"Non-Concept" of "No-Threshold": Chemicals in the Environment

Stochastic determinants impose a lower limit on the dose-response relationship between cells and chemicals.

Bertram D. Dinman

With the increasingly widespread concern about the environment, one also encounters with greater frequency the concept that there is no threshold below which a chemical agent does not induce a biological interaction. This concept is true in that, when one atom or molecule enters the reactive region of another, a chemical interaction will result. Unfortunately, this simple reality has been extended to imply that such an encounter has, of necessity, a deleterious impact upon the cell. I do not believe that such an extension of the no-threshold concept has a rational biological basis.

In part, this concept has been fostered by extrapolations (*1*) from the work of Beard and his co-workers (*2*). In brief, they found a linear relation between a dose of carbon monoxide and a response (that is, a decrement in temporal perception). Still other people have extrapolated this regression line downward so that the dose-effect curve relation goes through origin. Although there are no data points on which to base this extrapolation, the absence of such points below the observed dose-effect line is considered as representing the relative insensitivity of our measurement techniques. There is little consideration of the alternative proposition that, even with the ultimate in sensitive measurement capabilities, these biological effect points might not exist. Thus one arrives at the implication that the entrance of even one molecule into the "milieu interieur" will produce a biological effect that would necessarily be deleterious within a cell.

In any discussion the definition of the word "effect" should be clarified. For my discussion "effect" is a neutral word, implying neither benefit or harm. If the word "effect" is used in this strict sense, one has no difficulty in agreeing with the probability that the interaction of one pollutant molecule with another molecule of a cell component should result in an "effect." Confusion has arisen because of the frequent implication that an "effect" is per se deleterious. In fact it is my purpose to examine the validity of the concept that "effect" is necessarily associated with a biologically "untoward" or "deleterious" result.

The author is director, Institute of Environmental and Industrial Health, University of Michigan, Ann Arbor 48104.

The Cell as an Unstructured Reaction Mixture

Hutchinson in a perceptive essay (*4*) has proposed the concept that there might be a concentration limit below which biologically significant reactions probably would not occur.

If the hepatocyte is used as an example, and if such a cell is a homogeneous protoplasmic mass—for example, an average mammalian liver cell of 23.4 micrometers in diameter, with a volume of about 6700 μm^3, and a mass (the density being greater than 1) of about 7×10^{-9} gram—then, from the available analytical data, it is possible to estimate roughly the mean number of atoms per cell. These cellular concentrations are listed in Table 1 (as adapted from Hutchinson). Although better values have been obtained since 1964 for some of the elements in Table 1, the principle illustrated remains valid. From these data Hutchinson states, "The probability of an element having a function decreases with decreasing concentration" (*4*). Further examination of Table 1 demonstrates that the biochemistry of the cell's utilization of the elements therein contained must be reasonably specific. Stated in another fashion: The presence and abundance of a material in a cell does not constitute proof that it has any biological significance.

The question arises as to whether there are potentially more reactive sites for cobalt on various functional proteins, or whether such molecules (and reactive sites) are more abundant. Further there is the question of whether the binding of cobalt is so loose, except at a "utilization" site, that it "moves" more freely throughout the cell. Definitive answers—if indeed these are even the "right" questions—are not apparent. Nevertheless, the number of atoms of molybdenum, cobalt, and lead present in the cell is approximately equal, but only cobalt is biologically active at these "normal" concentrations. The implication is ineluctable that a prime determinant of biochemical activity is molecular specificity rather than the mere presence of an atom or molecule within the cell.

Another approach may be considered. Approximately 400 studies in vitro of enzymic inhibition for 100 different enzymes have been reviewed (*5*). The lowest concentration at which most sulfhydryl inhibitors acted are shown in Table 2. The agreement between these two different approaches is striking. It should be emphasized that the assumptions underlying the cell model discussed above is a rather gross oversimplification. As will be discussed below, the cellular structure does not permit a molecule entering the internal milieu to freely move through the cytoplasm. But as a first approximation only, these two approaches suggest that there are lower concentration limits for the occurrence of biologically significant intracellular molecular interactivity. However, that such inhibitors occur in vitro does not permit one to assume a similar dose level for inhibition in vivo, or indeed that in vivo inhibition occurs at all.

