Certain generalisations can be made from these observations.

1. Complexes formed by polyanions with cationic detergents are soluble in salt solutions at concentrations which are characteristic and widely different, according to the structure of the polymer.

2. Complexes of carboxylic polysaccharides are soluble in much lower concentrations of all types of salt than those of the polysulphates, with DNA and RNA[2] in the intermediary range.

3. The solvent action of the anion on polysulphate complexes is inversely related to its energy of hydration; the same applies (up to a point) for the cation. Ionic strength seems to be of greater importance in the prevention of polycarboxylate and nucleic acid precipitation, especially when the density of charged groups is low, as in pectin.

4. The bond between the quaternary ammonium ion and ester sulphate groups differs considerably from that derived from carboxylate, and is probably stronger.

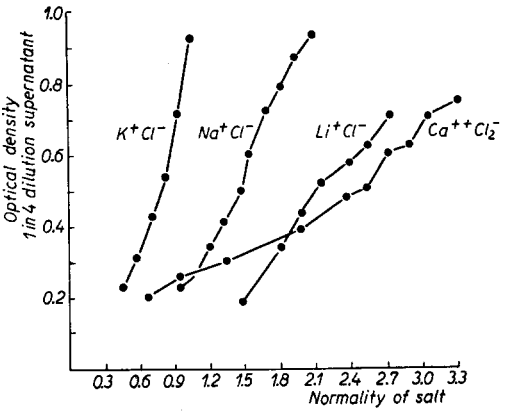

Fig. 1. The optical density was measured in 10 mm quartz cells at 260 mμ. A reading of 0.20 corresponds to complete precipitation, and 0.95 to no precipitation.

From the above and other experiments it is thought that two processes contribute to the formation of the precipitate.

1. Organic cations associate with the polyanion in solution displacing inorganic ions to some extent. When the energy of hydration of the polymer is insufficient to hold the long paraffin chains of the associated cetylpyridinium in solution, a new phase is formed.

2. Organic cations from solution exchange with inorganic ions remaining on the solid with release of energy, stabilising the precipitate.

Equilibrium in stage 1, between solid and solution phases depends on the ratio of the concentrations of quaternary ammonium and inorganic cations, their respective affinities for the anionic groups and on the hydration energy of the polymer, *i.e.* on its structure. The cation-anion affinity decreases with increasing solvation of both, *i.e.* is roughly proportional to the size, charge remaining constant (for review on ionic solvation see Ref. 3). This is probably because the strongest bond between cation and anion is an ion-pair, or a closely allied structure which can only be formed subsequent to the loss of water of solvation.

The affinity of COO^-, PO_4^-, SO_4^-, for a given cation seems to increase in that order; of cations for an anion, $Li < Na < K$; the order of increasing size. The hydrogen ion occupies a special place in such series because of its ability to form covalent bonds with anions.

The applications of these findings to biological materials, together with detailed interpretations and experimental data, will shortly be submitted for publication.

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Effect of salt concentration and pH on the rate of virus neutralization by purified fractions of specific antiserum

JERNE[1] and JERNE AND SKOVSTED[2] first reported that the rate of neutralization of bacteriophage by specific antiserum is considerably faster at low than at high salt concentration. This effect of salt concentration on the rate of virus neutralization has been confirmed by others[3,4]. The purpose of the present communication is to make a preliminary report of studies dealing

with the effect of salt concentration and pH on the kinetics of neutralization of bacteriophage T2r+ by purified fractions of specific antiserum.

A pooled, rabbit anti-T2 serum was separated into eight fractions of different mean isoelectric points by the method of electrophoresis-convection[5]. The material taken from the bottom reservoir of the electrophoresis-convection cell after the last stage of fractionation was further separated into a globulin and albumin fraction by salting-out with ammonium sulfate. All except the albumin fraction showed good virus neutralizing activity under physiological conditions of pH and salt concentration.

The kinetics of virus-neutralization by the various serum fractions indicate that the effect of salt concentration and pH on the rate of the neutralization reaction can be understood in terms of changes in the electrostatic interactions between specific antigen and antibody sites, modified by changes in collision frequency between virus particles and antibody molecules. The first-order rate of neutralization of T2 by the unfractionated serum at pH 7 is strongly dependent on the salt concentration of the reaction mixture. Thus, the rate of neutralization in $3 \cdot 10^{-3}$ *M* NaCl was approximately 30 times as fast as in 0.15 *M* NaCl. Whereas some of the serum fractions also showed a marked increase in rate of neutralization on lowering the salt concentration at pH 7, others showed no increase in rate, while with still others, the reaction was strongly inhibited at low salt concentrations. A correlation was found between the mean isoelectric point of the serum fraction and the effect of salt concentration on their rates of neutralization in neutral solution: the more acid the isoelectric point, the less the rate is increased on lowering the salt concentration, neutralization by the most acidic fractions being inhibited at low salt concentration. At pH values alkaline to the isoelectric point of the serum fractions, the net electrical charge carried by the viruses and the antibody molecules are of the same sign. It appears that if these net charges become sufficiently large, then the union of virus and antibody is inhibited at low salt concentration as a result of decreased collision frequency resulting from electrostatic repulsion between the particles. This explanation is supported by the observation that fractions showing inhibited or only slightly increased rates of neutralization in $3 \cdot 10^{-3}$ *M* NaCl at pH 7, show greatly enhanced rates at pH 5.4–5.6, which is acid to the isoelectric pH. The quantitative relationship between rate of neutralization and salt concentration, over the range 0.15 to $3 \cdot 10^{-3}$ *M* NaCl, has been determined for one of the fractions at pH values acid to its isoelectric point. Analysis of these results in terms of the BRÖNSTED-CHRISTIANSEN-SCATCHARD theory of the primary salt effect on reaction kinetics in solution, suggests that the increase in rate of neutralization on lowering the salt concentration results from increased coulombic attraction between specific antigen and antibody sites, *i.e.*, local areas on the respective particles where the specific reaction occurs. At these pH values the net charges on the virus particles and antibody molecules are also opposite in sign; however, the increased collision frequency at low salt concentration resulting from increased coulombic attraction between the net charges seems to be a second order effect.

