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Application of Synthetic Hydrated Aluminum Silicates as Orally Administered Absorbents of Ammonium Ion

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The adsorption of ammonium ion by three types of synthetic hydrated aluminum silicate (ZPC-10A, ZPC-50A and Molequlite 401) was studied to assess their possible utility as orally administered adsorbents for the treatment of uremia and ammoniemia. *In vitro* and preliminary *in vivo* studies suggest that ZPC-10A is promising as an adsorbent in terms of adsorption capacity, ion selectivity and resistance to acidity.

Keywords——zeolite; hydrated aluminum silicate; ammonium ion adsorption; ion exchange; uremia; ammoniemia; orally administered adsorbent

The removal of nitrogenous metabolites by means of various adsorbents has been attempted as an adjunctive therapy for kidney dysfunction.[1-3] The oral administration of adsorbents is desirable for uremic patients, since it would be much easier and cheaper than hemodialysis. Various kind of compounds have been reported as orally administered adsorbents, *i.e.*, oxystarch (dialdehyde starch),[4-6] encapsulated ethylene maleic anhydride mixed with urease, charcoal and ion-exchange resin,[7] tartaric acid microencapsulated with mineral "white" oil,[8] microencapsulated zirconium phosphate,[9,10] and natural saccharide.[11] Each of them, however, has problems regarding specificity or efficiency, and none has yet been used practically.

It is well known that urea in blood is excreted to the gut through capillaries, and metabolized to ammonium ion by microbiotics,[8,12,13] and that the removal of ammonium ion from the gut may reduce the blood concentration of urea. On the other hand, hydrated aluminum silicate (zeolite) is known to have a uniform structure with pores of constant size and is able to adsorb ammonium ion selectively through an ion-exchange mechanism[14,15] We focussed on the posibility of using zeolites as gastrointestinal adsorbents in the treatment of uremia or ammoniemia. This paper describes the physical characteristics of *in vitro* ammonium ion adsorption by three kinds of synthetic zeolites made for trial as orally administered absorbents, and discusses the feasibility of their application as adsorbents on the basis of the results of a preliminary *in vivo* study. Synthetic zeolites were chosen because their quality can be controlled more easily than that of natural zeolite. The synthetic zeolites studied consist of components similar to those of natural aluminum silicate and bentonite, which are commonly used medically. This suggests that these zeolites have no harmful effect on human beings.

Experimental

Materials——The zeolite samples used in this study were supplied by Shokubai Kasei Kogyo Co. (Tokyo). The compositions of these compounds are shown in Table I. Molequlite 401 (pore size: *ca.* 4.2 Å) is on the market and has been used as a chemical catalyst, while ZPC-10A (*ca.* 5.2 Å) and ZPC-50A (*ca.* 8.0 Å) were prepared for this study. The flameworks of ZPC-10A, ZPC-50A and Molequlite 401 can be considered to be Mordenite, Zeolite NaY and Zeolite NaA, respectively, all of which are known to be resistant to acidity.

Each sample was ground, washed with distilled water 5 or 6 times, collected on a membrane filter (Millipore HAWP 04700, 0.45 μm) and then dried at 110 °C for 48 h. After being ground again, they were dried at 110 °C for a further 3 h. The surface areas of ZPC-10A, ZOC-50A and Molequlite 401 were determined to be approximately 450 m^2/g, 500 m^2/g and 450 m^2/g, respectively, by the nitrogen adsorption method.

TABLE I. Compositions of the Zeolites Studied

Zeolite	Composition			Chemical formula	Adsorption capacity[a] (meq/g of zeolite)
	SiO_2	Al_2O_3	Na_2O		
ZPC-10A	10	1.0	1.0	$Na_8Al_8Si_{40}O_{96}\cdot xH_2O$	2.62
ZPC-50A	10	2.1	2.1	$Na_{58}Al_{58}Si_{139}O_{349}\cdot xH_2O$	4.43
Molequlite 401	10	5.0	5.0	$Na_{12}Al_{12}Si_{12}O_{48}\cdot xH_2O$	7.04

a) The theoretical amount of ammonium nitrogen adsorbed by zeolites, calculated from the aluminum (sodium) content in the molecule.

