Environmental Health Perspectives
Vol. 35, pp. 199-202, 1980

Summary and Future Research Needs

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Dr. Bromberg: This is a remarkable meeting in that, with very few exceptions, almost no mention was made of pulmonary mechanics and of gas exchange. That doesn't happen very often in meetings devoted primarily to lung research, but perhaps has been happening more frequently in the recent past and it may indicate a trend for the future. Another interesting note was that almost everyone is working or collaborating with an ultrastructuralist, or would like to have access to ultrastructural techniques. Dr. Reid, among others, gave us a very nice demonstration of how ultrastructural techniques can be combined with biochemical techniques in experimental studies of the reaction of the lung to insults. Dr. Satir presented most elegant ultrastructural biochemical data on the mechanism of ciliary motion.

The organization of the meeting, as I perceive it, looks at the lung as having three segments. There was a large area of attention to the epithelium, and at the end, there was a large area of attention to the endothelium. In the middle, I suppose appropriately, there were a number of speakers who talked about cells that more or less fit between the epithelium and the endothelium. Notably missing, or in large measure missing, were discussions of smooth muscle and of neural function at more than a passing level. The keynote of the epithelial sections was set by Dr. Boucher and Dr. Gatzy, who pointed to the barrier function of the epithelium and the attempts to quantitate the nature and the site of this barrier. They paid special attention to the intercellular junctions and the so-called tight junctions of the epithelium and defined their properties by electrical measurements and by using a variety of non-charged probe molecules.

Of particular importance are the macromolecules which may gain access to important cells residing deep in the epithelium. These cells were discussed by Dr. Bienenstock, Dr. Brain, and Dr. Wasserman. These powerful cells — Dr. Spitznagel's cell, the poly, the mast cell of Dr. Wasserman, and the "big Mac" of Dr. Brain — have an enormous armamentarium. It's important to consider how materials deposited in the airway may or may not be able to gain access to these cells, and how environmental pollutants and toxic agents might alter the barrier function of the epithelium and permit more ready access of other inhaled materials to these critical cells, which when turned on have the potential not only for protective action, but apparently for very damaging actions. We were shown by Dr. Wasserman in particular, and other speakers as well, the remarkable balance between active effects and mechanisms which repair or inhibit these effects.

This underlies a big problem that the EPA faces in trying to develop its research program. It may be relatively easy to show effects of one sort or another, but at what point do these effects go from the normal range or the range that can be dealt with by intrinsic mechanisms to the point where you have disease. To do this, one must bear in mind the intrinsic variability of the human subject. The fact that some of us have less hair than others and some of us have brown eyes while others have blue eyes is obvious. But there are many other differences of which we're perhaps less aware that make some of us more susceptible to certain insults than others, because our compensatory mechanisms are not as effective. A stress that can be easily tolerated by one individual may become a stress that produces overt disease in another. Even though the EPA is a regulatory agency and will continue to look on its role in research as developing data that directly abut on its regulatory function, the Agency must bear in mind that it is important to understand mechanisms to the extent that one can predict idiosyncrasies and unusual reactions that

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some people in the population will have to a given stress. To do this "blindly" using empirical protocols is impossible. There are too many agents to be tested and too many possible protocols. To do well-directed, intelligent, pertinent experiments, one has to have some reasonable conceptions of mechanism.

Another problem that the EPA in particular has to face is that the payoff is human effects. Yet, the experiments that can be performed in man are limited. At this symposium scientists have described to us systems that are very complex and far removed from the intact human being. But there are other techniques that are either directly applicable or potentially applicable to intact human subjects. We should make efforts to develop techniques that can be used in human subjects, and to apply them. For example, in studying tracheal tissue one can look at rats and hamsters, and so forth, but it is also possible with a fiberoptic bronchoscope to obtain samples of tracheal epithelium. Such epithelium can be cultured successfully. Dr. Collier has done so by using specimens that we have provided for him during ordinary bronchoscopies in clinical practice. He has been able to show by using such materials that these specimens can be maintained for many days, that they retain ciliary activity, that they can be infected by a variety of specific infectious agents, that characteristic morphologic pictures can be produced, and that these pictures are different from what is seen when one uses the same infectious agents in animal models. It's going to be important to try as much as possible to use human tissue, even for *in vitro* experiments. Dr. Boucher has shown us techniques that can be applied to intact animals, and a few steps have been made to apply them to intact humans, in whom one can measure one of the bioelectric parameters of the airways epithelium, and one can even induce local changes by the local application of very minute quantities of drugs. Other examples could be given. We also need to bear in mind the potential application of our techniques to human studies and the use of human tissue as much as possible in our experiments.

