PANEL DISCUSSION: THE ROLE OF ELECTRICAL POTENTIAL AT THE CELLULAR LEVEL IN GROWTH AND DEVELOPMENT

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DR. M. BENDER (*Fairleigh Dickinson University, Teaneck, N.J.*): I had actually wanted to ask Dr. Brick if he was relating adhesion to the surface potential. I am addressing this question to the panel and to Dr. Brick. Aren't the forces of adhesion so much stronger compared to the repulsion due to surface potential that we could explain the adhesion that occurred in that way? I am also thinking of Dr. Cone's work.

DR. WEISS: I will comment on this question. First, I am not sure that Dr. Brick was talking about adhesion. I think he was discussing cell separation, which is a process different from cell adhesion. Basically, you cannot learn about the forces of adhesion between the cells by pulling them apart, because you are probably separating them at a plane different from that at which they stick.

For example, if you want to know the forces of adhesion between a band-aid and your skin, you can't determine these forces by pulling it off, because the band-aid either tears off some skin or, alternatively, some of the band-aid remains on your skin. Do you understand my point about the different plane?

DR. BENDER: Yes, but didn't Dr. Brick show very rough surfaces approaching each other anyway?

DR. WEISS: That is a different matter. You raised a question about the forces of repulsion and attraction. Dr. Parsegian can talk about forces of attraction, but essentially they depend on the positions of the surfaces in relation to each other, because the forces of attraction and repulsion have different distance dependences. Therefore, before two charged surfaces can stick together, they must first, before their adhesive or short-range forces can come into play, run into a repulsion barrier. I am ignoring the "secondary attracted minimum," but the distance dependence has to be considered here.

DR. PARSEGIAN: I think you want to know at what point the force of repulsion is going to dominate the force of attraction. At a distance where the cells seem to be touching?

DR. BENDER: I am really looking for more enlightenment on that subject. Is there any information on the kind of role and the degree of energy involved in the repulsion as a result of the surface potential versus the attraction? I appreciate that it is a function of distance, but . . .

DR. PARSEGIAN: Let's ignore the distance and idealize the problem, such that the energy of bringing two surfaces to virtual contact runs into tenths of ergs per centimeter squared when you consider essentially the idea of jamming all that charge into a limited space.

To compare that with attractive forces is difficult, because we don't know what the attractive forces are at those distances. We do know that cells stick together in this manner very often, so these forces must be greater than the repulsion. I agree with Dr. Weiss in that we have to be very careful in defining the energies needed to rip the surfaces apart.

DR. BENDER: But, these are large particles, and therefore the radius of cur-

vature is large—more on the planar side. Also, the interface side is rather flexible and not very hard or rigid.

DR. WEISS: I think unless one is familiar with the units, one cannot very well comprehend what is involved. We did some computations a long time ago of the common question of how much electrostatic repulsion would tend to prevent a cell settling on a glass surface, the same culture. We reduced this term to relative centrifugal force and asked ourselves if a cell is placed in a glass centrifuge tube that contains medium, how much centrifugal force would be necessary to spin it to the bottom to produce physical contact with the glass?

The answer works out to be about 20,000*g* in some cases; for platelets to attach to the glass, the force is approximately 100,000*g*, and for them to attach to each other, about 200,000*g*. However, this occurs at a force of only 1 *g*, so something is wrong here.

DR. BENDER: I'm glad I'm not the only one who has observed this result. I've performed experiments with zinc sulfide with a fairly large zeta potential, and merely under the influence of gravity, 1*g*, the cells adhered to each other. In other words, after sufficient time elapsed to permit the cells to settle, they really stuck quite closely to each other, as seen through the glass.

DR. WEISS: This phenomenon is what is termed a triumph of biology over intellect.

DR. PARSEGIAN: I just thought I could put some numbers on the board about what Dr. Weiss and I are saying. If you look at the energy versus the distance between the two bodies, at very long distances the force is generally attractive but rather weak, and the potential minimum here is of the order of 10^{-4} ergs/cm^2. Bringing two planar surfaces together requires 10^{-4} dyn/cm^2, which happens to be roughly the same magnitude.

The barrier that Dr. Weiss was referring to relates to the fact that you run into an exponential repulsion between charged surfaces; our estimate was approximately 10^5 dyn/cm^2 for bringing two parallel surfaces together, not peeling them off the way the "band-aid" works.

There is a large force to overcome, at which point our theories become too complex, so we say something like this is predicted, some kind of dotted line, with a rather strong attractive force, which is the difference of an attractive charge fluctuation force in this particular case and a repulsive energy way up somewhere, say 10^{-4}, but a very strong repulsive force must be overcome to make this curve come down again. You should really think in terms of very great energies when you are talking about contact.

DR. BENDER: What are the distances?

DR. PARSEGIAN: The distance here is somewhere between 40 and 80 Å, and we are talking about a repulsion with a characteristic exponential decay of about 10 Å, that is, exponential to the minus distance over 10 Å. That is typical of physiologic saline.