Other chemicals used were of reagent grade.

Assay——The amount of ammonium ion adsorbed by zeolite samples *in vitro* was determined by the semimicro-Kjeldahl method (JP X) with some modification; 20 ml of 5% boric acid and 0.3 N sulfuric acid solution were used as the adsorption solution and the titrant, respectively. Potassium, calcium and magnesium ions were determined by ion chromatography (Dionex 2020i; HPIC-CG1 precolumn, HPIC-CS1 separator column, CSC-1 suppressor column). An aqueous solution of 5 mM hydrochloric acid and a similar solution containing 2.5 mM *m*-phenylenediamine dihydrochloride were used as eluents for univalent cations and bivalent cations, respectively.

The concentration of ammonium ion in blood samples was determined spectrophotometrically with the ammonia-test Wako (Wako Pure Chemical Industry) after centrifugation.

***In Vitro* Adsorption**——One gram of zeolite sample was added to 50 ml of ammonium chloride solution (pH 1 to 9, ammonium ion concentration 10—1000 mM), and shaken in a thermostat at 37 °C. At appropriate intervals, zeolite samples were collected on membrane filters (Millipore HAWP 02500, 0.45 μm) and washed with 5 ml of water. Ammonium ion adsorbed by the zeolite was determined as described above.

The ion selectivity of zeolites was studied with sodium, pottasium, calcium and magnesium ions. The zeolite sample was shaken in 3 M NH_4Cl solution at 80 °C for 24 h then collected on a filter, washed with distilled water and dried with silica gel under reduce pressure (20 mmHg) to constant weight. A 1 g of zeolite sample saturated with ammonium ion was added to 50 ml of various concentrations of cation solutions at 37 °C for 3 h. Ammonium ion remaining on the zeolite was determined as described above. The selectivity coefficient *K* was calculated according to Eq. 1,

$$K_{M^{n+}}^{NH_4^+} = \frac{[NH_4^+]_S^n \cdot [M^{n+}]_R \cdot \gamma_{M^{n+}}}{([NH_4^+]_R \cdot \gamma_{NH_4^+})^n \cdot [M^{n+}]_S} \qquad (1)$$

where [M], *n* and γ represent the concentration, the valence and the activity coefficient of cations, respectively.[16,17] The subscripts S and R stand for the solid and liquid phase, respectively.

The effect of cations on adsorption of ammonium ion was studied by measuring the amount of ammonium ion adsorbed by the zeolites in the presence of various cations (Na^+, K^+, Mg^{2+} and Ca^{2+}). A 1 g portion of zeolite was added to 50 ml of solution containing 1 meq/50 ml of ammonium ion and various amounts of cations. Ammonium ion adsorbed by the zeolites was determined as described above.

***In Vivo* Adsorption**——An azotemic dog model was produced by ligating both of ureters of mongrel dogs (8—13 kg). A 10 g portion of zeolite suspended in distilled water was administered to each adotemic dog through a catheter connected to the duodenum. The blood was taken from a foreleg vein 15, 30, 60, 120 and 180 min after administration of the zeolite, and the concentration of ammonium ion was determined as described above.