Structural Determinations of Cellular Reactivity

The possible actions of a "foreign" atom contacting or entering a cell should first be taken into account. One set of interactions produced by a "foreign" atom might involve a functional protein, whereas others might react with a structural protein.

In the case where a molecule, for

Table 1. Concentrations and estimated probability (P) of functionality of reactive atoms found in the hepatocytes. Elements *not* known to have functionality are indicated by italics. Elements known to have function in mammals—other than maintaining skeletal integrity—are listed in order of descending concentration throughout. [Adapted from Hutchinson (*4*)]

Element	Atoms in hepatocytes (No.)	P
H, O	$> 10^{14}$	1.0
C, N	10^{12}–10^{14}	1.0
S, P, Na, K, Mg, Cl, Ca, Fe, *Si*	10^{10}–12^{12}	~ 0.9
Zn, *Li, Rb,* Cu, Mn, *Al, Br, F*	10^{8}–10^{10}	~ 0.4
Sn, Ti, Mo, Co, I, *Pb, Ag, B, Sr, Ni, V, Sc, Cd,* Cr, Se	10^{6}–10^{8}	~ 0.3
V, Hg, Be plus 40 additional reactive natural elements probably in these two rows	10^{4}–10^{6}	~ 0.1 to ~ 0.1
Ra	10^{2}–10^{4}	
	10^{0}–10^{2}	

example mercury, enters the cellular milieu, it has the potential for binding with the rather abundant sulfhydryl (-SH) radicals, among others, associated with structural proteins such as those on the cell membrane. This binding may have no effect if there is no denaturation or alteration of tertiary relationships. Binding of lead at sites on the erythrocyte membrane sites may occur without any perceptible effect on the membrane's resistance to movement of potassium ion (*6*). Even if alteration of a protein molecule of the cell membrane is irreversible this need not necessarily mean that there is a deleterious consequence. To assume that the consequence is a harmful one is to ignore a biological reality; that is, all chemical constituents of the cell are constantly "turned over," they are being replaced and renewed. Hence, the qualitative loss of protein units of the cell membrane is not the question. Rather, concern should be directed toward the quantitative aspect, particularly whether the rates of such losses exceed the rates of replacement. It is also necessary to consider what the probability is that two mercury atoms will bind and denature two adjacent cell membrane protein molecules and then produce a significant gap in the functional cell wall at this point. On a stochastic basis it would be justifiable to propose that such probability is a function of the number of atoms of mercury introduced into the system. Finally, even if cellular death occurs, this is a normal event. Except in the central nervous system, all tissue components go through cycles of postmitotic growth and development, senescence, death, and replacement. Once more, it would seem that the deleterious consequence of introducing a foreign atom would again be a function of "dose," and would be measured by the number of cell deaths occurring beyond that expected from the normally recurring turnover rate.

However, the fate of a foreign element–protein complex is not clear. The binding constants between mercury and protein, for example, will obviously determine the subsequent fate or biological availability of mercury. If the binding constant is low, mercury could dissociate and yet again combine with still another sulfhydryl-containing molecule of the cell wall or cytoplasm. Or conversely, it might be bound so tightly that it is unavailable, whereupon it might be essentially sequestered or even discharged from the cell. If such an ion were subsequently found in a suitable milieu, for instance, as a consequence of renal dissociation, an enhanced biological availability may apply, as in the case of the mercury ion's first cellular interaction.

With respect to the interaction of a foreign molecule with a functional protein within the cell, similar answers suggest themselves regarding the relevance of dose to deleterious effect. Considering again an atom of mercury reacting with a sulfhydryl radical, several consequences may ensue. In view of the common occurrence wherein an enzyme's structure has numerous -SH groups, the question arises as to what the probability is that the Hg-SH binding will necessarily occur at the -SH in close spatial proximity to the reactive site. There is also the question of what the probability is that mercury will bind at the -SH sites that regulate the enzyme's tertiary characteristics. Unless either of these occurrences (among other possibilities) transpired, the binding of mercury might have no effect on the enzyme's functionality. Once again, it is obvious that the probability of these untoward effects occurring should increase with mounting numbers of mercury molecules within the cell, that is, with increasing dose.

