DISCUSSION

Dr. Maxwell Schubert: Dr. Katchalsky, you spoke at one point about calcium ions being bound much more firmly than you might have expected. I wasn't clear whether that applied just to the particular polymers that you dealt with or whether you thought it was general. Would you say a few words about that?

Dr. Aharon Katchalsky: The combination of calcium is of primary biological importance, and I am grateful for the question.

How do we know whether the extent of combination is the one we would expect? In the case of a purely electrostatic interaction of ions with polyelectrolytes one expects the osmotic coefficient to go down more or less linearly with the charge on the small ion. This means that if the charge is 2, the osmotic coefficient should go down to about a half its value for monovalent ions. This was observed in the case of magnesium. In the case of calcium, it was observed that the coefficient goes down by a factor of 10, which seems to indicate the existence of a much stronger combination. The reasons for this behavior might be a partial dehydration of the counterion, ion-pair formation, or the formation of stronger bonds. The exact nature of the bond formed is at present unknown.

A promising line of research which might lend deeper insight into the mode of combination of small ions with polyions is the study of the dielectric constants and dielectric dispersion. I have mentioned that the polyelectrolyte atmosphere has a very high polarizability. This high polarizability is due to the fact that the atmosphere is a mobile dynamic structure. It is many times higher than the polarizability observed in low molecules.

The work of Alpén, of Mandel, and of Eigen and Schwartz proved that polyelectrolytes very strongly increase the dielectric constant of the solutions. In some cases they observed that the increase in the dielectric constant of water was by a factor of 8. I leave it to your imagination to figure out what this means for the behavior of biocolloids and charged enzymes.

In the case of calcium, Mandel found also a very strong increase, but not as strong as one would expect for a freely movable atmosphere. This might indicate that there is a partial fixation of the counterions and formation of localized bonds.

In the case of proteins, it is known that calcium is bound to albumin in two different modes of bonding. Albumin has a hundred carboxyl groups, so that one would expect the maximal binding of calcium ions to be fifty ions per protein molecule. Various experiments have shown that about eight of the calciums are strongly bound and forty-two are weakly bound.

It seems that in this case, the firmly bound calcium is located in suitable local group configuration, say those including two carboxylic groups and one hydroxylic group, which permit the formation of true and firm complexes.

Question: In the case of binding between the polyelectrolytes, has any effect of pH been demonstrated on the strength of the binding or the degree of the binding, in other words, that the pH passes some critical level?

Dr. Katchalsky: The dependence of the energy of binding on pH is of fundamental importance. If increasing pH means increase in the degree of ionization, then the energy of binding per group increases with pH. We figured out that in some cases the binding energy reaches values of a few kilocalories per monovalent counterion.

In the very interesting presentation of Dr. Kauzmann, you heard that the strength of the hydrogen bond in aqueous solution is of the order of magnitude of approximately 1 kilocalorie. The electrostatic bonds for very highly charged polyelectrolytes in solutions of low ionic strength may reach values of 2 kilocalories. In natural polyelectrolytes, whose charge density is generally not very high, and in isotonic solution the energies of combination are much lower.

I might mention in this connection, a good rule of thumb for the relation between degree of ionization and degree of ion binding. If you plot the osmotic factor (φ_p) as a function of the degree of ionization (α), it goes down. Since the drop in φ_p is approximately parabolic $\alpha \cdot \varphi_p \simeq$ constant.

The meaning of this rule is that in a certain region, upon increasing the degree of ionization, the osmotic contribution does not change. In other words upon increasing the pH the degree of binding remains practically unchanged.

Dr. Seong S. Han (Ann Arbor): Would you mind elaborating on the method by which you introduce the polyelectrolyte to produce agglutination of the cells in your last illustration?

Dr. Katchalsky: The method was to add directly a polyelectrolyte solution to a suspension of cells. We considered it the onset of agglutination when over three cells did not separate from each other. Macroscopically, one could observe a precipitation of cells, yet this occurs generally either after longer times or at higher polybase concentrations.

Under the electron microscope, "agglutination" takes place not only between cells but in the cell surface itself.

The phenomena of agglutination occur at very high dilutions of micrograms or fractions of micrograms per milliliter.

It is remarkable that agglutination of cells does not occur when their electrophoretic mobility goes to zero. One would expect that the onset of agglutination should coincide with the moment when the cells do not repel each other. However, agglutination is already taking place when very small amounts of polyelectrolyte are adsorbed to the cell surface. The reason is apparently that there is no

direct attachment of one cell surface to another but that polyelectrolyte bridges keep the agglutinate together.

