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

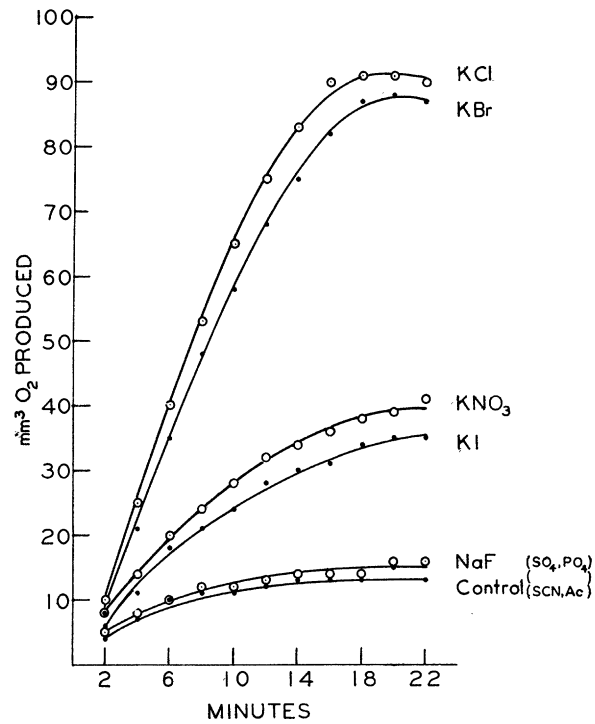

Fig. 1. Effect of anions (10^{-2} M) on oxygen evolution by illuminated chard chloroplasts. Reaction mixture: A chloroplast suspension containing 0.5 mg of chlorophyll, M/15 phosphate buffer, quinone as oxidant. Illumination at flask level approx. 28,000 lux, temp = 15° C. Other details of technique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

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.

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

their oxygen evolution under the influence of light was measured manometrically, by a technique similar to that used by Warburg and Lüttgens (*4*).

Our results disclosed important areas of agreement with those of Warburg and Lüttgens, as well as several differences. An analysis of both chloroplasts and cytoplasmic fluid showed no chloride in either, as would be expected in plants grown without chloride. Chloroplasts, even without washing, showed only feeble oxygen evolution. In our experiments, in contrast to those of Warburg and Lüttgens, the addition of cytoplasmic fluid failed to reactivate the chloroplasts, but as already noted, our cytoplasmic fluid contained no chloride. On the other hand, we fully substantiated the finding of Warburg and Lüttgens that addition of chloride brought about activation of chloroplasts, giving us stoichiometric yields of oxygen in relation to the oxidant used. The effect of chloride on the course of oxygen evolution by illuminated chloroplasts is shown in Fig. 1, which also confirms the

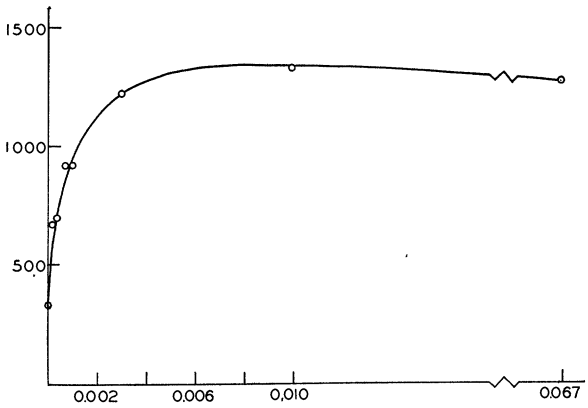

FIG. 2. Abscissas: KCl concentration. Ordinates: $Q_{O_2}^{chl}$. Effect of KCl concentration on rate of oxygen evolution by illuminated chloroplast fragments. $Q_{O_2}^{chl}$ = mm³ of oxygen/hr /mg of chlorophyll, computed from data obtained for the 6-min period from 1 to 7 min after turning on the light. Temp = 20° C. Conditions not specified were similar to those given in the legend for Fig. 1.

findings of these authors with regard to the influence of other anions on oxygen evolution. Bromide has an activating effect about equal to chloride; nitrate and iodide are much less effective; and sulfate, phosphate, thiocyanate, and acetate are without effect.

