them is.....there was a single paper that was published by Kimata (?) in Japan in which he reported that there were changes actually in four things ( I have only got two of them listed here) that are all basically inflammatory markers in response to chemical exposure in mcs patients. And so here you are taking a blood sample and measuring nerth (??) growth factor or histamine, and there are also two other inflammatory markers that he looked at. And all of those were increased in response to chemical exposure in the mcs group, but not in a normal group and also not in another group that had chronic inflammation but no chemical sensitivity. So this seems to be specific for mcs.

Milkfis (?) and her colleagues in Sweden have published a whole series of papers. I think there are about 8 of them now in which, she has been studying response to capseisin (?). Capseisin is the hot stuff in hot peppers....I don't know .....pepperoni? And what she and her colleagues have done is basically spray this into the upper respiratory tract and measure cough responses. And what she finds is that people with mcs typically have cough responses at very low levels of capseisin that normal people don't react to. So you have to use higher levels of capseisin to trigger a cough response in normal people.

So let me just say, I think this is a local response, OK? So people who don't have sensitivity in their upper respiratory tract will probably not react the way she has been measuring these things , but at least for those people who have local sensitivities there, this should be an effective specific biomarker test.

And let me say one other thing here. The pathway of action of capseisin here in producing cough responses is exactly the same pathway that I talked about before for organic solvents. You get a stimulation of the vanilloid receptor, and that in turn increases the NMDA activity, and that in turn produces a cough response. And that whole sequence is required then , so that if you lower the NMDA activity, you can lower the cough response. So I think that that is quite interesting.

There are some others, and I guess I have probably spent too much time on this already but there is at least one other type of study involving what is called nasal lavage, where you can actually measure changes in inflammatory markers in the nasal epithelia in response to low level chemical exposure in people with mcs. Again, there you are talking about a local response in the nasal epithelia. And that one I think has some particularly nice things but I don't really have time to talk about it.

OK. Let's go on and talk about therapy. All except one of the relevant clinical trials that have been performed on this group of illnesses have been done with either CFS or Fibromyalgia patients. There is only one that has been done with mcs. And so what I am going to do here is to talk about the data that has been accumulated with the CFS and Fibromyalgia patients because obviously it reflects what we think is the general mechanism that is involved here.

There are a number of studies in which antioxidants have been reported to be helpful in the CFS and Fibromyalgia group. Those include some flavanoids. They include equonia (?)kava extract, this is a brown elable (?) extract. They include (I am sorry I have got a spelling error) ascorbate,

vitamin C. And this is high dose ascorbate – and I will talk about that later . And so one has evidence that oxidative stress has a causal role in these illnesses. And let me just say there is also some fairly extensive data with an animal model for CFS, where there is also data that quite a number of antioxidants are helpful in the treatment of that animal model.

So we think that oxidative stress has a role. There is quite a bit of data on the use of NMDA antagonists and other agents that indirectly lower NMDA activity that seem to be helpful particularly with the Fibromyalgia group, where there has been a lot of study done on it, but also with the CFS group based on clinical observations. So we think that excessive NMDA activity has an important role .

There are agents that improve mitochondrial function that have been shown to be helpful, so we think that mitochondrial dysfunction has an important role here. And so immediately, what you are seeing here is different parts of the NO/ONO cycle that are implicated causally in these illnesses, simply based on clinical trial data. And that is obviously very important.

There is a nitric oxide scavenger which is hydroxycobalamin (?) . This is a form of vitamin B12 which has been shown to be useful in treatment of the CFS group. Here we are talking about quite high levels of this, which are required in order to get efficient nitric oxide scavenging . Levels that are able to provide a lot of B12 for simple vitamin B function are not adequate to produce this response, which is one of the types of evidence. And there are several types of evidence which suggest that this is acting as a nitric oxide scavenger and not acting simply to allay a B12 deficiency. That is obviously important.

Fish oil as well has been shown to be useful with I believe, both the Fibromyalgia and the CFS group and this has important anti inflammatory properties. So we think that there is at least some evidence that inflammation has a role. And I mentioned earlier about the inflammatory cytokines but a lot of the biochemistry of the NO/ONO cycle is inflammatory biochemistry. So the notion that anti inflammatories may be useful is perfectly consistent with the NO/ONO cycle mechanism.

