Water of such temperatures, or any other temperatures within this range, can be prepared by having constant level jars of cold and warm water and by regulating the flow from these jars into a mixing chamber where the desired temperature is attained. From the mixing chamber the water will flow into the trays or aquaria containing the experimental animals. Because the temperature of our cold water is very uniform, adjustments are seldom necessary to compensate for fluctuations.

Having running water of different temperatures offers an opportunity to experiment simultaneously with groups of organisms kept at such temperatures. For example, we used our facilities for observations on growth of adult oysters at temperatures of 10.0, 15.0, 20.0, 25.0, and 30.0° C; on development of eggs and growth of larvae of different mollusks; in studies of some phases of physiology of oysters and clams, such as gonad development and spawning; and in many other experiments.

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Toxicity and the Chemical Properties of Ions[1]

The mechanism of the toxic action of drugs has long been a controversial subject. Attempts at an elucidation of the problem include correlations between toxicity and molecular properties. The older physiologists and medical men are, in the main, opposed to any such correlation; the younger biochemists are for it. The more conservative investigators have much negative evidence to support their view, for usually when a drug is administered no one has the slightest idea what happens thereafter, other than the end result. Those who believe in an association between the physiological effect and molecular pattern of a poison have little evidence but much confidence to support their view—a view which they regard as the intelligent one, for, to what else can toxicity be due?

A study of the poisonous action of inorganic salts on slime molds should simplify matters, for the ion of a metal has a far less intricate structure than, for example, a molecule of cocaine, and a primitive form of life is devoid of the complexities of higher organisms.

The slime molds are Myxomycetes to the botanists, who regard them as plants, and Mycetozoa to the zoologists, who think these molds are animals. To medical men, slime molds are just protoplasm, far removed from the intricacies of the human body and therefore having little bearing on medical physiology. But perhaps the difference between the protoplasm of lowly organisms and that of higher forms of life is not always as great as imagined. Lacking the differentiations which tissues present, the protoplasm of a slime mold reveals correlations which are obscured in highly complex organisms. Furthermore, the visible effect of a toxic agent on a slime mold may be directly observed through the microscope.

The degree of toxicity of a poison acting on a slime mold is determined by a number of pathological changes which occur in the protoplasm; among them are the periods of time necessary to kill, to stop protoplasmic flow, and to produce injury. The degree and kind of injury are also significant criteria; among these are gelation, solation, syneresis, blistering, surface rupture, and general disorganization.

Results of studies on the poisonous actions of anesthetic agents, drugs, and metallic salts on protoplasm were reported at an Army Symposium in June 1948.[2] The discussion there, and later elsewhere, led to numerous comments and criticisms, both adverse and constructive. They are repeated here in the belief that they will prove of interest not only to toxicologists, biologists, and medical research workers, but to chemists as well, for they deal as much with the physical chemistry of solution as with toxicity.

I had found a correlation between the anesthetic effects of CO_2 and N_2O and their isosteric properties (*Science*, 1948, **107**, 15). The correlation was questioned because, so it was said, the protoplasm is not in contact with the gas, CO_2, but with carbonic acid. This is an old problem, and has long since been answered by both biologists and chemists. Of the several kinds of molecules and ions which CO_2 in water may present, it can be experimentally shown, by a process of elimination, that only the CO_2 molecule is responsible for anesthetic and other toxic effects. There are several ingenious experiments in physiology which demonstrate, by taste and color indicators, that an *acid* condition is established *within* living tissue when an *alkaline* solution is added outside, due to the rapid entrance of CO_2 as such. Few substances enter a cell as freely and as rapidly as does carbon dioxide, and it enters primarily as the CO_2 molecule.

The evidence from the physical-chemical side is of the same sort. H. B. Bull (*Physical biochemistry*, New York City: John Wiley, 1943) states that "at equilibrium, the amount of dissolved CO_2 is about 1000 times the amount of hydrated CO_2, i.e., of carbonic acid." This means that instead of having no free CO_2, there is some 99 percent of it when CO_2 is dissolved in water. The correlation between the isosteric and the anesthetic properties of the gas must, therefore, fall, if it is to fall, on other grounds.

