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.

### References


1. ARNON, D. I. *Amer. J. Bot.*, 1938, **25**, 322.
2. ———. *Plant Physiol.*, 1949, **24**, 1.
3. ARNON, D. I. and STOUT, P. R. *Plant Physiol.*, 1939, **14**, 371.
4. ARNON, D. I. and WHATLEY, F. R. *Arch. Biochem.*, 1949, **23**, 141.
5. HILL, R. *Proc. roy. Soc. Lond.*, 1939, **127B**, 192.
6. WARBURG, O. and LÜTTGENS, W. *Biochimia*, 1946, **11**, 303.


# 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.

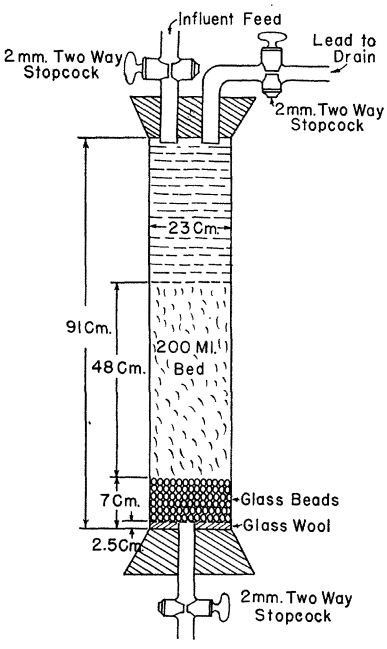


FIG. 1. Ion-exchange column. The Zeo-Karb-H column was initially conditioned with 400 ml of 5% NaCl, downflow at a rate of 13.7 ml/min, backwashed at flow rate to give 50% bed expansion for 5 min, regenerated with 450 ml of 0.407 *N* HCl at 17 ml/min, and washed with distilled $H_2O$ at the same rate 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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prove the ease of digestion and assimilation of milk through the softer curd obtained. Other investigators (*4*) have found that M.I.E. (mineral-ion exchange)-treated milk added to evaporated milk was capable of stabilizing the product against coagulation during sterilization at 240° F for 15 min. The M.I.E. milk can be used as a fluid, powder, or concentrate. Burgess[2] has a patent that covers the use of an artificial ion-exchange material for removing calcium ions more or less completely from milk. Otting (*6*) has discussed the problems which were surmounted in the application of the

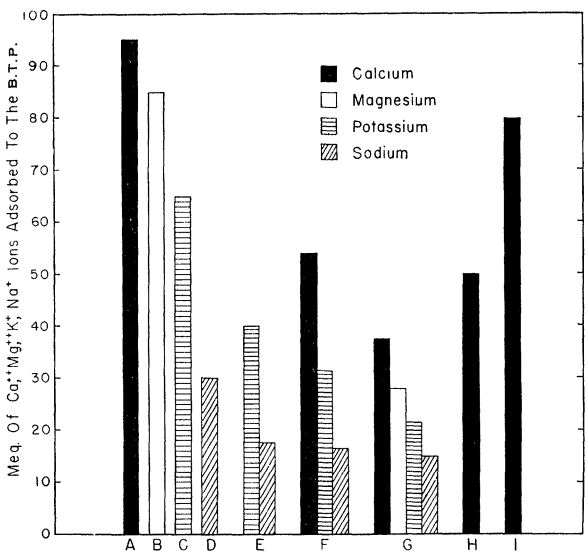


FIG. 2. Adsorption of cations to the B.T.P. by Zeo-Karb-H. Two thousand ml of the following solutions was passed through the column. *A*. $CaCl_2$, 100 meq./1; *B*. $MgCl_2$, 100 meq./1; *C*. KCl, 100 meq./1; *D*. NaCl, 100 meq./1; *E*. Binary soln. of $CaCl_2$ and NaCl, 50 meq. each/1; *F*. Ternary soln. of $CaCl_2$, KCl, and NaCl, 33.3 meq. each/1; *G*. Quaternary soln. of $CaCl_2$, $MgCl_2$, KCl, and NaCl, 25 meq. each /1; *H*. Binary soln. of $CaCl_2$ and HCl, 50 meq. each /1; *I*. Binary soln. of $CaCl_2$ and citric acid, 50 meq. each /1. Influent flow rate 50 ml/min; column regenerated with 450 ml of 0.407 *N* HCl at 17 ml/min, followed with 200 ml distilled $H_2O$ at the same rate, then a fast rinse at 50 ml/min for 10 min.

mineral-ion exchange principle in milk products, and the equipment and procedure to be used.