This is a very exciting era in lung biology; people with all kinds of background and training have been stimulated in a variety of ways to look at the lung as more than an organ that simply deals with shuttling air in and out, and transferring oxygen to the blood, and CO_2 out of the blood. I foresee that this kind of research is going to be of increasing importance to all aspects of government activity, to the NIH, NIEHS, and also to the EPA. It will take some ingenuity to do the right kind of experiments certainly, but it is going to take an attitude on the part of the EPA, in particular, not to disregard mechanism in favor of what may seem to be the short-term payoff of defining levels that are or are not "toxic." To do the latter job well will require some very clever application of the knowledge of mechanisms, and that is the plea that I would like to leave with you.

Dr. Menzel: We've seen here the beginnings of a new era in pulmonary research. It is directed to the definition of the normal physiologic state of the lung, emphasizing both respiratory and nonrespiratory metabolism. In defining the pathophysiologic state, mechanisms of action must be known so that alterations in particular values may be recognized as abnormal. Since biochemical measurements often are more sensitive than morphologic changes, connections between altered morphology and altered physiology must be sought. Unfortunately, the biology of the lung is but poorly understood. Much effort, then, is being expended on basic or baseline data to increase our surety that the measured effect on exposure to an environmental pollutant is indeed abnormal and hence toxic. To these ends our tools are still blunt and need to be honed to as fine an edge as possible.

Controversy surrounds the use of animal data in assessing human toxicity. Much of this discord results from the pioneer state of lung research. To be sure, there are differences between man and animals, especially in the morphology of the lung. But there are far fewer differences in the basic physiologic processes as seen here for ion transport, metabolism of xenobiotic compounds, and the uptake and metabolism of prostaglandins, angiotensin, and biogenic amines. On this scale the difference between animals and man is one of dose.

Morphology is highly important, as evidenced by the heavy collaboration between physiologists, pharmacologists, and morphologists. One approach has been to eliminate morphology by studying pure systems. Here the biochemistries of the hepatic and pulmonary systems of cytochrome P_{450}, for example, are remarkably similar. Tissue specificity is expressed in the molecular forms of cytochrome P_{450} present in the lung and the differences in inducibility by environmental agents. The uptake mechanisms unique to the lung are likely, however, to encourage recycling and higher concentrations of reactive intermediaries than in the liver. This may make the lung more susceptible to both environmental carcinogens and toxicants acting through activated intermediaries which bind covalently to cell macromolecules. The biochemistry and pharmacology of the lung has been aided by the isolation and culture of both organs and specific cells. Much more work is needed here to identify susceptible lung cells and to develop specific markers, enzymatic or glycoprotein, indicative of pulmonary damage. Release of marker enzymes by the liver and heart has been

established as a valuable tool for estimating hepatic damage by toxicants and myocardial damage by ischemia in the clinical assessment of man. Similar techniques are likely for pulmonary damage.

The development of *ex vivo* and *in vivo* perfused lung preparations has been accomplished in the last five years. It is now possible to study the transient events of prostaglandin metabolism which had been obscured by competing factors and compensatory mechanisms in intact preparations. Of particular note are the studies relating SO_2 to bronchitis and the consequences of aberrant prostaglandin metabolism in asthmatics. Perhaps here we will understand some of the more subtle effects seen in man on lifetime exposure to polluted air.

We cannot lose sight of the fact that regulation of pollution is the ultimate aim to which these studies will be applied. Therefore, it is important to define the point at which an effect ceases to become a normal response and becomes pathophysiologic. It is also necessary to attempt to extrapolate these measurements made in animals to the human condition in a manner which is defensible and at the same time sensitive. Certainly the question of the variability of man in regard to his response is particularly important. This integration is represented by the organization of this meeting. It represents the philosophy of those within the Health Effects Research Laboratory with whom I have had the pleasure to work, starting with Dr. Gordon Hueter, Director of HERL, and Dr. William Durham and represented at this symposium by Dr. D. E. Gardner and Dr. Ed Hu and their colleagues. Certainly, they recognize the need for the development of methods that are sensitive, the application of new methods, and the integration of these to toxic exposures which are relevant to the exposure of man in the environment and ultimately to the prevention of disease. This particular mission is deserving of the finest research that we can bring to the issue. I am very pleased to have been able to associate with you in demonstrating that it is possible to bring together high quality, thesis-directed research in the application to inhalation toxicology.