DR. CONE: With regard to the "fuzz" concept, what effect would it have on this classical analysis?

DR. PARSEGIAN: It moves this contact, which is related to the fact that you have a really thick fuzz layer that is not very sensitive to having more of it around. It just sort of saturates . . .

DR. CONE: You are moving far away from the idealized double-layer concept.

DR. PARSEGIAN: Oh, yes. And, of course, it also contributes to the attractive forces. But, mainly it changes the repulsion.

DR. CONE: The intermeshing of very long polymers might give a very complicated system.

DR. PARSEGIAN: That is probably what is really going on over here with the dotted line, which is what is really interesting. We have done a great deal of physical computations with these long distances and they provide insight only up to a certain point in talking about cell contact.

DR. A. R. LIBOFF: This may be an impossible task, but could the panel possibly direct its attention to the very specific question of whether some of the information that was presented this afternoon has any bearing on the kind of experiments in which electrodes are implanted in tissue or in which coils are put across tissue and changes occur in the material. Specifically, does the information that is accumulating with respect to the specificity of charged surfaces on cells affecting their properties have a bearing on the practical problems of the clinicians?

DR. JAFFE: I am uncertain in listening to the bone people to what extent it is established that these healing phenomena are really due to currents and to what extent they are due to electrode products. That they are due to electrode products is in a sense trivial—it is another matter entirely. That they are or that it may be demonstrated that currents through the fracture zone do really help healing seems to indicate that the most plausible mechanism involves a kind of electrotaxis. That is, the individual bone cells may be moving down a potential gradient. I think it is of interest that the current densities that we have measured as driven by cells through themselves are comparable to the current densities that have been applied by people working in the healing of bone, so I don't think it is implausible that such mechanisms really are involved. I urge that people who work with bones should really know that they are dealing with current-mediated phenomena and not consequences of electrode products.

DR. BECKER: I don't think that much doubt exists at present from some of the experiments, particularly those of Dr. Bassett, that this is, under certain circumstances, certainly purely a field or an electrical effect. I think Dr. Johnson will agree that it is an error to believe that we, and I am an orthopedic surgeon, stimulate bone cells to make more bone. If you look carefully at the work of all of us in this field, you will see that we are stimulating the bone marrow cells to convert into osteogenic cells, which is somewhat different than stimulating an already established cell line to merely reproduce. Dr. Johnson, would you care to comment on that?

DR. L. JOHNSON *(Armed Forces Institute of Pathology, Washington, D.C.)*: I certainly agree with you in part. However, when you have a healing fracture, a large change occurs in the circulation; that is, a substantial number of cells from other parts of the body drawn in by the circulation play an important role as do cells from the marrow, so this situation becomes much more complex to talk about.

DR. WEISS: In regard to Dr. Liboff's question about cell surface charge and other things, several bits of evidence now exist. We know, for example, that transmembrane movement of ions across a cell membrane is in some way linked to a surface potential. We also know, for instance, that if we remove sialic acid from the surfaces of a number of the cells, we effect both the net flux of ions across the membrane and the unidirectional fluxes of ions across the membrane. Bettinson and I showed this years ago. We know that the charge on a cell surface is in some complex way related to what the cell happens to be doing at that time. We often forget that the membrane is a dynamic part of the living cell, is wrapped around it, and mirrors what is happening in the cell in a way that is not well understood.

We know, for instance, that some cells, as Mayhew showed, have coupled to

mitosis an increase in surface charge density and that some cells don't exhibit this increase. It is known, for example, that the density of charge units associated with ribonucleic acid of the cell surface rises with increased metabolism. I think it is generally accepted in cell physiology that the cell membrane, perhaps by regulating movements of ions, does control cell metabolism. In a general way, then, I think there is good evidence in isolated systems that bioelectric phenomena do, or can at least, contribute to controlling cell growth and proliferation.

DR. C. P. RUBENSTEIN *(Prosthetics Research Study, Seattle, Wash.)*: Dr. Becker, what is the time element of the regenerative tissue responses in the current of injury before the hyperpolarization occurs that you have noted in your talk? Have you or anyone else done any work to determine how critical the time after injury and prior to hyperpolarization is to effect the regeneration of tissue growth? Specifically, I am asking how this might be applied to nonregenerative tissues.

DR. BECKER: I don't know if I understand the question exactly. Are you asking for a time relationship between the potentials that are observed and the biologic effects that are observed?

DR. RUBENSTEIN: No, you had indicated in the regenerative current of injury a kind of a depolarization and then a hyperpolarization.

DR. BECKER: Well, I don't know if you can use those terms, but I know what you mean.

DR. RUBENSTEIN: For the nonregenerative, there was no return . . .

DR. BECKER: No hyperpolarization, in your terms.

DR. RUBENSTEIN: No hyperpolarization. Did any critical time element prior to the hyperpolarization evidence itself such that you could indeed stimulate, for example, an hour, day, or month after a nonregenerative tissue exhibited a current of injury to effect a regenerative process in this normally nonregenerative tissue?