Results and Discussion

In Vitro Adsorption

Three kinds of zeolites were studied to establish their possible utility as orally administered adsorbents. Table I shows the compositions of the samples studied and the theoretical amounts of ammonium nitrogen adsorbed by the samples, calculated from the sodium ion contents in the molecules on the assumption that sodium ion can be completely replaced by ammonium ion. As shown in Table II, the amounts of ammonium nitrogen adsorbed from 1 M NH_4Cl solution by the zeolite samples ranged from 2.2 to 4.7 meq per 1 g of samples, which amounted to 66 to 85% of the theoretical values. Table III shows the amount adsorbed from 20 mM NH_4Cl solution, a concentration of which is considered to be close to that of ammonium nitrogen in the gut. The amount of urea nitrogen adsorbed by oxystarch, which has been reported as a nitrogen adsorbent, is also shown in Table III. The amount of ammonium nitrogen adsorbed by each zeolite sample was larger than that of urea nitrogen adsorbed by oxystarch. ZPC-10A adsorbed four times more nitrogen than oxystarch. Figure 1 shows the dependence of the adsorbed amount on the nitrogen concentration. Saturation of the adsorbed amount was observed with increasing nitrogen concentration for ZPC-10A and ZPC-50A, so the amount adsorbed by Molequlite 401 was the largest at concentrations above 40 mM. At lower concentrations, however, the amount of nitrogen adsorbed by Molequlite 401 was comparable to those by ZPC-10A and ZPC-50A. There should be no significant difference among the three samples in terms of ability to adsorb nitrogen in the gut, because the plasma concentration of urea in patients with kidney dysfunction is known to range from 15 to 35 mM (as nitrogen), which is 5 to 20 times larger than in healthy persons, and the concentration of ammonium ion in the gut is unlikely to be over 40 mM. The adsorption of ammonium nitrogen by each zeolite sample from 10 to 1000 mM NH_4Cl solutions reached equilibrium within 60 min at 37 °C (data not shown), so each zeolite appears to have enough time to adsorb ammonium nitrogen while it passes through the gut.

The ion selectivity of zeolites was studied with several cations. Table IV shows the

TABLE II. Adsorption of Ammonium Nitrogen by Zeolites from 1 M NH_4Cl Solution

Zeolite	Adsorbed amount (meq of ammonium nitrogen/g of zeolite)	Ratio to the theoretical value (%)
ZPC-10A	2.22	85
ZPC-50A	2.94	66
Molequlite 401	4.70	67

TABLE III. Adsorption of Nitrogen by Zeolites and Oxystarch from 20 mM NH_4Cl Solution

Zeolite	Amount of nitrogen adsorbed[a] (meq/g of adsorbent)
ZPC-10A	0.91
ZPC-50A	0.85
Molequlite 401	0.68
Oxystarch[b]	0.24

a) Ammonium nitrogen and urea nitrogen were measured for zeolites and oxystarch, respectively. *b*) Albumin-pretreated oxystarch having a degree of oxidation of 49.2%.

TABLE IV. Selectivity Coefficients[a] of Zeolites

Concentration of competitive cation (meq/50 ml)	$K_{Na^+}^{NH_4^+}$			$K_{K^+}^{NH_4^+}$			$K_{Ca^{2+}}^{NH_4^+}$			$K_{Mg^{2+}}^{NH_4^+}$		
	ZPC-10A	ZPC-50A	Molequlite 401	ZPC-10A	ZPC-50A	Molequlite 401	ZPC-10A	ZPC-50A	Molequlite 401	ZPC-10A	ZPC-50A	Molequlite 401
1	3.584	2.770	2.268	0.864	0.897	1.138	1.099	0.091	2.008	7.463	0.647	5.587
5	5.435	2.688	1.129	0.809	0.663	0.929	4.444	0.143	0.093	14.925	0.635	4.202
10	5.882	2.571	1.153	0.861	0.612	0.922	5.525	0.184	0.117	19.231	0.997	5.682
20	6.329	2.433	1.206	0.786	0.545	0.710	10.526	0.101	0.116	22.747	0.893	7.576
30	6.667	2.375	1.250	0.806	0.523	0.711	14.925	0.086	0.115	22.727	0.697	9.709

a) Calculated according to Eq. 1.

