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Comparison of a New Microcrystalline Aluminum Oxide Hydroxide and an Amorphous Aluminum Hydroxide for Binding to Phosphate, Proteins, Nucleotides, Lipids and Carbohydrates

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Aluminum hydroxide gel (ALG), which is currently used as a phosphate-adsorbent, dissolves in gastric juice and the eluted aluminum ion has been suggested to be a causative agent of osteomalacia and encephalopathy. A new type of microcrystalline boehmite compound of aluminum oxide hydroxide with high acid resistance (named PT-A), has been developed by the author's group as a possible replacement for ALG.

In the present study, the characteristic behaviors of PT-A and ALG toward various food components were compared at different pH's to get information on possible interference with phosphate adsorption onto the adsorbents by food components. The order of adsorption of food components per unit mass of PT-A was: proteins > nucleotides > lipids > carbohydrates. The order with ALG, with a minor exception, was the same as that with PT-A. However, the amounts of materials adsorbed on PT-A were generally much less than those on ALG. When phosphate was mixed with a synthetic milk as a model standard meal and exposed to the two adsorbents, phosphate-adsorption was entirely unaffected; however, the phosphate adsorption capacity of ALG was much less than that of PT-A. In conclusion, PT-A has a higher capacity for adsorbing phosphate, and adsorbs food components to a lesser extent than ALG. Phosphate-adsorption onto PT-A was not subject to interference by food components.

Keywords aluminum; phosphate; phosphate-adsorbent; boehmite; food component; milk

Hyperphosphatemia with secondary hyperparathyroidism has often resulted from long-term hemodialysis of patients with chronic renal disfunction.[1-4] Thus it is necessary to prevent hyperphosphatemia and its adverse effects on the parathyroid-vitamin D axis. To achieve this goal, various phosphate-adsorbent compounds have been clinically tested, including aluminum hydroxide gel (ALG),[5] calcium carbonate,[6,7] some amino acids[8] and other materials.[9,10] Among these preparations, ALG is the most potent phosphate adsorbent. However recent studies have suggested that aluminum released from ALG may cause osteomalacia,[11] dialysis-encephalopathy,[12] and possibly Alzheimer's disease.[13,14] Other phosphate adsorbent preparations have also been reported to have some disadvantages, or else their efficacy has been questioned.[8] Therefore, a stronger and safer phosphate adsorbent is needed. To utilize the beneficial properties of aluminum compounds as phosphate-adsorbents and to minimize undesirable effects associated with ALG, we have developed a new type of microcrystalline aluminum compound. This compound has been shown to adsorb much more phosphate and to release far less aluminum ions than ALG, a currently marketed preparation.[15] We have already reported[16] that macromolecules such as digestive enzyme proteins and bovine serum albumin (BSA) are adsorbed by a newly developed aluminum oxide hydroxide compound with boehmite structure. This new compound was tentatively named PT-A.[15,16] It is necessary to examine whether or not various kinds of materials in addition to proteins are adsorbed on PT-A. These materials include nucleic acids and phospholipids, both of which contain phosphate moieties, steroids and carbohydrates.

The purpose of this study was to compare the extent of adsorption of these materials on PT-A and ALG under fixed conditions and to establish whether or not food components interfere with the phosphate adsorption on either PT-A or ALG.

Materials and Methods

PT-A, a microcrystalline boehmite, is a product (lot No. L41122) of Tomita Pharmaceutical Co. Ltd. (Tokushima, Japan). The chemical formula of PT-A is $AlO(OH)\cdot nH_2O$. The characteristics, examined by X-ray diffraction, scanning electron microscopy, zeta-potential measurement, and gas adsorption/desorption isotherm studies, have been reported elsewhere.[16] ALG, a product (lot No. I40501) of the same manufacturer, was prepared to meet the specification of the Pharmacopoeia of Japan.