DR. BECKER: Well, I don't know. If you produce an injury in a nonregenerating animal, let's say you do amputation in the frog, you can produce a regenerate if you stimulate it in an appropriate electrical fashion. Dr. Smith will discuss this tomorrow.

I guess you want to know if there is a critical time? I don't think anyone has looked at that.

DR. J. F. GENNARO, JR.: This afternoon we have been concerned with individual properties of cellular systems. We have talked about cell surfaces, electrostatic potential, and about transcellular currents. As long as we confine ourselves to biologic systems, we can't really seek much refuge in computations of homogeneous or statistical kinds, because we are dealing in every case with inhomogeneity. Without inhomogeneity, there is no differentiation, movement, or selectivity, and I think we should relate inhomogeneity in biologic systems to cell surface charge or charge potential. Also, Dr. Jaffe spoke of the interesting potentials that occur periodically in the system and casually mentioned that this might be associated with vesicles that reach the cell surface. Do you know that they do? Have you seen these?

DR. JAFFE: We do know that in other growing tip systems, such as the growing pollen tube, there is an extraordinary accumulation of wall precursor vehicles. I suppose that in this slower growing system, the fucus egg, there is a comparable but less obvious accumulation.

DR. C. MINKIN: To respond to the first question, I think several talks today touched on the role of heterogeneity.

DR. PARSEGIAN: Yes, I don't think anyone underestimates the importance of heterogeneity. I think that the problem is to make the reality and the ability match. We don't have that matching. We are gradually introducing detail as we are able to

handle it and in the meantime learning order of magnitude properties of the systems from each new detail that is added. I strongly urge the addition of specific detail as soon as we can determine it.

DR. GENNARO: However, we should be discussing our neglect of a more selective approach to these problems. Dr. Weiss talked about the ions on the surface of fixed cells, I believe.

We found that concanavalin A binding sites are increased in fixed cells over those in nonfixed cells, whether you use red blood cells as an indicator or ferritin-labeled Con A. I think we have not actually determined the area that we want to attack, but we should be talking about how we can accomplish this.

DR. POHL: I shall turn that question around the other way and emphasize that electric fields can act in a very different manner if they are inhomogeneous.

A uniform field works on charged particles, whereas nonuniform fields can work on both charged and neutral particles. We needn't exclude electric field effects from acting on neutral particles as long as we have inhomogeneity. I will address this point in more detail in my paper. But, your question can be turned around and answered in the affirmative that it is very important that the inhomogeneity of the electric effect be looked at.

DR. JAFFE: From my efforts to read the theoretic literature on attractive and repulsive forces between cell surfaces, your comment seems quite pertinent.

I think that to view these two surfaces as simple sheets of charges is perhaps a very poor approximation and to consider them a mosaic that will fit itself somewhat in the manner that Steinberg has suggested for cell adhesion may really be very much closer to the truth. In brief, I think you are essentially correct, in that we should think not in terms of sheets of charge but in terms of the discrete charges that can interact with each other and move back and forth so as to optimize their interaction.

DR. BECKER: I suppose you have to start somewhere and as good an area as any are the simple, or evidently not so simple, phenomena of adhesion and contact.

I believe cells have much greater potential than most people do who work in my field, and I feel that the capacity of cells to alter their type, function, and morphology and to change their genetic operons around is tremendous. The information to these cells—that it is desirable for cell A to become cell B—must be delivered to the membrane in a form that is sufficiently sophisticated to carry the number of bits of information that are meaningful for this type of a signal.

DR. WEISS: The computations that have been made on flat membranes, for example, were never, in my opinion, intended to be definitive or taken too seriously. One merely sets up a model and then tests it; this is the procedure in every discipline that I am aware of, and I don't know of anyone who would have actually suggested that the cell surface consists of a homogeneously charged flat surface. This is so totally contradictory to what we see.

To study the question of heterogeneity, there are so many different approaches; my paper, in fact, was on the question of charged heterogeneity. I imagine that we will take such information, which is very recent, and try to make additional calculations to see whether the electron microscopic evidence can provide some computations with predicted value. If they are in the same ballpark, we will be getting closer to the truth. If they turn out to be tens of orders of magnitude out, we will have to try another method.

DR. CONE: I think the whole problem is one of continuous iteration between observation and hypothesis and observation and hypothesis, which arrives at a system that is ultimately reached in a certain stage in your hypothetical reasoning that

would be a substantial test of the hypothesis. I think the biologic systems in general are, as Dr. Becker said, as simple as adhesion mechanics, even in the simple physical realm, and still very, very poorly understood. With complex biopolymers, electric fields, and ionic double layers, for instance, the complexity is compounded enormously. So I don't think you can criticize anyone for not taking these factors into account; you just have to specialize your system to what can be meaningfully discerned.

DR. MINKIN: It is interesting to note that our long discussion has ended on an accusation and a reaffirmation of the scientific method.