Dr. Han: May I ask, in your case of the red blood corpuscle agglutination, what specific kind of polyelectrolyte you have used?

Dr. Katchalsky: We have used, first of all, polylysines of different molecular weights. Then, we used other polybases such as polyvinylamine, protamine, polyvinylpiperidine, and various polyvinylpyridines.

The general behavior is independent of the nature of the polymer. It was found that the higher the molecular weight of the polybase, the stronger is its agglutinating capacity.

The more highly charged the macromolecule is, the smaller are the amounts required for incipient agglutination.

During the intermission I was asked by one of our participants about the interaction of polyelectrolytes with enzymes. The interaction of polyelectrolytes with enzymes plays a tremendous role in biochemistry and biology. As shown by my brother, if you mix polysine with pepsin, the two interact violently as would two polyelectrolytes oppositely charged.

Some of the enzyme-polyelectrolyte complexes are inactive forms of enzymes and only when the polyelectrolyte is removed from the enzyme does the enzyme become active. A more general discussion of these interactions goes beyond the range of my talk and belongs to the general study of enzymatic behaviors.

Dr. William Regelson (Buffalo): We have been working with anionic polyelectrolytic tumor inhibitors (1–7) and we have the opposite phenomenon, that is, the opposite of agglutination produced by polylysine. With polyethylene sulfonate or polystyrene sulfonate, we get hydrophilic gels with nucleated cells. You need a nucleus. This gelation effect is blocked by the presence of deoxyribonuclease I. You can prevent the phenomenon if you have the enzyme present. With electron microscopy, we see no alteration in the nuclei of the cells—they appear essentially intact. However, when the DNAse I enzyme is added to the gel, it destroys the gel and destroys the cells at the same time (8).

Now, there is some specificity with regard to enzyme inhibition, in that we can inhibit, for example, with polyethylene sulfonate, DNAse II, and not DNAse I (2, 9). We can get degrees of specificity, depending on the molecular weight of polycarboxylic ethylene maleic anhydride copolymers *in vivo* in the mouse (3). With the low molecular weight amide-acid copolymers being the more potent tumor inhibitors (3) from the point of view of therapeutic index, one can work with these polymers, getting significant tumor inhibition at least in the mouse. Clinically, you get into a lot of problems which can be characterized as macromolecular disease: shock, severe pain in the tumor, cytopenia, and such things (5, 7).

Dr. Katchalsky: It is a very good remark, and I am grateful for your information.

REFERENCES

1. REGELSON, W., and HOLLAND, J. F., The anionic polyelectrolyte, polyethylene sulfonate, as a new anti-neoplastic agent, *Nature*, 1958, **181**, 46.
2. REGELSON, W., TUNIS, M., and KUHAR, S., The inhibition of tumor growth and deoxyribonuclease II by anionic polyelectrolytes, *Acta, Unio Internat. Contra Cancrum*, 1960, **16**, 729.
3. REGELSON, W., KUHAR, S., TUNIS, M., FIELDS, J., JOHNSON, J., and GLUESENKAMP, E., Synthetic polyelectrolytes as tumour inhibitors, *Nature*, 1960, **186**, 778.
4. FELTZ, E., and REGELSON, W., Ethylene maleic anhydride copolymers as viral inhibitors, *Nature* 1962, **196**, 642.
5. REGELSON, W., and HOLLAND, J. F., Effect of an anionic polyelectrolyte (polyethylene sulfonate) in patients with cancer, *Clin. Pharmacol. and Therap.*, 1962, **3**, 730.
6. CARRUTHERS, C., and REGELSON, W., Molybdenum, copper and zinc contents of mouse liver and Sarcoma 180 treated with molybdenum compounds, *Oncologia*, 1963, **16**, 101.
7. MIHICH, E., SIMPSON, C. L., REGELSON, W., and MULHERN, A. I., Pharmacological study of ammoniated ethylene maleic anhydride polymer MW 20,000–30,000 (AEMA 20–30), *Fed. Proc.*, 1960, **19**, pt. 1, 142.
8. TUNIS, M., JOURNEY, L., and REGELSON, W., The gelation effect of sulfonated polymers on nucleated cells, to be submitted for publication.
9. TUNIS, M., and REGELSON, W., A comparative study of the inhibiting effects of anionic polyelectrolytes on deoxyribonuclease, submitted for publication.