Pepsin, trypsin and BSA were purchased from Sigma Chemical Co. Ltd. (St. Louis, U.S.A.). Herring sperm DNA and adenosine triphosphate (ATP) were obtained from Wako Pure Chemical Co. Ltd. (Osaka, Japan) and Sigma Chemical Co. Ltd., respectively. Phosphatidic acid prepared from egg yolk lecithin was obtained from Sigma Chemical Co. Ltd. Powdered milk was obtained from Meiji Milk Products Co. Ltd. (Tokyo, Japan).

Radiolabelled [2,4-^{3}H(N)]cholic acid (specific activity; 0.93 TBq/mmol), [7-^{3}H(N)]cholesterol (740 GBq/mmol), [2-^{3}H(N)]D-glucose (1.08 TBq/mmol), [^{14}C]starch (55.5 MBq/g, obtained from *L. nicotiana tobacum*), and [^{3}H]dextran (3.7 TBq/g, molecular weight, 70000) were purchased from either Daiichi Chemical Co. Ltd. (Tokyo, Japan), or Muromachi Chemical Industry Co. Ltd. (Tokyo, Japan). Other chemicals used were commercial products of special reagent grade.

Unless otherwise stated, for adsorption tests, 0.2 g of PT-A or ALG was immersed in 1 ml of H_2O (NANOpure II, Barnstead Co. Ltd., Dubuque, U.S.A.) overnight at room temperature, then a test material was added, and the volume was adjusted to 25.0 ml with H_2O. The mixture was mechanically stirred for 10 min at room temperature. The PT-A was separated from the solvent by appropriate means such as centrifugation, filtration through a disposable column, or aspiration through a chemical membrane filter. The initial concentration of each test compound is given in the figure legends. This was selected on the basis of a series of preliminary examinations to obtain the concentration at which the equilibrium between adsorbed and unadsorbed test compounds was established for a fixed time. The pH value of the incubation medium was adjusted by the addition of either 0.1 N HCl or 0.1 N NaOH.

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It was confirmed that the pH remained constant within the range of experimental error before and after the reaction. This was also true in the experiment with milk. The residual test compounds in the medium were measured by appropriate procedures. Water-insoluble materials such as cholesterol were suspended in an aqueous phase by sonication and subsequent procedures were the same as for water-soluble compounds.

When radioactive materials were tested, the amounts adsorbed by PT-A or ALG were estimated by measuring radioactivity. In these experiments, aliquots of separated adsorbents were placed in a vial containing 5 ml of scintillator solution, consisting of 4 g of 2,5-diphenyloxazole and 0.1 g of 2,2′-*p*-phenylenebis(5-phenyloxazole) in 1 l of toluene. The radioactivity of ^3H or ^{14}C was measured in a liquid scintillation counter (Aloka type LSA-703).

Inorganic phosphate was measured by the method of Bonting *et al.*[17] Phosphatidic acid in the filtrate was measured in terms of phosphorus content after complete ashing of the lipid with concentrated HNO_3, 30% H_2O_2 and 6 M $HClO_4$.[18] Protein determination was carried out by Lowry's procedure.[19] All experiments were carried out with a set of five identical samples and the results were expressed as the mean ± S.D. Student's *t* test was used to analyze the data statistically.

Results

Adsorption of Peptides It has been suggested that PT-A adsorbs phosphate in the stomach and upper intestinal areas, where a variety of digestive enzymes are present in addition to protein components ingested in foods. Therefore, the profiles of protein adsorption onto PT-A or ALG were examined. The adsorption profiles of pepsin[15] and BSA[16] have already been reported in previous papers. Here, in addition to the profiles of these two proteins, the adsorption of trypsin by PT-A or ALG was examined and compared with those of other proteins. Adsorption of pepsin was quite distinct at acidic pH's (pH 1—4), but at neutral pH (pH 7.0) or higher, the two adsorbents showed reduced adsorption efficacy and adsorbed the protein in equal amounts. The maximal adsorption of pepsin on either adsorbent was observed near the isoelectric point (pH 4.4) of the protein. Less BSA was adsorbed by PT-A than by ALG over the whole range of pH tested (Fig. 1b). Maximal adsorption of BSA was observed at pH 6.6, which is near its isoelectric point. Amounts of trypsin adsorbed on PT-A and ALG were equal over the range of pH examined (Fig. 1c). However, the adsorption increased with increase in pH. The maximal adsorption of trypsin appeared at pH higher than 7. This seems reasonable, because the isoelectric point of trypsin is 10.1.