A further criticism, directed against physical-chemical interpretations of the toxicity of metallic salts, involved the validity of the assumption that the metal ions are present as free ions. That hydrates and complexes are formed in solution is, of course, well known. Salts such as those of Al and Zn are particularly bothersome from

[1] A number of chemists have contributed to these comments, some of them unknown to me, speaking in group discussions. Rather than attempt to select those whose comments should be acknowledged, I shall express my indebtedness to all collectively, and disclaim for myself any credit for the more fertile suggestions. I need only add that the physiological work on the toxicity of salts reported here was done in my laboratory by myself and my assistant, whose presence I owe to the Sloan-Kettering Institute for Cancer Research.

[2] Army Chemical Center, Edgewood Arsenal, Maryland.

this point of view and are therefore frequently omitted from toxicity series. They not only hydrolyze readily, forming weak bases, but produce soluble complexes, leading to such ions as $[Al(H_2O)_4(OH)_2]^+$.

The case of mercury is a special one. $HgCl_2$ is but slightly dissociated in solution, and the concentration of Hg^{++} is correspondingly low. But mercury salts of oxygen acids are capable of giving high Hg^{++} concentrations. Mercury poisoning by $Hg(NO_3)_2$ is therefore due to free mercury ions.

$AgNO_3$, which I had regarded as a clear case of simple dissociation, was said to be another "troublemaker," an appellation which I had given to $ZnCl_2$ and $AlCl_3$. But this does not appear to be true of $AgNO_3$. The extent of hydrolysis of any given salt in aqueous solution can be calculated from the strength of the base, the metal hydroxide, and the acid. In the case of $AgNO_3$, hydrolysis and complex formation, if any, are negligible, and the solution can be considered as one of Ag^+ and NO_3^- ions.

As for $ZnCl_2$, it, too, in dilute solution, consists largely of the simple ions Zn^{++} and Cl^-. Hydrolysis, however, is great.

Accepting, therefore, as we apparently must, the fact that certain metals assumed to be responsible for the toxic effects of their salts are actually present as free ions in solution, we may seek in the physical and chemical properties of these ions an explanation of their toxicity.

A toxicity series such as $Na^+ < Ba^{++} < Au^{+++} < Th^{++++}$ is readily explained in terms of valence and charge. That such an analysis has some meaning is seen in the relative capacity of these ions to precipitate colloidal suspensions. But there are striking exceptions to a valence interpretation of toxicity. Monovalent Ag^+ is highly toxic, far more so than divalent Ca^{++} and trivalent La^{+++}. Very significant also is the fact that within any one valence group there are pronounced differences in toxicity. Thus, among the divalent cations, Ba is far more toxic than Ca.

Ionic series are frequent in chemistry and biology. The seriation is based on various reactions, on protein coagulation, sensory responses (FRINGS, H., *J. Comp. physiol. Psychol.*, 1948, **41**, 25) cell permeability, adsorption, zeta potentials, and colloidal precipitation (FREUNDLICH, H., *Kapillarchemie*, Leipzig; Akademische Verlagsgesellschaft, 1934). Usually these lyotropic salt series are presented in one continuous line, but this is not always feasible, for there is frequently much overlapping; furthermore, the less toxic mono- and divalent ions often constitute a clear-cut series of increasing toxicity, whereas the more poisonous metals form a heterogeneous group. I prefer several distinct series, and I prefer, too, a staggered arrangement, as it better illustrates the experimental facts. For the protoplasm of slime molds, the complete toxicity grouping:

$$Li^+ < Na^+ < K^+ < Rb^+ = Cs^+$$
$$Ca^{++} < Mg^{++} < Sr^{++} < Ba^{++}$$
$$La^{+++} < Pb^{++} < Au^{+++} < Ag^+ < Th^{++++}$$

The hydrogen ion, itself highly toxic, is not involved in the toxicity of the salt solutions here mentioned. The pH of all solutions used was near neutrality, and slime mold protoplasm thrives well at any pH above 4 and below 8.