How should these results be interpreted? The intact plant is able to carry on normal photosynthesis without chloride, as judged by its excellent growth despite absence of this ion either in nutrient medium or in leaf tissue. Yet when chloroplasts are isolated from the same plant, they require chloride for vigorous progress of the photochemical reaction. One explanation would be that chloride acts in the leaf as a micronutrient, and that minute amounts of chloride which escape detection by usual chemical analysis may nevertheless be present in the nutrient medium as an impurity and reach the leaf. This explanation, although it cannot be ruled out entirely, is rendered unlikely by the data presented in Fig. 2. In this chart, the rate of oxygen evolution by illuminated chloroplasts ($Q_{O_2}^{chl}$) is plotted against chloride concentration. It will be seen that, whereas small additions of chloride brought about appreciable activation, a fairly high concentration, around 0.007 M, is required for full activation. This is in agreement with the value of M/150 KCl, reported by Warburg and Lüttgens as necessary for full activation in their experiments. Such relatively high concentrations of chloride are not uncommon in soil-grown plants, but there is strong evidence from these and numerous other experiments that plants can make excellent growth without the presence of measurable amounts of chloride either in the nutrient medium or in the plant. The other anion capable of giving full activation of

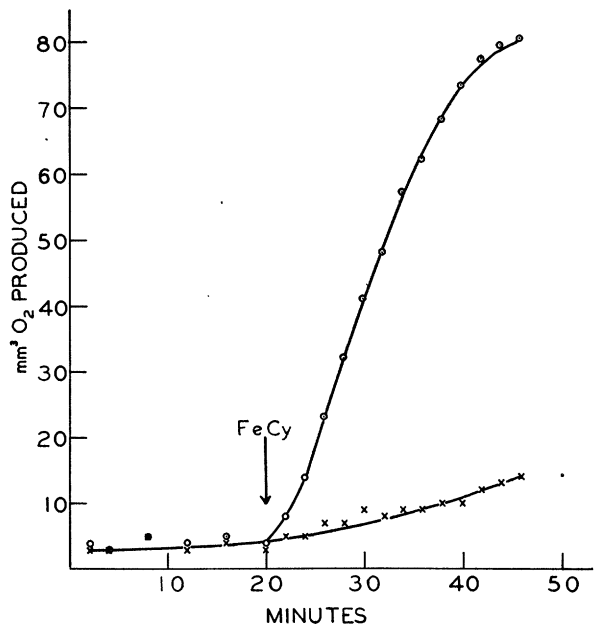

FIG. 3. Protective effect of chloride on illuminated sugar beet chloroplast fragments. *Circles*—illuminated for 20 min in the presence of chloride. At $t = 20$ min, the oxidant (ferricyanide) was tipped into the manometer vessel. *Crosses*—illuminated for 20 min in the absence of chloride. At $t = 20$ min, chloride and ferricyanide were added simultaneously. The concentration of chloride was 0.01 M KCl. A quantity of 1.5×10^{-7} moles of $K_3Fe(CN)_6$ was added to each vessel. Conditions not specified were the same as those given in legend for Fig. 1.

photochemical oxygen evolution, bromide, although readily absorbed and tolerated by plants in appreciable amounts, is not a common constituent of plants or soils, and there is even less reason for suspecting it as being essential for plant growth.

If the view that chloride or bromide is a coenzyme of photosynthesis *in vivo* is to be abandoned, how can the effect of these anions *in vitro* be explained? We have formulated the hypothesis that in the intact green cell photosynthesis goes on without participation of either chloride or bromide, but once the cell is broken there is a rapid light-induced deterioration of some cellular substance essential for the photochemical evolution of oxy-

gen by chloroplasts. Chloride or bromide is able to protect this substance against inactivation, but the intact cell accomplishes this in some other manner. This would explain the superfluousness of the halide *in vivo* as contrasted with its requirement *in vitro*.