TABLE V. Inhibition of Ammonium Nitrogen Adsorption on Zeolites by Cations[a]

Concentration of competitive cation (meq/50 ml)	Percent inhibited (%)											
	Na^+			K^+			Ca^{2+}			Mg^{2+}		
	ZPC-10A	ZPC-50A	Molequlite 401	ZPC-10A	ZPC-50A	Molequlite 401	ZPC-10A	ZPC-50A	Molequlite 401	ZPC-10A	ZPC-50A	Molequlite 401
1	7.69	19.57	12.33	21.78	19.51	17.65	18.89	23.53	10.29	9.89	18.83	0
5	26.37	45.65	41.10	50.46	64.63	57.35	30.00	64.71	44.12	15.38	47.06	0

a) Concentration of ammonium ion: 1 meq/50 ml.

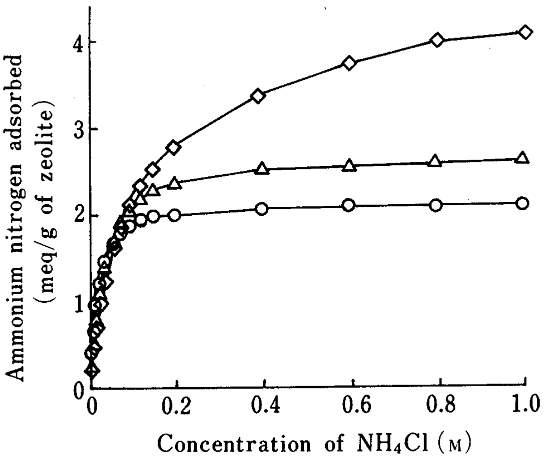

Fig. 1. The Adsorption of Ammonium Nitrogen by Zeolites from Various Concentrations of NH_4Cl

○, ZPC-10A; △, ZPC-50A; ◇, Molequlite 401. The amount of zeolite: 1.0 g.

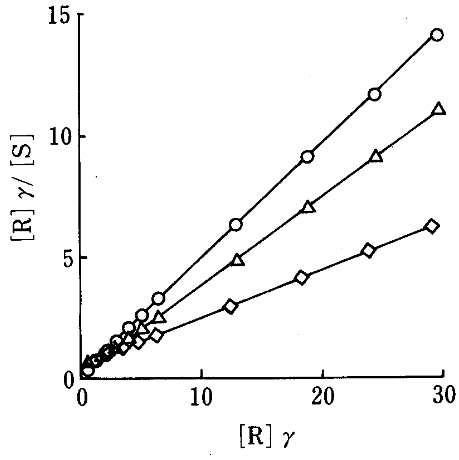

Fig. 2. Langmuir Plots of Ammonium Nitrogen Adsorption by Zeolites

○, ZPC-10A; △, ZPC-50A; ◇, Molequlite 401. The amount of zeolite: 1.0 g.

Table VI. Effect of pH on the Adsorption of Ammonium Nitrogen

pH	Amount of ammonium nitrogen adsorbed (meq)					
	Concentration of NH_4Cl (meq/50 ml)					
	1	5	1	5	1	5
	ZPC-10A		ZPC-50A		Molequlite 401	
1.00	0.80	1.69	0.60	1.13	0.66	1.84
4.26	0.81	1.68	0.69	1.91	0.68	1.95
5.90	0.80	1.67	0.70	1.87	0.62	1.85
7.53	0.80	1.68	0.70	1.89	0.60	1.87
8.75	0.79	1.67	0.69	1.89	0.57	1.86

Table VII. Adsorption of Ammonium Ion[a] and Desorption of Sodium Ion[b]

Concentration of NH_4Cl (meq/50 ml)	ZPC-10A		ZPC-50A		Molequlite 401	
	NH_4^+	Na^+	NH_4^+	Na^+	NH_4^+	Na^+
1.00	0.91	0.90	0.85	0.84	0.86	0.67
3.00	1.60	1.58	1.73	1.70	1.58	1.54
5.00	1.80	1.78	2.11	2.13	2.11	2.10
8.00	1.91	1.89	2.30	2.28	2.58	2.57
10.00	1.95	1.93	2.38	2.36	2.77	2.74

a) The amount of ammonium ion adsorbed when the zeolites were shaken with various concentrations of NH_4Cl (meq). *b*) The amount of sodium ion desorbed from the zeolites (meq/50 ml).