There have been clinical trials done on high dose ascorbate and also folic acid, both of which help to restore tetrahydrobiopterin (?) levels, and I'll talk about that a little more later. But we think that tetrahydrobiopterin depletion probably has a role based on this information. In fact there is some other evidence that that is true, which I won't discuss here.

So basically we have evidence, simply based on clinical trial data on the CFS and Fibromyalgia group that each of the important parts of the NO/ONO cycle seem to have a role causally in producing these illnesses and therefore, by improving those you can get measurable clinical responses in these patients.

The only one of these that has ever been tested as far as I can figure out with mcs, is the high dose ascorbate, and I'll talk about that in a little bit, later on. But Hoyser (?) and one of his colleagues have published a clinical trial on that with the mcs group.

## Pall conference Part 5

In my book, Explaining ‘Unexplained Illnesses’, I discuss five different protocols that have been developed by different physicians and scientists to treat this group of illnesses, that are complex protocols. And these all involve from 14 to 18 different agents or classes of agents which I predict should act to downregulate different aspects of the NO/ONO cycle. These are complex protocols, and I would argue that the reason that the people came up with these protocols that involve so many agents that are predicted to downregulate the NO/ONO cycle, I am arguing that is probably not coincidental, and that that is more evidence that the cycle, in fact, is causal here.

One of these I had a role in here. I worked on with Doctor Graceen (?) and that is the only one actually that has been tried, of those five, on chemically sensitive patients. And the description of the responses of those patients is in chapter 15 in my book, so I am not going to try to discuss that here.

Subsequently I have developed another protocol. This is entirely over-the-counter based, based on nutritional supplements. It contains 22 different agents predicted to downregulate different aspects of the NO/ONO cycle biochemistry. I don’t know that the numbers here are so important, but perhaps the fact that we are trying to use various parts of the cycle, to downregulate different parts of the cycle, to try to get better clinical responses.

What I am going to do is to describe to you, and these are simply based on unpublished observations, most of them have been communicated to me by physicians, of their clinical observations. So these are clinical observations and anecdotal reports, so basically you should maintain, obviously, a high level of skepticism about this. But this is sort of my perception of what the response has been to this approach. And let me just say this, the protocol is available in the EU as well as in Canada and the US.

So, what we are seeing roughly is, and let me just say I am a PhD not an MD, OK? I just want to emphasize that, and that nothing I say should be viewed as medical advice. About 80-85% of the sufferers appear to respond positively to the protocol. And that percentage is roughly similar at least with CFS, with Fibromyalgia, with mcs. There doesn’t seem to be a big difference among them, going from one to the other. Which is a little bit surprising because, you know, the perception has been that mcs is the hardest of these to treat, and yet at least roughly speaking the mcs patients seem to be responding about equally well to this treatment.

Now, obviously, there is a percentage that don’t respond positively. I’ll talk a little bit about those as well. But generally one sees improvements that are maintained by those staying on the protocol. Relapses are rare, OK? So the first thing is that if you have a patient typically who responds well to the protocol, they tend to **keep** responding well to it. And that is perhaps surprising. Nevertheless, these people are still sensitive to stressors. So the chemically sensitive people do respond to chemicals, with a worsening of symptoms. Many of these people respond to infections and to other stressors. And I may talk a little bit about some of the other stressors that come into this. So they are not asymptomatic. And I will tell you some of the ways in which we see this.

The extent of the improvement varies a lot among the sufferers. Some of them respond extremely well in a short time period. Within weeks. And those include, interestingly, people who have been

ill for two decades or longer, in some cases. Who respond within weeks to this protocol and in some cases it is really surprising that that is true. The person that I have been in contact with who has been ill the longest claims to have been chemically sensitive for over 50 years, I mean basically his whole conscious life, and he has responded surprisingly well to this therapy. And maybe we can talk a little bit about that.

One thing that seems to be true is that people who have high levels of mercury in their bodies do not tolerate this protocol.

The protocol has seven different pills, basically, that people take. And four of the seven contain alfalipoic acid (?) which is an important antioxidant. But it has a property, that it can mobilize mercury in the body. And so we think that probably what is happening is that people with very high levels of mercury have to undergo detoxification before they can tolerate this protocol. That is a relatively small group, but obviously for them it is very important. And so that is one group that this does not work well on, at least as long as they have the high levels of mercury.