Molecular interpretations of lyotropic series have been numerous. Valence and atomic weight are the first obvious correlations to be made with toxic effects. From top to bottom and left to right in the periodic table is the general rule for the increasing toxicity of the elements.

The unexpected position of certain ions in the third of the foregoing groups is more difficult of interpretation, but there are a number of atomic properties which appear to bear on the anomaly. The position of the ion in the electromotive force series correlates with toxicity to some extent. Five highly toxic elements, Cu, Ag, Hg, Pb, and Au are grouped together in the emf series. Of possible significance is the second electronic ring, that adjoining the outer valence orbit. Thus, Ag, Pb, and Au are all highly toxic and all have 18 electrons in the second orbit. The meaning of such a correlation would lie in its influence on the activity of the ions, on their tendency to form bonds with atoms which are part of the protoplasmic structure. Resonance is another property of molecules which has come into the biological picture of late. L. Pauling (*The nature of the chemical bond*, Ithaca, N. Y., Cornell Univ. Press, 1945) calls attention to resonance as a likely factor in physiological activities, to which W. T. Astbury (chapter in *The structure of protoplasm*, Ed. by William Seifriz, Ames, Iowa: State College Press, 1942) adds, "It may well be that resonance and the hydrogen bond are of more importance to physiology than any other two facts in chemistry."

It is upon still another property of ions that I wish to lay particular emphasis. There is a very close correlation between the toxic action of ions and their hydration. Protoplasm is largely water and lives in an aqueous medium. Hydration must, therefore, play a major role in physiological reactions. The order of hydration of the monovalent ions is: $Li > Na > K > Rb = Cs$; for the divalent metals it is: $Ca > Mg > Sr > Ba$. These are both the order of increasing toxicity. Relative hydration, therefore, correlates perfectly with degree of toxicity.

Some questions have arisen over types of hydration, over arrangement and density of the hydration layer. I therefore quote J. D. Bernal and R. H. Fowler (*J. chem. Phys.*, 1933, **1**, 515) who state simply that Cs^+ is not hydrated and Li^+ is highly hydrated.

Water is an associated molecule, tetrahedral in structure. Bernal and Fowler compute ionic hydration from the maximum packing of the tetrahedrons, and find the order of hydration of the monovalent cations to be as given above, which agrees with results from all other sources (HARNED, H. S. and OWEN, B. B. *The physical chemistry of electrolytic solutions*, New York City: Reinhold, 1943.)

Harned and Owen point out that the ionic field is $\frac{1}{p^2}$, and hydration is, therefore, in inverse ratio to the ionic radius. The radius of the Li^+ ion is small enough to attract water molecules, whereas the large Cs^+ ion does

not permit the ion to hold a water layer. Thus do ionic radii, fields, hydration, and toxic effects run parallel to each other.

The role of hydration is a shielding one. It determines whether or not a toxic ion can exert its effect, but it does not determine the innate toxicity of the ion; i.e., if an ion is in itself not toxic, the absence of a protective water layer cannot make it so. A hydrated layer can protect but it cannot activate. Mobility is a measure of the activity of the ion and therefore must determine, in part, the nature of the reaction between ion and protoplasm.

