Large collections from the Cretaceous and Tertiary deposits of western North America have recently been restudied; criteria have been established for separating fossils of the three genera, and for setting up recognizable species.

During Cretaceous time, and at the beginning of the Tertiary period, *Metasequoia* was abundant at high northern latitudes. Its deciduous habit was well suited to a regime of summer rainfall and lowered winter temperature. With gradual reduction in rainfall and temperature. *Metasequoia* and its associates are recorded from successively more southerly stations, as far south as California; near the end of the Tertiary period it became extinct in North America, probably as a result of a shift to winter rainfall and summer drought. Its failure to survive in the southeastern United States along with *Taxodium*, which is also deciduous, cannot now be explained.

The geologic history of *Sequoia* has been somewhat different because of its evergreen habit. Never as abundant as *Metasequoia*, the ancestors of the California redwood were in past ages largely limited to foothills and to the borders of ancient seas. With the change in rainfall regime during later Tertiary time, *Sequoia* was restricted to the borders of the Pacific; in regions with summer fog it still survives. *Metasequoia* is known to exist only in the interior of China, where heavy summer rainfall is combined with moderate temperatures. Here in valleys so remote that they have not yet been completely deforested by land-hungry farmers, the redwood of Asia appears to be living the last chapter of its hundred-million-year existence.

TECHNICAL PAPERS

Mutual Interaction of Polyelectrolytes[1]

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Polyelectrolytes (*1*), even in the most dilute solution, give regions of high charge density in the neighborhood of each polyion, as a simple consequence of structure. We might therefore expect a strong electrostatic interaction between the fields of polycations and polyanions, which would lead to mutual precipitation. Results are herewith presented which verify this prediction.

In one series of experiments, portions of a dilute solution of sodium polyacrylate[3] were added to a solution of poly-4-vinyl-*n*-*N*-butylpyridonium bromide. Flocculent precipitates formed immediately; at concentrations as low as 10^{-5} normal in bromide ion (about 10^{-8} molar in polyelectrolyte), a distinct turbidity was still visible. The precipitation was followed quantitatively by measuring the turbidity of the mixtures with a Phoenix light-scattering photometer. Fig. 1 shows the results of a typical experiment in which successive portions of 10^{-3} normal polyacrylate solution were added to 40 ml of 25×10^{-6} normal polypyridonium bromide. Up to the equivalence point, the turbidity τ increased linearly with the amount of polyacrylate added; just beyond equivalence, the turbidity increase accelerated sharply, and after a ratio of about 1.5, slowly decreased with further addition of polyacrylate. Fig. 2 is a log-log plot of the turbidity obtained when 1.5 equivalents of polyacrylate were added to 1.0 equivalent of polypyridonium salt, the latter at varying stoichiometric bromide concentrations, *N*. It will be noted that the points fall on a 45° line, showing that the amount of precipitate is proportional to the concentration of the polypyridonium solution from which it was precipitated down to below millionth normal in bromide ion (approximately 10^{-9} molar in polybromide or 0.2 ppm by weight). The lowest concentration corresponds to the present limit of sensitivity of our optical equipment.

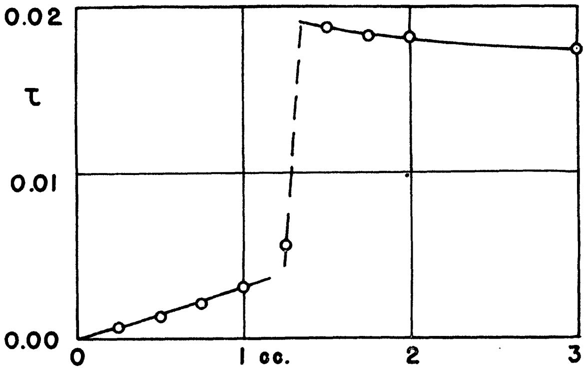

FIG. 1. Turbidimetric titration of polyvinyl-butyl-pyridonium bromide (40 cc of 25×10^{-6} normal) with sodium polyacrylate (10^{-3} normal).

These results may be interpreted as follows: When polyacrylate is added to polypyridonium salt, the latter is initially in excess. Normally, each polyacrylate ion is surrounded by an atmosphere of sodium counter ions; when a polyacrylate ion-plus-ion cloud encounters polypyridonium ions, the strong attractive field between poly-

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[3] We are grateful to the Minnesota Mining and Manufacturing Company for a sample of polyacrylic acid and to the Dow Chemical Company for a sample of sodium polystyrene sulfonate.