There are some others that basically do not respond either positively or negatively to it. And we are not sure why that is but I can think of some possibilities that might explain them.

Basically what I have done is to suggest that with these seven different supplement combinations ... that people take one for three days and see if they tolerate it well, and then take another one for three days and so forth. If a particular supplement is not tolerated it can be dropped out from the protocol and people can go on with the others.

I have even.....and I even ran into one person who said 'I can only tolerate one of the seven'. She was still getting a positive response to that one, perhaps surprisingly. But I don't know why. Generally, as you might expect, the mcs group has more trouble tolerating these than the others, but many of the mcs group tolerate the whole bunch. So that is perhaps surprising.

In terms of a general approach to therapy, what you need to do, I think, is you need not only to use agents that downregulate the cycle, but you also have to avoid upregulating the cycle. And there are a number of different stressors that will upregulate the cycle, chemical exposure, in the case of the mcs group, being the most obvious, because we have talked about how that can occur. But I think that the same thing is true for instance with exercise in the CFS group, it is true for exposure to allergens, including food allergens, which – I think you know – food allergy problems are very common with this group, and then there may be some others as well. So psychological stress may come into this.

I think that in general it is important both to avoid stressors that will upregulate the cycle and to use agents that we use that will downregulate the cycle.

You know, one of the things as a scientist that you have to do is you kind of have to be your own worst critic. And it seems to me, if we understand this mechanism well enough, we should be able to start curing people. We are not at that point. And so the question I ask myself is – how can we improve this to the point that we start seeing cures and not just clinical improvements?

Obviously there are several possible reasons why we might not be seeing cures. One of them is maybe we don't understand this well enough. The other one might be , well, the whole NO/ONO cycle is a bunch of hooey and it is not right. Obviously I am not going to agree with that one!

## **Pall conference Part 6**

And a third might be that there is some important aspect to this that we are simply not treating well enough. So I want to consider that third aspect with you. And that is that the real core of the cycle that we talked about before is this relationship between peroxynitrite and tetrahydrobiopterin depletion, OK? Peroxynitrite oxidizes tetrahydrobiopterin and that in turn gives you this uncoupling which produces more peroxynitrite.

So this is probably the real core of the cycle, and because it is the real core it is particularly important that we treat it well. That we lower this effectively. And if that is true, OK, so why are we not already treating it well. Well, we have a number of agents in there that are peroxynitrite scavengers and we also have some agents, and I talked about them briefly before, that will help restore tetrahydrobiopterin pools. But it is not at all clear under the conditions that we are using, that any of these work very well. So, one can at least make a case that this couplet, this central couplet, of the cycle is not being treated effectively. So the obvious question that one asks then is how can we treat it better. What agent or group of agents can we use to lower this central couplet. And my candidate for that is something that actually may surprise you, and that is high dose ascorbic acid, high dose vitamin C, given IV, intravenously.

So, why do I think that that is a good candidate? We are talking here about high dose ascorbate given intravenously and why do I think that is important. First of all, ascorbate is a peroxynitrite scavenger. That is clear. But it is not clear that it works very well at the normal levels that you have in the blood. In fact it is pretty clear that it doesn't work well at the normal levels that you get in the blood, even the levels that you typically get from oral supplements. So the argument is that when you use IV ascorbate, you can get levels that are 30 times or even 70 times higher than the normal levels that you normally get. And at those very high levels one would predict that the IV ascorbate might, must, would be, vastly more effective as a peroxynitrite scavenger. Ok, so that's number one.

Number two is that when peroxynitrite oxidizes tetrahydrobiopterin, so here you have got BH<sub>4</sub>, the first oxidation product is BH<sub>3</sub>. So, for those of you who remember your chemistry, that is the one electron (?) oxidation product. And BH<sub>3</sub> can then be reduced back to BH<sub>4</sub> by ascorbate, by vitamin C. So vitamin C is a reducing agent and it can then convert BH<sub>3</sub> back to BH<sub>4</sub>. The problem here is that BH<sub>3</sub> is itself unstable and so it tends to oxidize further, and so that may be a reason why you would want to have these very high levels of ascorbate to get this reduction, because this stuff doesn't stay around very long and so you have a short, sort of, time window to reduce it back. So it may be useful to have a lot of ascorbate to reduce the BH<sub>3</sub> back to BH<sub>4</sub>.