Numerous other investigations have been made on the use of ion-exchange substances in the separation of cations from anions (*7–10*), in the separation of amino acids, purine, and pyrimidine bases, alkaloids, and many other substances (*2, 3, 11*).

The present paper describes briefly the action of a representative pair of commercial anion and cation exchange resins on the various salts known to occur in milk. The salts were prepared in water solutions containing one, two, three, or four of the cation or anion constituents found in milk, in equivalent concentrations, and then given appropriate ion-exchanger treatment.

[2] Burgess Zeolite Co. Ltd. British patent, No. 542,846 (1942). C. A. 36: 4217–4.

Zeo-Karb-H and De-Acidite[3] were the exchangers employed. The columns were set up as shown in Fig. 1. The air-dry exchanger was added to the column until a 200-ml backwashed and drained volume was obtained. Before using the columns in experimental procedures, each was put through two cycles of exhaustion and regeneration in order to condition the beds.

The break-through point (B.T.P.) was designated as that point at which the concentration of a given ion in the effluent reached a value which was 5% of the initial concentration, while 95% was still being adsorbed by the exchanger. The results were expressed in terms of milliequivalents adsorbed to the B.T.P.

The increasing order of removal for the cations studied, either from individual ion solutions or from mixtures of all four, was from sodium to potassium to magnesium to calcium. The type of opposite ion present in solution was found to be a factor in some cases, since calcium ions were found to be more completely removed by the cation exchanger if the anion present was citrate, than if it was the chloride ion. This is shown in Fig. 2.

When the B.T.P. has been reached, the concentration of the ion in the effluent rapidly approaches the initial concentration, and in the solutions containing two, three, or four of the cations, it has been found that some of them may surpass their initial ion concentration.

The cations present in a complex solution that appear to be least adsorbed by the exchanger were found in reality to be removed during the first part of the exchange run, then released later by the regeneration effect of the other cations in the solution which were preferentially adsorbed. Thus, the hydrogen ion from the exchanger in the acid cycle is not always exchanged each time a cation enters the exchanger, since the entering cation may replace a previously adsorbed cation. The hydrogen ion-cation exchange for solutions containing one cation was found to be quantitative.

When single, binary, and ternary solutions of hydrochloric, citric, and phosphoric acid were passed through a bed of an anion exchanger, De-Acidite, it was found that the order of removal for acids in mixtures was different from the order in trials with solutions containing but one acid. Thus, the order of removal of the acids from individual solutions increased from hydrochloric to citric to phosphoric, but in a solution containing all three acids the order of increased removal was from citric to phosphoric to hydrochloric.

A 0.6% solution of urea, creatine, and creatinine, each being present in 0.20% concentration, was subjected to cation-exchange treatment. A 100% removal of these substances was effected from the first 400 ml of effluent. A 50% removal still occurred after 1600 ml of solution had passed through the bed. These substances were not adsorbed from solution by De-Acidite, an anion-exchange resin.

From the experimental results obtained in this study on less complicated true solutions, and from unpublished data on the action of ion-exchange resins on skim milk, whole milk, and deproteinized milk, ion-exchange ma-

[3] Manufactured by the Permutit Company, New York City.

terials appear to offer a variety of possibilities for modifying the mineral components of milk, either by removal of certain ions, by substituting other ions for normal ions present, or by both operations. Unpublished work, by one of the authors, E. F. Almy, on the calcium removal from cation-exchanged skim milk, shows that removals for calcium start at 83% and drop to 39% as the exchange run progresses, whereas the adsorption for phosphorus remains constant at approximately 7–8%.

Applications of ion-exchange milk to produce smoother ice cream, to improve the quality of baked goods made with milk, and to improve various other dairy products have been suggested, but await further investigation. A local company[4] has patent applications filed covering complete demineralization of cheese whey for the production of lactose with low mineral (ash) content, and for the production of the M.I.E. milk previously mentioned. It also has filed patent applications covering the production of a powdered cream which has been rendered heat-stable by ion exchange to lower the calcium ion content before drying.