The order of mobilities of five monovalent ions is (BRIGGS, D. R. *J. phys. Chem.*, 1928, 32, 1646):

Li	Na	K	Cs	H
33.4	43.5	64.6	68.0	315.0

and this is the toxicity order. But mobility is itself dependent upon hydration. The order of ionic mobility parallels that of hydration and therefore of the toxicity of the mono- and divalent ions. Cs^+ is about twice as mobile as Li^+. Li^+, as $Li^+ \cdot nH_2O$, becomes then the ion with the smallest radius, greatest field, maximum hydration, minimum mobility, and least toxic ion in the series. Cs and Rb, with numerous electron shells and therefore large radii, have weak fields, low hydration, and high mobility. They are, consequently, more toxic, being more active and having more direct contact with the protoplasm. The difference in the fields of Cs and Rb is slight; one would, therefore, expect that there would be slight difference in their toxic effects, and this proves to be true—it is difficult to distinguish the poisonous action of the two.

The third group of ions listed above is a heterogeneous one. The highly toxic Th^{++++} ion is of large size, heavy weight, great charge, and low hydration; Au^{+++} is the same; Pb^{++} also, but to a lesser degree. Their toxicity therefore correlates with their physical qualities. The toxicity of La^{+++}, however, is surprisingly low for a trivalent metal, being less than Ba^{++}. Trivalent Au^{+++}, on the other hand, is highly toxic. Hydration helps in part to set the matter straight. If we compare Ba^{++}, La^{+++}, and Au^{+++}, we find that Ba^{++} is highly toxic for a bivalent metal; its charge is not excessive but its hydration is low. La^{+++} is a heavy metal ion of large size and great charge; it should therefore be quite toxic, but it is much more heavily hydrated than Ba^{++}; the protoplasm is consequently protected against it. Au^{+++} has all the properties of La^{+++} except its hydration; it should therefore be highly poisonous, and this it is.

Ag^+, because of its position in the third group with other exceedingly toxic elements, presents a special case. It is a large ion, and not highly hydrated, but these two facts alone are not enough to establish its high toxicity. The reaction between protoplasm and an ion is determined by the thermodynamic properties of the ion and by the selectivity of protoplasm. The former include the several properties so far presented, but they are not equally effective. Mobility and hydration dominate among the lighter elements. These two properties become increasingly less significant with increase in atomic weight. Among the heavier metals, mobility and hydration play a lesser part.

Electronegativity, indicated by position in the emf series, determines the force with which a metal attracts electrons and therefore the ease with which it forms combinations with other substances. In the present case, these other substances are the protein constituents of protoplasm. Thus may we say that ionic mobility is a primary factor in determining toxicity among the lighter metals, whereas electronegativity is primarily the factor in determining the toxicity of the heavy metals. The long-recognized correlation between toxicity and atomic weight is here realized. Mobility decreases with atomic weight, but electronegativity increases, being greatest in the noble metals, among which Th, Ag, Au, Hg, etc., are the most poisonous of elements.

My interest in those physical properties of metals and organic compounds which explain their toxicity has centered primarily in the hydration and mobility of ions and in the structural patterns of molecules; but the further one looks into other properties the more nearly perfect the parallelism becomes. This is seen in the isosteric properties of CO_2 and N_2O (LANGMUIR, I., *J. Amer. chem. Soc.*, 1919, 41, 1543). It is also obvious in a number of other properties of those elements considered here. The coagulating power of ions is of the same order as their ionic mobilities (PAPPADÀ, N. *Kolloid Z.*, 1909, 14, 56). Furthermore, an ion which moves into an interface readily, because of high mobility, will for the same reason lower the potential and the surface energy.

Adsorption, likewise, nicely parallels both the physical and the physiological properties of ions. G. W. Scarth (*General physiology*, New York City: John Wiley, 1930) points this out in listing the order of adsorbability, which for the monovalent and divalent ions is that of the toxicity order given above. For certain ions, including some of those in the third heterogeneous group, the order of increasing adsorbability by charcoal is: $Na < K < Ca < Zn < La < Cu < Hg < Ag$. Adsorbability is thus seen to increase with valence, mobility, and capillary activity among the lighter elements. Among the heavier metals, electronegativity, i.e., position in the emf series, is the dominant factor in determining adsorbability. The nobler the metal the more strongly it holds its electrons, the greater it is adsorbed, and the greater is its toxicity.