The third mechanism which I think may also be important here is that it is known that high dose ascorbate in the body generates hydrogen peroxide. And hydrogen peroxide will induce the first enzyme in the pathway for the synthesis of tetrahydrobiopterin. So this is what is known as the denovo (?) pathway for the synthesis of BH<sub>4</sub>. That is this enzyme GTP cyclohydrolase one (?). And so by inducing that what are you going to do? You are going to get more BH<sub>4</sub> being produced. So let me just say that hydrogen peroxide, even though it induces this enzyme, it does not oxidize BH<sub>4</sub>. Unlike peroxynitrite which does, hydrogen peroxide does not. So it does not destroy the BH<sub>4</sub> but it will induce increased amounts of synthesis. And so that is another activity that could be useful.

So I think that is as far as I am going to go here, and we have got about a half hour for questions and I hope that if we have questions from people, you know, that you want to give in Italian, we might be able to find someone to translate so that I might actually be able to answer them.

Sorry we have a .....

? .....We have many questions of the many substances/circumstances(?) I am sorry we have had .  
... inaudible.... Many things .

I want to introduce our group. We are ...Belgian medicines....for thirty years now we are very active here in Rome. We were .... By the association of those patients with mcs syndrome for the purpose just to measure the volume of ..of,,of the toxic lines(?) .. but since we are looking for the markers for the diagnostic and prognostic markers in plasma and culled(?) blood of the oxidative stress and in particular of this cycle you so brilliantly described to us , the ATP the nitrisolative(?) stress , the esuberan(?)site/cycle(?) production and so on and so forth, we just, it's a very good introduction. Because neither doctors, nor patients, they just understand nothing about our explanation. Why they should control first before being prescribed first of all this ni.... pro oxidants ..inaudible.

But my question is whether you have some information about another group which makes this kind of analysis of markers of oxidative stress generalized. Not only in the brain or in the skin but through the less traumatic ways to measure the oxidative stress by ...inaudible.. Not all markers but only through size/signs(?) markers like peroxyxynitrite, like nitric oxide production, like (inaudible) proteins, like nitrates (inaudible) and so on.

This is my first question. But you can find somebody to collaborate with .

PALL: Let me just say, I met Doctor Takenaju (?) who is a scientist in New Jersey in the United States, at a meeting last summer, and he is doing some very nice measurements, this is with the CFS group, on some of these markers. And he has measured, for instance, threenitrotyrosine (?) , which is thought to be a marker for peroxyxynitrite. And he has also measured a marker for nitric oxide.

Now let me just say something about this because I think that one of the problems that you have in looking at these is the question of specificity. And the problem basically is that we have so many different chronic inflammatory diseases in human populations that will elevate all of these things. And so generally, to be considered to be abnormal, the levels have to be extreme, OK, they have to be rare. And yet there are many people with chronic inflammatory diseases so obviously the levels that they have are considered normal, even though they are produced by having chronic inflammatory diseases. So, many of the levels that one sees with these groups of illnesses, where they have been studied, are in fact measured in the normal range. But that does not mean that they are not elevated by the mechanism, OK? And so that is a problem when you do these. So what Takenadu(?) , what he sees is that there is a much higher frequency of people in the abnormal range for these markers , but all of the people with CFS are not in the abnormal range. I don't think that is surprising at all but it is something that basically says that you cannot use these as specific tests because they are not specific and you can't use them as.... But you can use them to show that people have , you know, that there is a much higher frequency of abnormal levels.

I mean we measured a marker of nitric oxide which was elevated in the CFS group , but again it was a statistically significant difference, but a lot of the people with CFS are still in the normal range.

## **Pall Conference Part 7**

Q ...can understand is that this is also clear for us that this cycle you describe ... inaudible ...and we can do nothing about it. But not prescribing these high doses of ascorbic acid. It is really very dangerous. I think so. Because for example it is a very big threat to prescribe ascorbic acid to people with oxidative stress diseases such clear oxidative stress diseases like thalassimia. And it was a lot of harm to these people done. I am an ....