Whereas, the rate of virus neutralization in 0.15 *M* NaCl is insensitive to the hydrogen ion concentration over a rather wide range of pH, the rate is extremely sensitive to hydrogen ion concentration in $3 \cdot 10^{-3}$ *M* NaCl. For example, at low salt concentration the rate of neutralization by one of the fractions increases as much as 45-fold on going from pH 7 to 5.5. At lower pH values, however, the neutralization reaction is strongly inhibited, the rate decreasing 45-fold on going from pH 5.5 to 4.5. The increase in rate on going from pH 7 to 5.5 appears to be due to increased coulombic attraction between specific antigen and antibody sites as a result of binding of protons by some group or groups, presumably imidazole, in the immediate vicinity of one of the sites. That binding of protons by these groups is not, in itself, essential for the reaction is indicated by the fact[6] that the rate of neutralization at high salt concentration is insensitive to pH over the range 5 to 10. Inhibition of the neutralization reaction at pH below 5.5 is attributed to the binding of protons by one or more carboxylate groups in one or both of the sites. That ionization of these carboxyl groups is essential for the antigen-antibody reaction is indicated by the fact[6] that neutralization is also inhibited below pH 5 at high salt concentration.

Control experiments have shown that the antibody does not lose its specificity under conditions of salt concentration and pH which yield maximum rates of T2 neutralization, and that normal serum proteins do not neutralize the virus under these conditions.

In an earlier communication[3] the authors reported measurements on the effect of salt concentration on the rate of virus neutralization by unfractionated antiserum. It was found that under certain conditions the neutralizing activity of the antiserum changed in a rather complex fashion during incubation at low salt concentrations. This led us to conclude that the effect of salt is on one or more of the components of the antiserum and not directly on the neutralization reaction itself. However, the results of the present experiments using fractions of antiserum show quite clearly that this is not the case. The primary effect of salt concentration is on the neutralization reaction itself, although prolonged incubation of the serum fractions in media of low salt concentration does result in some loss of neutralizing activity. Effects such as slow desorption

of bound ions from the antibody molecules might account for the slow increase in neutralizing activity of unfractionated serum previously observed during early stages of incubation at low salt concentration.

Finally, the differences in the behavior of the virus-neutralizing activity of the various serum fractions with respect to changes in salt concentration of the antigen-antibody reaction mixture at pH 7, shows that the antibody molecules in the different fractions are different physical entities. This result is not surprising, since electrophoresis-convection separates proteins on the basis of differences in their electrophoretic properties.

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The assay of soluble hydrogenase

CURTIS AND ORDAL[1] have shown that the soluble hydrogenase from *Micrococcus aerogenes* is inactivated by methylene blue, which is the dye most commonly used as hydrogen acceptor in the enzyme assay. Benzyl viologen is less inhibitory, while methyl violet proved least injurious of the dyes tested.

We have obtained analogous results for two other hydrogenation systems. Working with the soluble hydrogenase from *Desulphovibrio desulphuricans* (SADANA AND JAGANNATHAN[2]), we find that dilute solutions of the enzyme do not take up hydrogen in the presence of methylene blue. Concentrated solutions of the enzyme will transfer hydrogen to methylene blue for several minutes, followed usually by a sharp decline in activity. With benzyl viologen or methyl violet 3B we have observed little or no inactivation of the enzyme.

For comparison we have been studying the transfer of hydrogen to acceptors in the presence of one of the relatively simple organo-metallic complexes which, in aqueous solution, react with molecular hydrogen. The catalytically active complex is formed by addition of a large excess of potassium cyanide to cobaltous chloride solution in the absence of air (IGUCHI[3]). We find that H_2 uptake is inhibited by dyes in the increasing order methyl violet, benzyl viologen, methylene blue (Fig. 1).

There can be little doubt that the explanation of the inhibition is the same for both the hydrogenase and the cyanide systems. In each there are two opposing reactions:

(i) reaction of the catalyst with H_2;

(ii) reaction of the catalyst with oxidised dye.

The first is followed by donation of hydrogen to the dye, thus freeing the catalyst to undergo either (i) or (ii). The second leads to inactivation. Although it is a relatively slow reaction, it brings about a decline in the number of effective catalyst molecules. The degree of inhibition by each dye is a measure of the rate at which it can oxidise the catalyst and is determined not only by redox potential, but also by the mode of reaction.

This explanation suggests that the site in soluble hydrogenase which is capable of reacting directly with H_2 is readily accessible for collision not only with H_2, but also with any other solute. The active site is believed to consist of either one or two metal atoms. (The reasons are discussed in a forthcoming review.) On collision with an oxidant, such as methylene blue, an electron is transferred to the dye, while the metal atom passes to a higher oxidation state (*e.g.* $Co^{II} \rightarrow Co^{III}$). In the cobalt cyanide system the oxidation cannot be reversed, and this may well be true for hydrogenase.

With particulate hydrogenase, dyes have no inactivating effect. It is conceivable that the active site in particulate hydrogenase is screened by protein or lipid which, although it is readily permeable to diatomic gases, hinders the diffusion of larger molecules.