That these considerations are not theoretical is indicated by evidence that sulfhydryl or other radicals may be bound without producing functional aberration. Swenson and Boyer (*7*) have shown that ten sulfhydryl groups can be blocked in aldolase (by an SH-blocking agent) without any loss of enzymatic activity. Segal and Boyer (*8*) have reported that iodosobenzoate can react within three of the five sulfhydryl groups of glyceraldehyde-3-phosphate dehydrogenase without binding the two sulfhydryl groups at or near the active center of this enzyme. In yet another connection, Massey (*9*) indicates that at least one-half the iron in succinate dehydrogenase can be removed by phenanthroline without loss of activity.

That alterations of molecular configuration may occur without any apparent functional significance is clearly indicated by hemoglobin's polymorphism. Though we are aware of approximately 100 different variations in the amino acid sequence of hemoglobin, only a few of these are associated with aberrations in hemoglobin function. A possible cause for such alterations in structural configuration is that an environmental chemical or physical agent might have in the past caused a change in the DNA base sequence (such as deletion, substitution) which specifies hemoglobin's amino acid sequence. Yet even with such a significant effect transmitted over an evolutionary time scale, structural change has not necessarily implied significant functional concomitants.

Furthermore, if function and structure considerations are combined, it should be noted that a blocking agent may react with one enzyme in prefer-

Table 2. Lowest concentrations at which inhibitors act on three selected enzymes.

Enzyme	Inhibitor	Concentration		Inhibition (%)
		mM	Molecules per cell	
Glutathione reductase	Hg^{2+}	0.000067	2.5×10^{5}	15
Creatine kinase	*o*-Iodosobenzoate	0.00001	4×10^{4}	13
Arginine kinase	*p*-Mercuribenzoate ion	0.00001	4×10^{4}	27

ence to another, depending on the location within the cell and the affinity for the agent. Whether a "biologically significant" effect occurs, then, depends on the "importance" of the enzyme in the cell's economy or on the existence of an alternate metabolic pathway.

Finally, changes in structural or functional proteins might never occur if we accept the possibility that mercury might as readily bind with an interfering molecule, for example, it may bind with substrate. While such binding may make such substrate unavailable for utilization, its biological significance should again be a function of the number of substrate molecules made unavailable, that is, a function of dose.

At present, we have insufficient information on which to build a stochastically sound structural-functional model that could adequately predict the lower limits of molecular concentrations that are sufficient to produce a significant biological effect. As an example of lack of knowledge on which to predict mercury's potential cellular interactions, the spatial distribution of -SH molecules is unknown for most enzymes. As alluded to above, even if one assumes passive diffusion, the flow through a cell is not random. If one conceives of the ergastoplasm as an invagination of the cell of plasma membrane, which penetrates the ergastoplasm, it would be reasonable to propose that a foreign molecule's first significant cellular encounter might be with this structural component. It follows that the probability for mercury binding with, for example, the -SH group could be a function of the abundance of -SH groups on the ergastoplasm. If -SH groups were abundant on this structure, there would be a greater probability of interaction on this membrane rather than with an enzymatic protein within the cell. Again, this model becomes convoluted both by the fact that we do not perceive the distribution of -SH groups in the cell and that -SH groups are not the only binding site for mercury.

Obviously the construction of a satisfactory stochastic model is beyond our present capabilities. But if we use the approach suggested by Hutchinson, it would apparently be reasonable to suggest that, "there might be too many commoner, accidentally and potentially interfering materials (or sites?) around in the cell for any very important substance to work practically at a concentration of less than 10^4 atoms or molecules per cell" (*4*).