PALL: Yes but thalassemia , you have got a big problem with iron and that's of course yeah,

Q: So prescribe it if you have clear indication this person has for example very low level of transition metals because if it has for example iron, free iron in the blood, and you don't know it because you don't control it then with ascorbic acid it will be like an atomic bomb with explosion of oxidative stress and he will die. He will not support it any more. So either you can control it before very strictly, because ascorbic acid has double face, it is double faced molecule. It is a very strong pro oxidant and it is sometimes antioxidant . And if you think about danger of peroxyntirite, there are many different molecules which are very more specific for peroxyntirite. For example selenium, organic selenium, ..

PALL: But we are using those already. But the problem is yeah...

Q: But ascorbic acid is absolutely not specific for peroxyntirite. If you don't have any symptoms of peroxyntirite increase in these mcs patients, why should he or she be exposed to such a harm of or such a risk of ascorbic acid in reaction?

PALL: Well,let me respond to your point, OK? I think, first of all, there is no question that if you have very high levels of iron, as you do in the patients you were referring to earlier, there is no question that there are problems. What I think is true is that there have been four clinical trials done with the CFS group in Japan, all of them have reported favourable responses to high dose IV ascorbate . There have been no examples of the sorts that you were concerned about. There has been one clinical trial that has been done with the mcs group, and again, the responses were quite favourable.

I think that we need to cure these people. And so far, based on those clinical trials, those data look favourable. Now my suggestion is that the high dose ascorbate be used along with all the other agents , and only after they have been used for a period of time, and including some of the other antioxidants.

But, you know, we can discuss these issues, about what the best approach is, but this has been used already with a substantial level of safety.

Q: This is not established. This has not been published.

PALL: Yes, it has been published. There are four trials that have been done in Japan and there is one in the US and those have all been published.

Q: OK, we will extend our literature. And can you just , in your protocols there are many flavanoids prescribed . Have you published a review of this effect of flavanoids on the detoxifying enzymes which supposed to be very effectful (sic effective?) and very (inaudible) in these mcs patients? When you give a flavanoid in different situations then you are also at risk to compromise further the activity of the detoxifying enzymes like GFT(CGST?), like cytocomb??450. So please, just be very careful about it. Some flavanoids are good, some flavanoids like arseyse(?) , also it belongs to the same flavanoid group, but some of them they really inducers of this effect, but some of them are very strong inhibitors . So if we ..... them , we are looking for polymorphisms (?) of the defect ive/in GSTU/GSTT and so on and when we give the people the problems which inhibit the level it just makes really a disaster. So just my message is since these patients

are really at very great risk of different external signals like odours, like chemicals, flavanoids are so ....?  
And other presumed antioxidants like alphalicoic (?) acid, they are also chemicals, they are also

PALL: Well of course they are, yeah.

Q:....a little bit.....(?) so I ...on your side. I agree that it could be , the cause could be oxidative stress, probably local oxidative stress, .....(?) probably generalized, but to prescribe these things it is another question. It is completely, we don't know anything about the disease, and starting to prescribe them something wh

Q2 I would like to say something about mcs in inaudible.....I would consider that this pathology seems like many other pathologies,like protocolik (?) syndrome.....so the expression of (?)fundamental aggression (?) .....inaudible.....expression.....

But it seems like in mcs the initial damage is at the level of blood/brain barriers, because in these patients .....inaudible..... But it is like .....inaudible.....this patient is .....

.The difference is the target , the initial target . .....didn't .....

And so .....the patients, are protected (?)...... And it is probably the best of mcs patients is their inability, they are unable to produce more.....

Q1 But many of these persons , they don't penetrate the blood/brain barrier. And that is also my point. How could be improved the clinical situation when none of these problems, like polymers(?)....and so on , they don't penetrate.

PALL: Well, I don't think that is really true. You get clear effects on brain function for instance, with...

Q Culture(?) Yes yes that's true with culture (?)

PALL: No, no I am not talking about culture . You can get with ginko extract there is a fairly extensive literature on improved brain function with some of these diseases. So it obviously has to get in. Alfalipoic (?)acid gets through the blood/brain barrier, that's one of the arguments that's been used strongly to use it , and obviously ascorbic acid does.

You know I think first of all it is important, if I have had any effect on this at all, it is to show how these various aspects can interact with each other. And while the oxidative stress issue is probably the most important of the cycle, we also have problems with its cytotoxicity/exito(?)toxicity, with mitochondrial disfunction , with inflammatory biochemistry. I mean all of those things come into this. And I think also with tetrahydrobiopteren depletion. Those are all things that are very important. And I think it's a mistake just to look at one aspect of it. The only thing I would argue is that, and you know I am happy to discuss with you the issue of whether we are using the best flavanoids that we should for this, and I am delighted to get information that we are not doing that as best we can. But most of the people are responding well to the protocol and most of the people are responding well to the flavanoids .