Adsorption of Nucleotides Nucleotides contain phosphate moieties. It is of interest to see whether or not PT-A or ALG can adsorb organic phosphate compounds as well

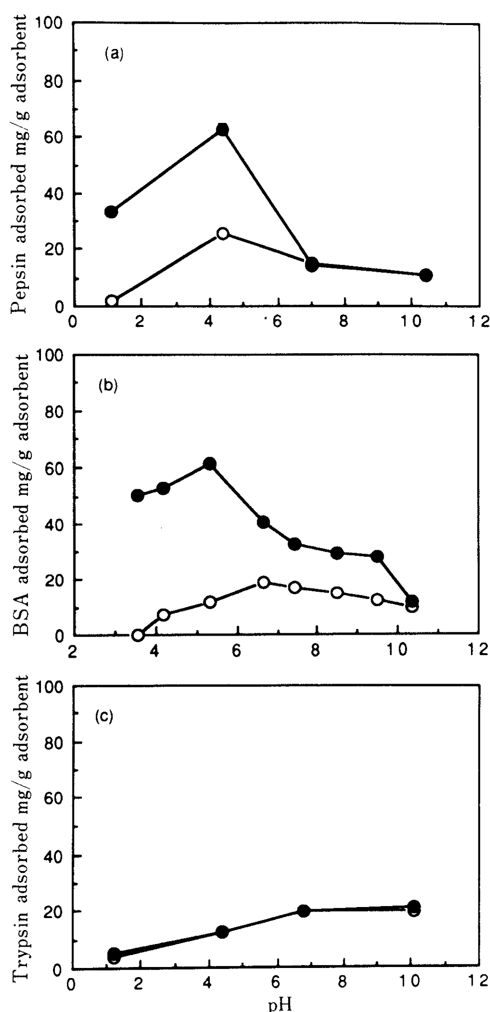

Fig. 1. Adsorption of Proteins on PT-A (—○—) and ALG (—●—)

The initial concentrations of test compounds were 20 mg of pepsin, 10 mg of trypsin and 20 mg of BSA in 25 ml of the medium. For details, see Materials and Methods. Each value is the mean ± S.D. of five samples. The S.D. lies within the symbols if not shown. (a) pepsin, (b) BSA, (c) trypsin.

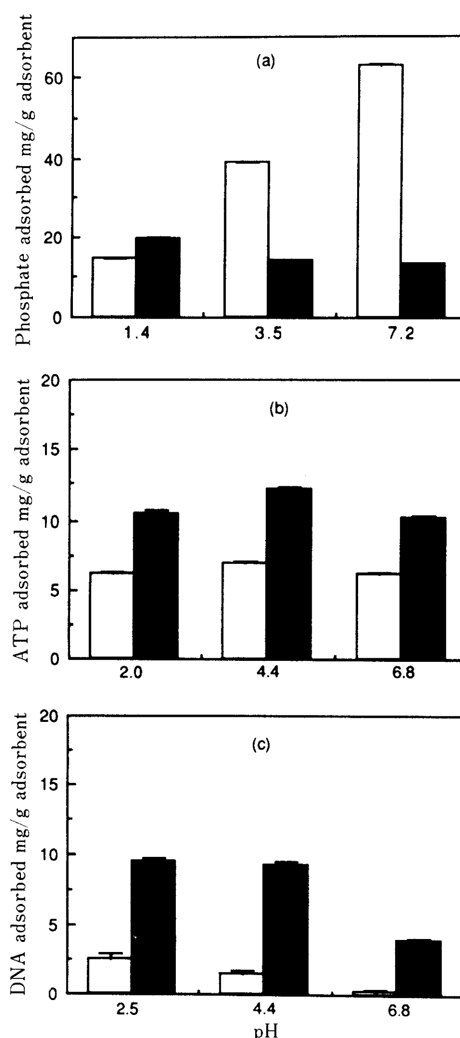

Fig. 2. Adsorption of Inorganic Phosphate and Nucleotides on PT-A (□) and ALG (■)