Homeostasis: Its Necessity, Its Costs

Radiobiologists, in their studies of accelerated aging, have pointed out the possibility that environmental stresses "speed-up" the processes that represent the end result of a lifetime of biological activity involved in tissue replacement or energy production. It is difficult to conceive of an existence free of any displacement from the steady state, for even the assimilative process implies a "new" cellular amino acid, hexose, or fatty acid environment. Yet without such process there is no life. Therefore the living organism must—and does—possess the ability to permit limited displacements from homeostasis. Do such costs involved in reestablishing the steady state necessarily impose an indelible decrement on a fixed lifetime quanta of energy generation? This assumption implies that biological systems operate within exceedingly narrow if nonexistent rate limits. Even minimally, that there is such a concept as "rate limits" strongly suggests the hazard of ignoring quantitative parameters when dealing with any life process. It is for this reason that I deplore the use of the pure qualitative descriptor inherent in much of the thinking of those who equate "effect" with "deleterious."

Such thinking also ignores the ability of the organism to accommodate. The finding that DNA repair can occur after ultraviolet irradiation (*10*), or that bacterial cells can utilize an "abnormal" carbon source by, for example, enzyme induction (*11*), belies the concept that biological systems have exceedingly narrow ranges of response to environmental challenge.

Finally, we are faced with an ultimate question of whether an organism would live forever if cells did not require replacement, and if some optimum nonstressing nutrient input were provided to metabolic systems for the makeup of nondegrading structural elements and enzymes. Such an organism could not face any perceptible stimuli, since these also produce a displacement from the aforementioned steady state, such a creature would have a Faustian life span but could receive no new knowledge, an existence barely removed from that of the amoeba in its ability to experience the world around it. The danger that a world ablated of sensory inputs imposes has been clearly demonstrated by sensory deprivation experiments. It seems that man is destined to fail and challenge, to perceive and appreciate his world, but at the cost of mortality. Otherwise, what an immortality, what a life!

Summary

Present confusion that equates the presence of a biological effect with a deleterious implication ignores several concepts. To believe that a single molecule's presence in a cell implies a definite potential for deleterious effect disregards stochastic considerations. To believe that such molecules cause an undesirable effect disregards the presence of multiplicity of interferring substances. Such thinking also does not take into account the fact that the dose of a foreign atom may be related to the probability of its interacting with an available active site, or that similar probability governs the answers to the question of whether interactions will occur at discrete topographical loci upon a structural or functional molecule (or on a possible precursor). While the construction of stochastically sound model is remote, the reasonableness of the hierachy of cellular element concentrations as these relate to metabolic function suggests that a threshold for biological activity exists within a cell at 10^4 atoms.

The cellular organism operates within a quantitative rate limit that transcends any statements having only qualitative bases. Thus concepts concerning encroachments on response capabilities over a lifespan are inadequate descriptors of biological activity in the absence of quantitative qualifiers.

References

1. "National Primary and Secondary Air Quality Standards," Code of Federal Regulations 42, chap. 4, part 410; also *Federal Register* **36**, No. 84, 8186 (30 April 1971).
2. R. R. Beard and G. A. Werheim, *Amer. J. Public Health* **57**, 2012 (1967).
3. *Webster's New International Dictionary of the English Language* (Merriam, Springfield, Mass., ed. 2, unabridged, 1958).
4. G. E. Hutchinson, *Proc. Nat. Acad. Sci. U.S.* **51**, 930 (1964).
5. J. L. Webb, *Enzyme and Metabolic Inhibitors* (Academic Press, New York, 1966), vol. 3.
6. H. Gregarzik and H. Passow, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere* **267**, 73 (1958).
7. A. D. Swanson and P. D. Boyer, *J. Amer. Chem. Soc.* **79**, 2174 (1957).
8. H. M. Segal and P. D. Boyer, *J. Biol. Chem.* **204**, 265 (1953).
9. V. Massey, *Biochim. Biophys. Acta* **30**, 500 (1958).
10. J. E. Donnellan, Jr., and R. S. Stafford, in *Biochemical Responses to Environmental Stress*, I. A. Bernstein, Ed. (Plenum, New York, 1971), p. 38.
11. R. P. Mortlock and W. A. Wood, *J. Biol. Chem.* **204**, 1 (1953).