Some of them are not. There are some people that I know of that don't tolerate that part of the protocol. So they drop it out. So obviously if we can improve that I would be delighted to get some information from you on that.

Q1 And the outcomes, you control only the clinical outcomes? You don't have any biochemical, any immunological symptoms of the program?

PALL: Any what improvement?

Q1: The clinical improvement. Or let's say the opinion of the patient or the opinion of the clinician.

PALL: Well, let me just communicate with you about a particular patient, OK? So you can kind of see where I am coming from on this. Because it is one thing to talk about these things in generalities, and another thing to look at a specific patient, ok? But I have been in contact with a number of people who have real scientific background and come to their interpretation with healthy scientific skepticism. So when I interact with them I have much more confidence in the accuracy of what they are seeing.

One person that started this protocol, I guess about ten months ago, was a woman whom I met actually at a meeting, who lives up in Anchorage, Alaska. And she has given me permission to talk about her case. She had been diagnosed as having mcs from chemical exposure. She used to be a science teacher in school, and from chemical exposure, apparently. And she has been severely ill with mcs for about twenty years. –She also has a lot of fatigue and had a lot of cognitive dysfunction, but she did not have post exertional malaise, which is considered to be the characteristic symptom for CFS. So she doesn't meet the criterion for CFS although she has a number of the symptoms of CFS.

She also has had post traumatic stress disorder. So she has been severely ill, ill for a long time, and she has symptoms, at least characteristic of more than one of these illnesses. And she was very skeptical about it because she tried so many things that didn't work, for her. She had been to DeLay's (?) clinic and she had tried quite a number of nutritional supplements and so forth, and basically nothing had seemed to help her. She goes on the protocol, and she emails me about 2.5 weeks later that she is amazed at the response she is getting. She's not even on the whole protocol yet and she's already getting an amazing response.

Her cognitive function now is normal. She has essentially no pain problems. Her fatigue is gone. She is still somewhat chemically sensitive. Not as sensitive as she used to be but she is still somewhat chemically sensitive, so when she goes out into an environment where she will be chemically exposed, she reacts. But instead of being ill for a week or two afterwards, she is ill for about a day and she is much less ill than she was before. Her friends and her husband have both noticed tremendous obvious improvement just looking at her.

My question to myself was why is she responding so well? I would have thought she would have been among the least likely people to respond because she'd been ill for twenty years, she'd been severely ill, she apparently has symptoms of more than one of these illnesses. On all of those criteria I would have thought that she would have been among the least likely people to respond, and yet she is responding so well. She, by the way, tolerated the whole protocol, so that is a plus for her in my judgement.

I think the answer why she responded so well is actually very simple. She weighs about 27 kilos, so she was tiny. And I asked her, well, what did she weigh before she became ill and she had weighed about 40 to 43 kg, something like. So she had lost about a third of her normal body weight as a function of the illness. And why is that important? Because she was getting a much higher dose per unit body weight than most of the other people.

So what I inferred from that was the dosage that I suggested to the other people was inadequate. And in fact there are quite a number of other people who now have tried higher doses and they have communicated to me that they get better responses. So my suggested dosage now has increased as a consequence of that. And it is at least to some extent based on body weight.

I can tell you some other stories about people who responded well ..

Q1 Testimonial based medicine..... Believe only this clinical types of experts.....

PALL: So you are saying we need to do clinical trials. I agree with that.

Q1 One patient responded it's OK but.....

PALL: Well, what I am telling you is about 80 to 85% of the people responded OK? So that is not just one patient. I am not saying that that figure 80/85% is written in stone it may be that when we get more information that will change, but most people seem to respond well to it.

You had a question.

Q3 inaudible

PALL: We are trying to get a clinical trial going in France right now. I am working with a mcs support group there. They are very much interested in doing that. Whether we will actually be able to do it or not is another issue. I don't know yet whether we will be able to do it.

Q3 ....inaudible....taking place in the united states.