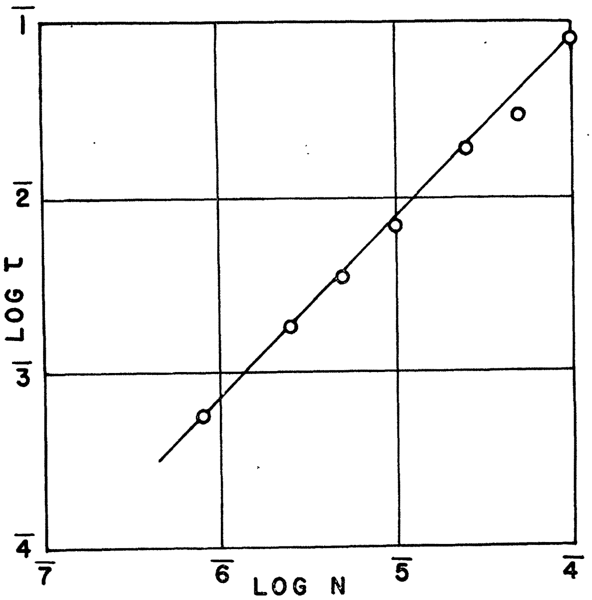

FIG. 2. Test of proportionality between turbidity and concentration of polyelectrolyte solution.

anion and polycations causes the polymeric ions to approach, and many of the monovalent gegen ions are displaced. The resulting aggregate is a compact insoluble cluster, cross-linked by electrostatic forces. We naturally do not expect perfect pairing of acrylate and pyridonium ions; those unpaired will be accompanied by their corresponding gegen ions. Since the pyridonium salt is in the medium into which the acrylate is poured and is initially in excess, the exterior of the cluster will be mostly polypyridonium chains. Then when excess acrylate is added, more polyanions attach themselves to the polycationic exterior of the precipitate particles, displacing both sodium and bromide counter ions until the excess charge is compensated. This hypothesis accounts for the sharp rise in turbidity just beyond the equivalence point followed by a leveling off at a fairly well-defined degree of excess. The subsequent slow decrease of turbidity probably is due to an electrostatic contraction of the particles of precipitate in the presence of excess ions, or else to coalescence of original particles. We plan to determine particle size.

In another series of experiments, solutions of sodium polystyrene sulfonate were mixed with polypyridonium bromide solutions. Again, flocculent precipitates formed. These experiments were carried out at higher concentrations of polyelectrolytes, so that conventional analytical methods could be used. The precipitates were centrifuged out, dried and weighed, and bromide ion in the supernatant liquid was determined by potentiometric titration. The results of a typical series of experiments are given in Table 1, where the polybromide solution (B) analyzed to 0.0180 normal in bromide ion. The concentration (approximately 0.005 normal in sulfonate) of the polysulfonate solution (S) was not accurately known, because the starting material contained some insoluble cross-linked products which were removed by centrifuging before the solution was used. (There also appeared to be some impurity of low molecular weight present.) In all the experiments of Table 1, polybromide was in excess. The first column gives the volume and sequence of reagents; e.g., "5 B: 20 S" means that 5 cc of polybromide was poured into 20 cc of polysulfonate. The second column gives the weight (in mg) of precipitate found, and the third, the mg of precipitate per unit volume of polysulfonate solution. The fourth column gives the number of milliequivalents of bromide ion used, and the fifth, the deficiency of bromide ion in the supernatant liquid after mixing with polysulfonate and centrifuging out the precipitate. This deficiency corresponds to bromide ions which accompanied the precipitate as neutralizing counter ions for the dangling polypyridonium chains.

TABLE 1

PRECIPITATION OF POLYVINYL-BUTYL-PYRIDONIUM BROMIDE WITH SODIUM POLYSTYRENE SULFONATE

Expt.	Ppt. in mg	mg/cc	Meq. Br^-	ΔBr^-
5 B : 20 S	47.9	2.40	0.090	0.0090
10 B : 20 S	40.1	2.00	0.180	0.0151
10 B : 10 S	21.6	2.16	0.180	0.0163
20 B : 10 S	21.2	2.12	0.360	0.0184
20 S : 5 B	37.0	1.85	0.090	0.0081
20 S : 10 B	25.9	1.30	0.180	0.0111
10 S : 10 B	14.9	1.49	0.180	0.0125
10 S : 20 B	16.8	1.68	0.360	0.0168

An average of 1.58 mg/cc precipitate was obtained when polysulfonate was poured into polypyridonium salt. Here the polycation was always in excess, so each polyanion formed the nucleus for a precipitate particle which contained more than a stoichiometric equivalent of polypyridonium, due to its dangling chain ends. When polybromide was poured into polysulfonate, however, an average of 2.17 mg/cc sulfonate solution was found. This result seems reasonable on the basis of our picture of the mechanism of precipitation; at first, polyanions were in excess, and each polycation added was the nucleus for a precipitate particle which contained dangling polyacrylate chains. Further addition of polycation added to the polyanionic chain ends, thereby increasing the weight of the precipitate which had formed initially. As seen in Table 1, the bromide ion deficiency was correspondingly greater for this sequence of mixing.

These precipitates are inherently different, both from familiar electrolytic precipitates like silver chloride and from the colloidal coagulates, such as those obtained from mixing suspensions of arsenious sulfide and of ferric hydroxide. In all three cases, electrostatic forces cause the approach of particles of opposite charge; with silver chloride, a substance of low intrinsic solubility is formed from silver ion and chloride ion, while with the colloidal precipitates, rigid, relatively massive particles coalesce. But with the polyelectrolytes, the individual charges are normal in their behavior; it is their high local concentration which brings about expulsion of gegen ions and solvent, and leads to precipitation. (Both sodium acetate and sodium benzene sulfonate, for example, will precipitate the polypyridine salt only at very high concentrations.)