selectivity coefficients of ammonium ion against the cations. ZPC-10A showed high selectivity for ammonium ion among Na^+, Ca^{2+}, and Mg^{2+}. However, the selectivity coefficient of ammonium ion against K^+ was close to 1, which indicates that the amount of K^+ adsorbed by ZPC-10A was almost same as that of NH_4^+. This is relevant to all of the zeolites studied and may result from the fact that the size of hydrated K^+ is about 3 Å, which is close to that of hydrated NH_4^+. For Molequlite 401 and ZPC-50A, the selectivity coefficients of ammonium ion were less than 1 against Ca^{2+}, and against Ca^{2+} and Mg^{2+}, respectively. The ion selectivity of zeolites was further studied by using a different parameter from the conventional ion selectivity coefficients shown in Table IV. The amount of ammonium ion adsorbed by zeolites from a 20 mM NH_4Cl solution was determined in the absence or presence of various amounts of cations. Table V shows the inhibition of ammonium ion adsorption caused by coexisting cations. The ammonium ion adsorption by ZPC-10A was inhibited to the smallest extent by coexisting cations, and the amount of ammonium ion adsorbed by ZPC-10A was reduced only to 50% even by a 5 times larger amount of K^+.

Table VI shows the effect of pH on the adsorption of ammonium ion by zeolites. The amount of ammonium ion adsorbed by ZPC-10A or Molequlite 401 was constant in the pH range of 1 to 9, while that by ZPC-50A was decreased at pH 1. This may be due to the solubilization of adsorption sites on the surface in a strongly acidic solution. The decrease in the adsorbed amount at lower pH, however, was not observed in the case of Molequlite 401, which is soluble in acidic solution due to the high content of aluminum in the molecule. This phenomenon can not be explained at present. Table VII shows the amount of the sodium ion desorbed from zeolites by shaking with an NH_4Cl solution, determined by ion chromatography, in comparison with the amount of ammonium ion adsorbed by zeolites. The former was found to be close to the latter, and this suggests that ammonium ion is adsorbed by zeolites through an ion-exchange mechanism. As shown in Fig. 2, Langmuir plots of ammonium ion adsorption show good linearity for all the zeolites studied.

In Vivo Adsorption

Table VIII shows the ammonium ion concentration in the blood taken from dogs after administration of zeolites into the duodenum. The blood concentration was decreased significantly 15 and 60 min after administration of ZPC-10A, and of Molequlite 401 and ZPC-50A, respectively, and this suggests the effectiveness of zeolites as orally administered adsorbents. ZPC-10A caused the largest decrease in the blood concentration among the zeolites studied. The plasma concentration of sodium ion was found to be constant (data not shown), and the effect of sodium ion desorbed from zeolites on the total blood concentration may be regarded as negligible.

Conclusion

In vitro and preliminary *in vivo* studies suggest that ZPC-10A is most promising as an orally administered adsorbent of ammonium ion from the viewpoints of adsorption capacity, ion selectivity and resistance to acidity. ZPC-10A is a hydrated aluminum silicate compound, which is insoluble in the gut, so that it is unlikely that ZPC-10A is adsorbed by the

TABLE VIII. Decrease in Plasma Concentration of Ammonia after Administration of Adsorbents[a]