PALL: No there are no clinical trials currently occurring with this protocol. Let me just say with regard to the other protocols that I talked about. The five that I discussed in my book. Two of them have been tested in clinical trials. One is Jacob Teitelbaum's protocol. That is a very complex one because he uses a different protocol for each patient depending on what the test results are and so it's a complex approach. But they have done a placebo controlled trial, actually two of them. One with the CFS group and one with the Fibromyalgia group, and they have gotten positive results with both.

The other one is the protocol that is developed by Garth Nicholson (?) which is mainly designed to improve mitochondrial function. And he also has reported statistically significant improvements with that protocol. That's in the US. Both Teitelbaum's and Nicholson's were in the US.

Q4 I have 2 questions. Maybe I'm wrong but when you write ascorbate maybe you don't mean simply ascorbic acid. Maybe you mean magnesium ascorbate (?) or potass(?) ascorbate .

PALL: Well , I don't know. I mean as a chemist, ascorbate is just the anion(?) so yes, you are asking what the cation (?) would be. I'd have to look up the individual trials that have been done to see what they use for that. I don't know. But yes, generally you don't want to use a highly acid ph for these infusions.

Q5 Is it possible to.....inaudible.....one to one ..... ascorbic acid.....ph 1.5.....some kind of.....forever .....calcium or magnesium or something . It should be neutralized otherwise it's impossible you would get .....burn.....patient

Q4 That .....question. Because .....if you prepare (prefer)?.....ascorbic acid with the magnesium or potassium you cannot.....a real molecule of ascorbate for just for some hour (how)? The question is that. How can you make .....this problem. You have .....in fact using ascorbate after two hours ,.....action of ascorbate and simply using a product .....

Q5 inaudible

Q4 Yes using ascorbate after two hours in the operation. This was a .....action of ascorbate and see if it....using a product

Q5 It's very unstable of course

Q5 Yes it is very difficult

Inaudible discussion.

Q4 And the second question is about poly.....

PALL Yes cytokines. P450s is it?

Q4 Have you seen some difference between the control population and mcs population. Do you have some evidence of the difference?

PALL: No. I mean there is some of the genetic evidence. One of the genes that are predisposed towards increased susceptibility, was the P450 gene. But no. In fact it is difficult to look at that. I mean the nitric oxide effect on cyclone (?) P450 is in inhibition, it's not an inactivation. So if you, of course you can't take brain tissue anyway, and measure it but if you could take tissue out, say, in peripheral tissue, and measure cytokine (?) P450 the prediction is it would be normal. Because at that point the nitric oxide would dissociate and you wouldn't see any inhibition.

In fact, I believe there was a study done of that sort where they showed it was normal but I don't know that that means anything. So it's a difficult thing to test because you are looking at nitric oxide as an inhibitor of cytokine P450 and of course when you isolate tissue that inhibition will be gone because nitric oxide is unstable.

Q6 .....the patient will come to me.....in relation to .....in the expression of some genes.

For example the genes of the detoxifying enzyme (?) like PT ??? or PT??? or C??? or different....

The expression to be affected by.....polymor..... Therefore when the doctor ask me about polymorphisms(?) I am a little bit, it's a big job you know

It's a big effort but then the result .....the patient does not .....in Sweden and then they cannot see anything. Why is something wrong .....and no clear evidence

The inhibition of expression at the gene level or at the .....translation of

**But then I can see the point of using different substances to ameliorate the patient. But this is genetically.....**

Any genetically, every genetically

We just looking for any genetic mechanism .....mcs people

Inaudible

Background noise

Q1 .....and so its not possible for.....and then there is.....it is clear that.....another one it is.....

Discussion

PALL: Well but those , the heterogeneity in terms of sensitivity may simply be due to the polymorphisms that we have already talked about

Q1 Find out the molecular basis for this, try just to investigate whether it is at genic level or at epigenic level

PALL: Well that is

Q1 .....can substitute something

PALL: Well this is not entirely genetic or epigenetic. The point is that these are initiated by chemical exposure, and the point is that these people respond to treatments that will not change the genetics and probably won't change the epigenetics either, although I am not convinced that there is any real epigenetic role here at all. I could be wrong about that. I think that the point is that we do have responses to these clinical treatments and so there is no point in tearing our hair about, you know... The genetics is simply understood in terms of susceptibility. Let me just say there is another gene that has been implicated, which influences the NMDA receptor activity indirectly.