When numerous physicochemical properties correlate with toxicity it is impossible to know where to lay the emphasis. All play their parts in different ways. The important point at the moment is that all correlate with the relative toxicity of the ions.

Toxicity, in the last analysis, involves an interaction between the poison and a specific component of protoplasm. Selectivity between ions and certain chemical groups is recognized. J. Northrop (*J. gen. Physiol.*, 1928, 11, 480) has shown that Cu combines primarily with the NH_2 groups of gelatin whereas La combines at some other point. Particularly important in the present discussion is the knowledge that the heavy metals, such

as Th, Ag, Pb, and *to a lesser extent*, La, show a pronounced tendency to form covalent bonds, especially with atoms of sulphur (FEIGL, F. *Specific and special reactions*, New York City: Elsevier, 1940).

That the correlation between toxicity and ionic properties is not limited to metallic ions but holds well for certain organic molecules, is to be seen in the perfect parallelism between the poisonous action and the molecular pattern of the barbiturates (SEIFRIZ, W. and POLLACK, H. *Arch. d. Sci.* (Swiss), 1949, 2, 9). The highly polar sodium pentothal is the most toxic, and the weakly polar barbital the least toxic. With decreasing toxicity, the molecule becomes smaller, the carbon chains progressively shorter, and the molecule less polar.

Opposition to molecular interpretations of toxicity is not, I believe, so much a question of lack of evidence as a fear of complexity. A request for a brief account of the mechanics of kidney function was refused on the ground that the kidney has one million tubules, each functioning differently. Just what a million differences in kidney tubule activity might be I cannot imagine. Surely, there is an over-all picture of the functioning of the kidney which, taken in its entirety, presents a single mechanism. In a similar way there may be one over-all picture of the toxicity of metallic ions, with each ion possessing not so much individual characteristics as an emphasis on one property over others. The individual differences will be relative—distinctions in solubilities, equilibria, and reaction rates, determined by electron pattern and ionic behavior.

The opinion expressed above is that of the pharmacologist, A. J. Clark (*General pharmacology*, Berlin: J Springer, 1947; lithoprinted Ann Arbor, Michigan: J. W. Edwards, 1944) who says that to regard drug response as an expression of individual variation is a wholly unfruitful view. To this I should add that the surprising feature of drug action is not the variation but the clear-cut correlations. W. T. Astbury (in W. E. Clark and P. B. Medawar [Eds.] *Essays on growth and form*. Oxford: Clarendon Press, 1945. P. 309) agrees in saying that the general shape of a molecule can often be surprisingly effective in determining certain biological reactions, "from which fact there is emerging a clearer idea of families of molecular structures." L. Pauling (*Amer. Scientist*, 1948, 36, 51) is of a similar opinion, that chemotherapeutics, enzymology, catalysis, chemical kinetics, and immunological reactions depend for their solution upon an understanding of molecular pattern. A. Szent-Györgyi (*Nature of life*, New York City: Academic Press, 1948) introduces his lecture on muscle with the statement that "Life, however varied in its appearance, is always built on the same simple principles," and he cites the similar reactions of different tissues to caffeine and veratrine.

After all, intelligent speculation involves no more than thinking about one's data, which is the way of science.

Aside from all speculation—even aside from an interpretation of data—our conclusion, reduced to its minimum irrefutable facts, forces us to concede: that it is the CO_2 molecule as such which enters cells and causes anesthesia; that we may still regard free metallic ions as responsible for the toxic effects of their salts in solution; that the poisonous action of metallic ions on the protoplasm of Myxomycetes is, in general, directly proportional to atomic weight, valence, electric charge, surface activity, adsorption, ionic diameter, hydration, mobility, and electronegativity. The present work shows the order of the toxic effects of ions on slime mold protoplasm to be the same as the order of their physical properties, certain of these properties, such as hydration and mobility, playing greater part among the lighter metals, and certain others, such as electronegativity, being dominant among the heavier noble metals.

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