The medium (25 ml) contained 3.75 mg of ATP and 3.75 mg of DNA. For phosphate-adsorption, 100 mg of $Na_2HPO_4 \cdot 12H_2O$/dl was incubated with 1 g of pre-soaked PT-A or ALG. Other details are the same in Fig. 1. (a) inorganic phosphate, as PO_4^{3-}, (b) ATP, (c) DNA.

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as inorganic phosphate. ATP was chosen as a nucleotide and herring sperm DNA as a high-molecular-weight DNA. The adsorption profiles of these organic phosphate-containing compounds were compared to that of inorganic phosphate. Phosphate was adsorbed more efficiently by PT-A than by ALG at pH values of 3.5 and 7.2 (Fig. 2a). The amount of phosphate adsorbed by PT-A was 3- to 4-fold more than that by ALG. ATP was not adsorbed in large quantities by either adsorbent (Fig. 2b). However, the adsorption pattern of ATP by either PT-A or ALG was less significantly affected by pH change than that of phosphate. Of the three ligands, DNA was least well adsorbed by both adsorbents (Fig. 2c). At all pH values, the amount of DNA adsorbed by PT-A was lower than that adsorbed by ALG. DNA precipitated at lower pH than 2.0, so its adsorption was examined at pH's 2.5, 4.4 and 6.8. The amount of DNA adsorbed by ALG was 4-fold more at lower pH's and 10-fold more at higher pH's than that adsorbed by PT-A. The amounts of nucleotides adsorbed by either PT-A or ALG were significantly lower than the amounts of proteins.

Adsorption of Lipids Cholic acid is a component in bile, while cholesterol is amply present in foods. Less than a few percent of available cholic acid or cholesterol was adsorbed by PT-A and ALG at any pH tested. Although the amount was small, the adsorption of cholic acid on PT-A or ALG was pH-dependent; the amounts adsorbed by ALG were greater at lower pH, whereas the amounts adsorbed by PT-A increased at higher pH's (Fig. 3a). Cholesterol, which is structurally similar to cholic acid, showed different adsorption profiles from those of cholic acid; the amounts adsorbed by PT-A and ALG gradually increased with increasing pH and the amounts adsorbed by PT-A were greater than those by ALG at pH 4.2 and 6.8 (Fig. 3b). The adsorption of phosphatidic acid by PT-A or ALG was substantially greater than those of cholic acid and cholesterol. However the extents of adsorption by PT-A and by ALG were about the same (Fig. 3c), in contrast to the adsorption of steroid compounds by PT-A and ALG.

Adsorption of Carbohydrates D-Glucose and starch, an α(1-4)-linked polymer of D-glucose, were tested as a