Q1 Not easy

PALL: No, it's not

Q1 Nobody has an evidence of this.

PALL: What do you mean there is no evidence for it? It has been published. You are saying, what are you saying, that the published evidence is wrong? Or what, what are you saying?

Q1 inaudible

PALL: I can't hear you, I am sorry.

Q1 If it is genetic, a trait then we have to change our .....for our directions of the treatment

PALL: Yes but these things are never, these are not either genetic or non genetic. The genetic role is to increase susceptibility and that doesn't mean that the disease is inherently different when it occurs. It may mean that it is more difficult to treat if there is a susceptibility problem.

I have to say in the rare case, in fact I only have one case where there is pretty good evidence for a major genetic susceptibility role with some of the patients who have gone on our protocol, and they seem to respond quite well to it. Despite the fact that they are probably carrying a susceptibility gene. So I am not sure that the genetics necessarily implies that the approach to treatment is necessarily going to be different.

Q7 I think it is very difficult to find a

## **Pall Conference Part 8**

Q7 I think it is very difficult to find a very common trait because they are all multifactorial pathologies. When we have ten patients we are seeing ten (?) mcs. One patient can have an overload from toxic ... virus, psychological stress and so on. The second can have the same but it is a different chemical substance, a completely different virus, and so on. ...Its impossible to ..

Q1 Heterogenic group

Q7 Its impossible to go straight to one only factor, as nitric sodium (?). This is very important to have this basic biochemical grounds, because this is atleast, and finally a common basis we have it until now. And on this common basis then when we have to go say to each patient with each different work (?), each patient is different we

Q1 ....the characteristics of this patient. Not only clinical

Q7 This is the characteristic, biochemical...

Q1 Yes, yes, yes I know

Discussion

Q1 this is I am speaking about, that you have to check every patient

Q7 Yes, but

Q1 For this characteristic. Because the person just presented us, his hypothesis is from biology not, from the general biology, not from the patient. That my question was, were the patients controlled for these parameters.

Q7 Very difficult because one patient can heal only with psychotherapy, I ..... One patient only with another... significant therapy....because each patient, on the basis of this very important, then ...goes to one personal

Q1 Q7 discussion

PALL: I am afraid I have to leave because we have to go to the airport and I'm not sure how we get a taxi here.

Taxi discussion

So I'm going to be about two weeks travelling before I get home but I'd be happy to interact with you over email and we can discuss things at that point

Discussion leaflet distribution title of book Explaining 'Unexplained Illnesses' Francesco Pesce, neurourologist introduces himself, "as I treat a lot of patients with .....cystitis, pelvic pain I also see patients with fibromyalgia, Chronic fatigue, and I am following up some mcs patients because they seem to have also lower urinary tract symptoms for some reasons, is your experience also?"

PALL: Well I am a Phd so I don't treat patients but I know of some that have urinary tract problems and you now I think that what you see with this whole group is that they have many chronic inflammatory problems.

Pesce: IBS, for example.

PALL: And some, perhaps many, of those may also be NO/ONO cycle diseases of their own and located in different things, and if that is true then they should all respond to the therapy. But that's what we don't know yet, whether they will or not.

Pesce: And I was interested because we mentioned the alfalipoid test (?) because I am using sometimes for neuropathic inflammation , impression (?) of the pudendum(?) ....etc . And I also read something about the possibility of mobilizing mercury.

PALL: Mercury? Yes.

Pesce: This is established is it?

PALL: Well, I don't know that literature, but my understanding is that it is. That's my understanding. And so basically it's the reduced form that mobilizes the mercury. So the alfalipoic acid that you use, of course when it gets transported into the cell, it rapidly gets reduced to dihydrolipoic(?) and it is that...

Pesce: So I tried to avoid in patient with some dental

PALL: Oh with mercury fillings?

Pesce: Yes

PALL: I'm not sure that it's a problem with most of them. My impression is that it is only a problem with people who have very high levels of mercury and they...

Pesce: In the blood or in the tissues?

PALL: In the tissues. And often I think, and again I am not an expert on this,

Took away the microphone

END OF RECORDING

**P.S. (Trascrizione della registrazione audio della conferenza (Roma nov.2008) del Prof. Pall effettuata dalla mamma (inglese) di una paziente CFS)**