	Blank		ZPC-10A			ZPC-50A			Molequlite 401		
(min)	NH_3	S.D.	NH_3	S.D.	S	NH_3	S.D.	S	NH_3	S.D.	S
0	208.4	(8.6)	228.4	(20.8)		205.8	(38.8)		240.0	(22.9)	
15	209.0	(8.0)	185.6	(20.0)	S	180.6	(46.9)	NS	221.0	(33.3)	NS
30	210.2	(9.7)	160.2	(17.0)	S	164.6	(18.4)	NS	208.8	(31.0)	NS
60	210.4	(8.9)	141.6	(9.6)	S	154.4	(28.7)	S	184.6	(30.4)	S
120	212.0	(8.7)	121.6	(13.9)	S	144.4	(20.3)	S	166.0	(30.6)	S
180	216.2	(7.0)	104.2	(11.8)	S	129.0	(25.9)	S	156.6	(29.7)	S

a) The amount of adsorbents administered: 10 g. *K*: in plasma concentration of ammonia after administration of adsorbent (μg/100 ml). S.D.: standard deviation. S: significant ($p \leq 0.05$). NS: not significant.

gastrointestinal membrane. Furthermore, since ZPC-10A adsorbs ammonium ion through an ion-exchange mechanism, nutriment may not be removed from the gut by ZPC-10A.

On the basis that ZPC-10A shows an adsorption capacity of 2 meq/g for ammonium ion, the administration dose is theoretically estimated to be 400 g to adsorb 7 g of ammonium ion, which is the amount that should be removed from the blood of uremic patients per day. Therefore, ZPC-10A may be useful just as an adjunctive therapy. On the other hand, only 12 g of ZPC-10A is theoretically required to lower the blood ammonium ion level of ammoniemia patients (0.06 meq/100 ml). Thus, ZPC-10A may be available as a new type of formulation in the treatment of ammoniemia, being potentially more effective than conventional formulations such as arginine hydrochloride, sodium glutamate, neomycin and lactulose.

References

1) H. Yatzidis, *Nephron*, **1**, 310 (1964).
2) T. Manis, S. Zeig, E. I. Feinstein, G. Lum, and E. A. Friedman, *Trans. Am. Soc. Artif. Intern. Organs*, **25**, 19 (1979).
3) T. T. Blaney, *Trans. Am. Soc. Artif. Intern. Organs*, **12**, 7 (1966).
4) C. Giordano, R. Esposito, and M. Pluvio, *Proc. Eur. Dial. Transplant Assoc.*, **10**, 136 (1973).
5) T. Shimizu, Y. Yamazaki, M. Ueda, I. Honda, Y. Tanaka, and K. Tsuda, *Kobunshi Ronbunshu*, **39**, 629 (1982).
6) T. Shimizu, K. Shimada, Y. Tanaka, K. Sasaki, K. Tsuda, T. Akizawa, and S. Koshikawa, *Kobunshi Ronbunshu*, **39**, 637 (1982).
7) D. L. Gardner, R. D. Falb, B. C. Kim, and D. C. Emmerling, *Trans. Am. Soc. Artif. Intern. Organs*, **17**, 239 (1971).
8) W. J. Asher, T. C. Vogler, K. C. Bovee, P. G. Holtzapple, and R. W. Hamilton, *Trans. Am. Soc. Artif. Intern. Organs*, **22**, 605 (1976).
9) D. L. Gardner, T. S. Emmerling, K. D. Williamson, W. C. Baytos, and C. R. Hassler, *Kidney Int.*, **7**, S-393 (1975).
10) C. Kjellstrand, H. Borges, C. Pru, D. Gardner, and D. Fink, *Trans. Am. Soc. Artif. Intern. Organs*, **27**, 24 (1981).
11) H. Yatzidis and D. Koutsisos, *Dial. Transplant.*, **9**, 313 (1980).
12) D. O. Castell, *Gastroenterology*, **60**, 33 (1971).
13) A. Vince, *Gut*, **14**, 171 (1973).
14) N. Hara and H. Takahashi, "Zeolite," Kodansha, Tokyo, 1980, p. 61.
15) M. A. Weber, K. A. Barbarick, and D. G. Westfall, *J. Environ. Qual.*, **12**, 549 (1983).
16) I. Goto and M. Ninaki, *Nogakushuho*, **25**, 164 (1980).
17) H. Hisayama, "Kireitojushi, Ionkoukanjushi," Kodansha, Tokyo, 1976, p. 158.