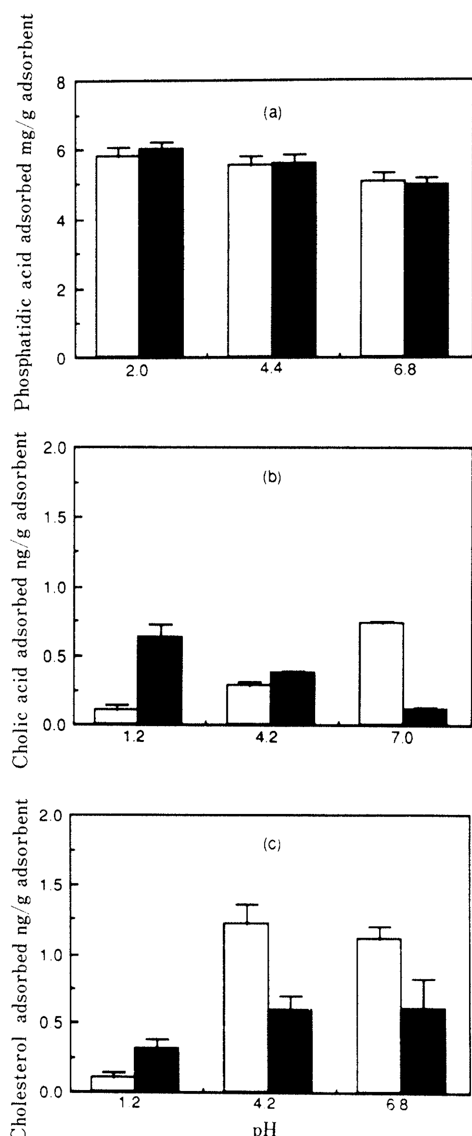

Fig. 3. Adsorption of Phosphatidic Acid and Steroid Compounds on PT-A (□) and ALG (■)

L-a-Phosphatidic acid (2 mg), radioactive cholic acid (4 ng) or radioactive cholesterol (4.75 ng) was added to 25 ml of the medium. Other details are the same as in Fig. 1. (a) phosphatidic acid, (b) cholic acid, (c) cholesterol.

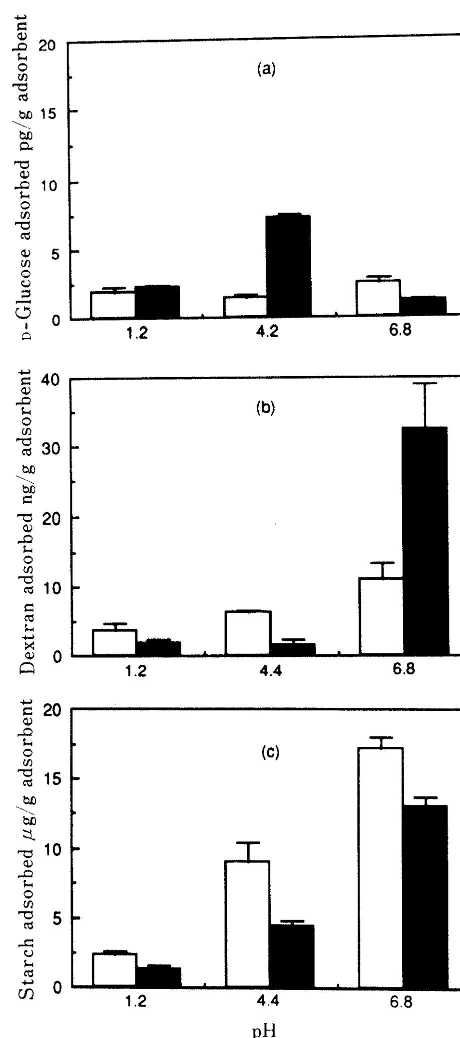

Fig. 4. Adsorption of Carbohydrates on PT-A (□) and ALG (■)

The initial concentrations were 1240 pg of D-glucose, 2000 ng of dextran and 100 μg of starch in 25 ml. Test compounds were all radiolabeled. Other details are the same as in Fig. 1. (a) D-glucose, (b) dextran, (c) starch.

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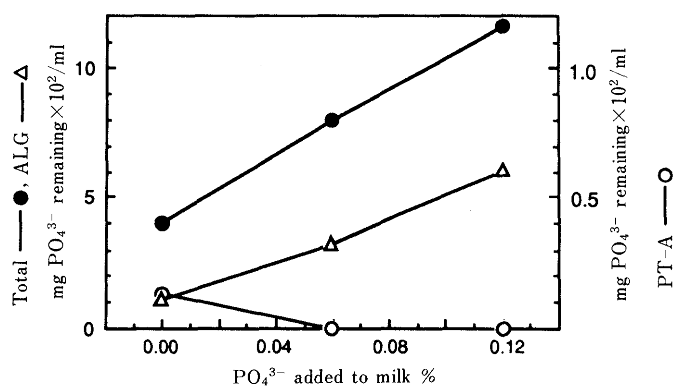