The hypothesis was tested in the following manner. Isolated chloroplast fragments were illuminated without, however, adding the oxidant (in this case ferricyanide) which is necessary to bring about the evolution of oxygen. In one instance, chloride was added to the illuminated chloroplasts; the control contained no chloride. After 20 min of preexposure to light, the oxidant was added and the photochemical oxygen evolution was measured manometrically. To the chloroplast suspension which was exposed to light in the absence of chloride, this anion was added simultaneously with the oxidant. The results are shown in Fig. 3. The preexposure to light in the absence of chloride inactivated the oxygen evolution system of the chloroplasts. This inactivation was nearly irreversible. The subsequent addition of chloride had only a slight reactivating effect. On the other hand, a vigorous oxygen evolution, resulting in stoichiometric yields, was given by the chloroplasts which had received added chloride during their exposure to light. Thus chloride appeared to protect some essential photosynthetic substance which in the absence of this anion was irreversibly destroyed by light. Chloride also seemed to exert some protective action on the chloroplasts in the dark. There was evidence of inactivation from shaking chloroplasts in the manometer vessels at 15° C, for a period equal to the light exposure. Inactivation in light, however, was much more pronounced. The identification of this substance would be of great physiological interest. Experiments along this line have been under way in our laboratory, but no conclusion is possible at this time.

The proposal that chloride is a conenzyme of photosynthesis would have endowed chlorine with the status of an essential element for growth of higher plants. It would also have been the first instance in the history of plant nutrition where the essentiality of an inorganic element was established by the discovery of its biochemical function, in the absence of corroborative evidence from growth experiments according to specific criteria of indispensability (*3*). Our results, which speak against the role of chloride as a coenzyme in photosynthesis, also illustrate the contribution which growth experiments can make in evaluation of biochemical data bearing on the essential status of an inorganic element in nutrition of higher plants.

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The Action of Mineral-Ion Exchange Resins on Certain Milk Constituents

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With the discovery in 1936 by Adams and Holmes (*1*) that certain artificial resinous materials possess the ability to act as ion exchangers, new interest was aroused in this field. A large number of such synthetic ion-exchange materials are now commercially available, and various laboratories have been experimenting with their properties when used to treat milk. It was felt that some fundamental studies should be made also on the action of typical anion-exchanger and cation-exchanger resins with simple solutions of the known major inorganic milk constituents, at concentrations as they normally occur.

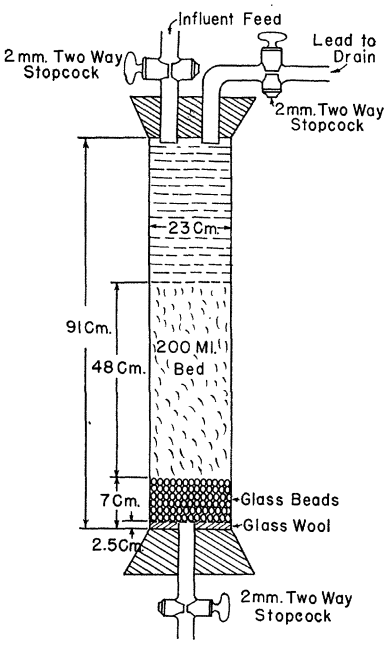

FIG. 1. Ion-exchange column. The Zeo-Karb-H column was initially conditioned with 400 ml of 5% NaCl, downflow at a rate of 13.7 ml/min, backwashed at flow rate to give 50% bed expansion for 5 min, regenerated with 450 ml of 0.407 *N* HCl at 17 ml/min, and washed with distilled H_2O at the same rate until free of acid. The De-Acidite column was exhausted with 2,000 ml of 0.100 *N* HCl, backwashed, regenerated with 280 ml of 0.75 *N* Na_2CO_3 at 4.5 ml/min, and washed free of alkali.

In 1933, Lyman and co-workers (*5*) discovered that by the action of certain natural base-exchange materials called zeolites, the mineral constituents of milk could be modified, chiefly by decrease in calcium ion, so as to im-

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