When the charge density is as high as that corresponding to vinyl polymers such as we used (where an ionogenic group is attached to every second carbon atom of the chain), we would expect that any polycation will precipitate any polyanion. On the other hand, by preparing copolymers (*2*) with controlled spacing of charges, it might be possible to obtain polyelectrolytes which show a selective precipitability, according to whether opposite charges can be paired off geometrically or not. It might thus be possible to make a model similar to those postulated (*4*) for immunological reagents.

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Is Chloride a Coenzyme of Photosynthesis?

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One of the serious obstacles in the experimental study of the mechanism of photosynthesis has been the impossibility of separating the process from the activities of intact green cells. The recent work of Hill (*5*) makes it possible, however, to investigate outside the living cell the reaction most characteristic of photosynthesis in green leaves: photolysis of water resulting in the evolution of gaseous oxygen. The oxygen-liberating system resides in the chloroplasts, and remains functional when fragments or whole chloroplasts are removed from green leaves.

The photochemical evolution of oxygen by chloroplasts isolated from sugar beet and spinach was recently investigated by Warburg and Lüttgens (*6*), who reached the rather striking conclusion that chloride ion was a coenzyme essential for photochemical reactions in photosynthesis. That such a simple yet important fact had escaped the notice of all other workers in this field was indeed cause enough for Warburg and Lüttgens to remark how rash were all previous theories on the mechanism of photosynthesis. The evidence which led these authors to conclude that chloride is a coenzyme of photosynthesis was as follows. Isolated chloroplasts lose their capacity for oxygen evolution after several washings in water. They can be reactivated, however, by adding cytoplasmic fluid. The factor in cytoplasmic fluid responsible for reactivation of the chloroplast was found to be heat-stable. An analysis disclosed that cytoplasmic fluid contained chloride in 0.08 molar concentration. Addition of chloride alone as M/150 KCl brought about complete reactivation. Of the other anions tried, bromide was almost as effective, iodide and nitrate much less so, and fluoride, sulfate, thiocyanate, phosphate, and all the cations tried were without effect. Since chloride was the effective anion found in sufficient concentration in cytoplasmic fluid, Warburg and Lüttgens concluded that it was the natural coenzyme of photosynthesis.

Impressive as this chain of biochemical evidence is in support of chloride as a coenzyme of photosynthesis, it poses at once a rather perplexing physiological problem from the standpoint of plant nutrition. Chloride is not generally regarded as an essential element for growth of higher plants. Is it then possible that plants can get along in nutrient solutions without a coenzyme required for photosynthesis, a process indispensable for growth? The fact that Warburg and Lüttgens found appreciable amounts of chloride in their plants is not surprising. Chloride is widely distributed in soils and readily absorbed by most plants. Its presence in the plant, however, was hitherto regarded as incidental.

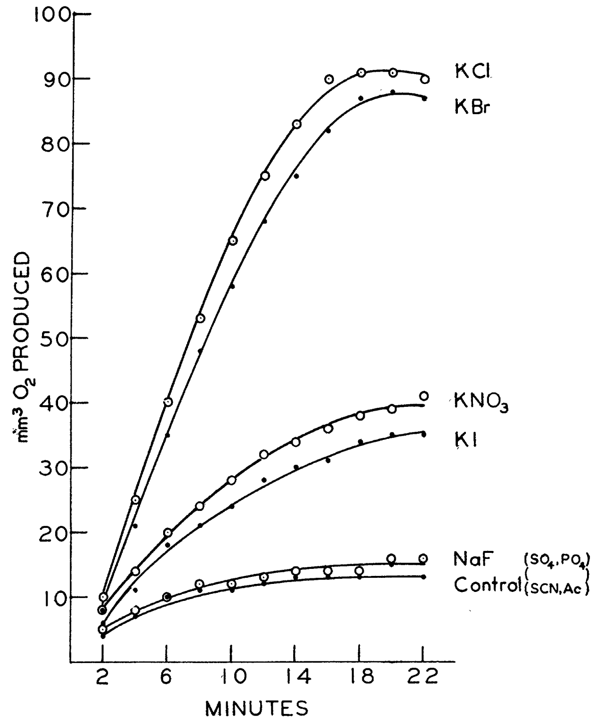

Fig. 1. Effect of anions (10^{-2} M) on oxygen evolution by illuminated chard chloroplasts. Reaction mixture: A chloroplast suspension containing 0.5 mg of chlorophyll, M/15 phosphate buffer, quinone as oxidant. Illumination at flask level approx. 28,000 lux, temp = 15° C. Other details of technique were similar to those previously described (*4*).

We undertook to investigate the problem by growing sugar beet and chard in nutrient solutions without chloride. Plants were grown in a nutrient solution supplemented with the micronutrients B, Mn, Cu, Zn, and Mo in amounts and from sources previously described (*1*), except that $MnSO_4$ was substituted for $MnCl_2$. As was expected, the plants made excellent growth in the nutrient solution to which no chloride was added. The chloroplasts from these plants were isolated (*2*) and