Fig. 5. Phosphate-Adsorption of PT-A and ALG in Milk

Each value represents the mean of three samples. The S.D. lies within the symbols. Neither addition of phosphate nor elimination of phosphate by adsorption on the adsorbents caused any detectable pH change in milk.

representative sugar and a highly polymerized carbohydrate, respectively. Dextran, a non-digestable α(1-6)-linked polymer of D-glucose, was also tested. D-Glucose was adsorbed by PT-A and ALG in picogram quantities (Fig. 4a), whereas dextran and starch were adsorbed by PT-A and ALG in nanogram and microgram quantities, respectively (Figs. 4b and 4c). None of them was adsorbed to an extent greater than 0.1% of the total amount added. Although the magnitude of adsorption was small, the adsorption of the two polymers on both PT-A and ALG was pH-dependent, increasing at higher pH.

Adsorption of Phosphate in the Presence of Milk If PT-A is administered right after eating a meal, the phosphate-adsorbing potency of PT-A could be affected by food components in the alimentary tract. The adsorption profiles of phosphate by PT-A and ALG were therefore examined in a synthetic milk. An equal amount of PT-A or ALG was added to the milk, which contained 4.01×10^{-2} mg/ml phosphate. Figure 5 shows that phosphate remaining in the medium was 1.07×10^{-2} mg/ml for ALG and 0.13×10^{-2} mg/ml for PT-A. When the total phosphate content was increased 2-fold by the addition of phosphate salt, ALG could not adsorb all the phosphate present and consequently phosphate remaining in the medium increased. However, even if the amount of phosphate added to the milk was increased 3-fold, PT-A could adsorb all of it and virtually no phosphate remained in the medium.

Discussion

ALG, an amorphous type of aluminum preparation, has been used clinically as a phosphate-scavenger.[20] When ALG is administered orally, aluminum ion is liberated in the acidic gastric solution and passes into the duodenum. Although ionic aluminum increases the potency of phosphate-adsorption at neutral pH,[21] it is absorbed readily into the circulation and precipitates in tissues, causing serious side-effects.[22] PT-A is also an aluminum preparation, but its crystalline structure is completely different from that of ALG. PT-A barely dissolves in acid, and has a higher adsorption capability for phosphate than ALG. Thus, PT-A is expected to be a potent and safe phosphate-adsorbent.

Early physico-chemical studies have elucidated the structures of PT-A and ALG.[16,23] The efficacy of phosphate adsorption of PT-A has been explained in terms of its porous structure with numerous micro-pores (maximal radius $r_{max} = 0.7$ nm, range of radius distribution < 1.0 nm). Such a structure has a very large surface area for phosphate adsorption. However, macro-molecules such as proteins can not enter the micro-pores. In PT-A the internal surface area accounts for 74% of the total surface area ($230\,m^2/g$). On the other hand, ALG has an amorphous structure with much larger pore sizes ($r_{max} = 2.5$ nm, range of radius distribution 2nm <) with a smaller total surface area ($40\,m^2/g$) than PT-A. These structural differences may account for the characteristic difference in binding various compounds between the two adsorbents.

In the present study, the adsorptions of several biological materials on PT-A or ALG were compared. The amounts of these materials adsorbed on the bases of weight per unit mass of adsorbent at the pH of maximal adsorption for PT-A were as follows: pepsin = BSA > trypsin > ATP = phosphatidic acid > DNA > starch > dextran > cholesterol > cholic acid > D-glucose. The order for ALG was pepsin = BSA > trypsin > ATP > DNA > phosphatidic acid > starch > dextran > cholesterol > D-glucose. The only difference between the two adsorbents was in the order of DNA and phosphatidic acid. However, a marked difference was seen in the amounts of various biological materials which were adsorbed per unit mass of the adsorbents.