### References


1. Adams, B. A. and Holmes, L. E. *J. Soc. Chem. Ind.*, 1935, **54**, 1.
2. Cannan, K. R. *J. biol. Chem.*, 1944, **152**, 401.
3. Cohn, W. E. *Science*, 1949, **109**, 376.
4. Josephson, D. V. and Reeves, C. B. *J. dairy Sci.*, 1947, **30**, 737.
5. Lyman, J. F., Browne, E. H., and Otting, H. E. *Ind. eng. Chem.*, 1933, **25**, 1297.
6. Otting, H. E. *Ind. eng. Chem.*, 1949, **41**, 457.
7. Runeberg, G. and Samuelson, O. *Svensk Kemisk Tidskrift*, 1945, **57**, 250.
8. Samuelson, O. *Svensk Kemisk Tidskrift*, 1947, **59**, 14.
9. *Ibid.*, 1946, **58**, 247.
10. *Ibid.*, 1942, **54**, 124.
11. Sussman, S., Mindler, A. B., and Wood, W. *Recovery of alkaloids by ion exchange.* Reprint. New York: Permutit Co., 1946.


## The ΔG/ΔP Ratio after the Administration of Dextrose as an Index of Insular Function


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In the course of some investigations on the decrease in serum inorganic phosphorus produced by the administration of glucose, it was found useful to relate increase in blood sugar values to decrease in inorganic phosphate.

It is well known that deficiency in insulin production is accompanied by high blood sugar levels as determined a half hour after administration of the carbohydrate, and vice versa, that increased insulin secretion leads to low values. However, there are factors, such as rate of glycogenolysis, secretion of epinephrine, storage ability of the tissues, and different rates of intestinal absorption, that may easily alter blood sugar levels.

The decrease in serum inorganic phosphorus which follows dextrose administration (*1*, *5*, *7*) depends upon the liberation of insulin; in fact, it does not take place in the pancreatectomized animal (*2*). Epinephrine also produces a similar decrease, but only in animals with intact pancreas (*8*). Insulin and epinephrine produce the same changes in inorganic phosphorus, insulin acting by itself (*9*), and epinephrine through the discharge of insulin induced by the elevation of blood sugar. The decrease in inorganic phosphate determined by glucose is not affected by liver deficiency (*2*).

TABLE 1

The ΔG/ΔP Ratio, in Normal and Alloxan-treated Dogs

| Group | Number of animals | ΔG/ΔP Ratio averages ± standard errors |
|---|---|---|
| Normal dogs | | |
| Staub effect present | 26 | 60.4 ± 18.2 |
| Staub effect absent | 17 | 105.9 ± 53.5 |
| Alloxan-treated dogs | 9 | 316.8 ± 75.2 |

TABLE 2

Statistical Significance of the Differences between Means*

| | *p* Values |
|---|---|
| Normal dogs, Staub effect present, vs. Staub effect absent | 0.40 |
| Normal dogs, Staub effect present, vs. alloxan-treated dogs | < 0.01 |
| Normal dogs, Staub effect absent, vs. alloxan-treated dogs | 0.02 |

* Student's test (*3*).

These facts show the importance of studying systematically changes in serum inorganic phosphorus during the glucose tolerance test, in metabolic clinics, and in research laboratories. The results of such investigations are herewith reported.

The work was carried out in apparently normal dogs and in alloxan-treated dogs. Some of the normal dogs were in poor nutritional condition. A fasting sample was taken from the saphena vein, and immediately 1 ml/kg of body weight of a 50% glucose solution injected into this vessel; 30 min later a second sample was taken and the injection of dextrose repeated; 30 min later the last sample was drawn. The blood sugar determinations were carried out in duplicate, according to the Nelson method (*6*) and the serum inorganic phosphate determinations were also done in duplicate by the Fiske-Subbarow procedure (*4*). The ΔG/ΔP ratio was calculated in the following manner: the difference between the blood sugar value at 30 min and the initial value was divided by the difference between the initial serum phosphate and its value at 30 min. Results are shown in Tables 1 and 2 and Fig. 1.

From these results some conclusions can be established.


[1] With the technical assistance of Miss Jeanne Lopez, Miss Luisa Maria Andueza, and the cooperation of Dr. Alfonzo Podrizki.


[4] M. & R. Dietetic Laboratories, Columbus, Ohio.