Proteins are the most readily adsorbable materials on PT-A or ALG. As can be seen in Fig. 1, the adsorption of pepsin, BSA and trypsin on either PT-A or ALG was apparently pH-dependent, but the amount adsorbed on PT-A never exceeded that on ALG at any pH examined. Since proteins are unable to enter the small pores in PT-A, the differences observed are presumably due to the surface properties of PT-A and ALG (for example, surface area, surface charge and so on); zeta-potential measurement showed a negative charge on the surface of PT-A in the range of pH higher than neutral and a positive charge at pH lower than neutral.[16] This is considered due to dissociation–association of protons of hydroxyl groups of aluminum oxide hydroxide in PT-A. The surface of ALG is substantially dissolved at acidic pH's, resulting in an increase of its surface area, so that more proteins can be adsorbed on ALG than on PT-A under acidic conditions.

Nucleotides are probably the second most adsorbable materials to PT-A or ALG, though the adsorption profiles of ATP and DNA are different from that of inorganic phosphate (Fig. 2). The adsorption of ATP on PT-A was low and pH-independent. Thus, the adsorption of ATP on PT-A was not due to electrostatic force, but rather due to a physical association of the ligand with PT-A, *e.g.* by diffusion into its internal structure. DNA was adsorbed to a much lesser extent by PT-A than ALG. Since PT-A is supposed to be positively charged in acidic pH's, it should have adsorbed more DNA, which has negative charge, but the results of this study did not show this. This is probably due to the size of DNA, which can not enter the pores of PT-A. However, the amount of DNA adsorbed by PT-A is pH-dependent, with more DNA adsorbed at lower pH's. Some denaturation of DNA by the acidic environment might have occurred. Even though electrostatic repulsion is expected, DNA was adsorbed in larger amounts on the

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surface of ALG at lower pH's, indicating that partial denaturation of DNA may also have facilitated its adsorption on the ALG surface.

PT-A and ALG substantially adsorbed phosphatidic acid with similar profiles at all pH values tested. This indicates that adsorption of phosphatidic acid by the adsorbents does not require dissociation of aluminum ions. Since the amounts of phosphatidic acid adsorbed on both PT-A and ALG were pH-independent, the adsorption may be physical, rather than electrostatic. This adsorption profile of phosphatidic acid is very different from that of inorganic phosphate, so a phosphate moiety in highly hydrophobic compounds clearly behaves differently from inorganic phosphate.

Cholic acid and cholesterol were chosen as representative steroid compounds. The amounts of adsorbed materials were at most a few percent of the available amounts. When the pH is low (pH 1.2), ALG binds 10-fold more steroids than PT-A does, but at high pH's (pH 7.0) steroids bind more to PT-A than to ALG. Steroids may not have a strong affinity for the aluminum compound, suggesting that PT-A will not adsorb large amounts of bile acids after ingestion.

Carbohydrates are one of the major components in food and will come into contact with phosphate adsorbents in the gastro-intestinal tract when the adsorbents are administered at or after meals. Glucose, dextran and starch had no marked affinity for either PT-A or ALG at any pH tested. Presumably this is due to mutual repulsion by hydroxy groups of sugars and PT-A or ALG. Dextran is a non-digestible polysaccharide in humans. In contrast to glucose, dextran seemed to have been adsorbed in slightly higher amounts by PT-A than by ALG. This trend, however, was reversed at pH 6.8. Therefore pH-dependent interaction between dextran and each adsorbent has to be considered. Moreover, it was found that there was 1000-fold difference in the adsorbed amounts of dextran and starch (Figs. 4b and 4c). The reason for this is not known, but must involve configurational differences due to α (1—4) or α (1—6) linkages. On the assumption that phosphate-adsorbents would be taken during or after a meal, phosphate adsorption by PT-A or ALG was examined in powdered milk, which was chosen as a model of a routine meal because it contains numerous food components. The rate of adsorption of the phosphate is shown in Fig. 5. Essentially all the available phosphate was adsorbed by PT-A and 70% by ALG. These values are about the same as those observed for phosphates in an aqueous medium. Powdered milk was fortified by the addition of more phosphate, but still all the phosphate was adsorbed by PT-A. The phosphate-adsorption capacity of PT-A is not impaired by the presence of various dietary components. Therefore phosphate in the alimentary tract should be efficiently adsorbed by PT-A even in the presence of other major components in meals.

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