

ABSORPTION OF PYRIMIDINES, PURINES AND NUCLEOSIDES BY
Li-, Na-, Mg-, AND Ca-MONTMORILLONITE (CLAY-ORGANIC STUDIES XII).

by

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ABSTRACT

The absorptions of the biologically important purines adenine and hypoxanthine, the pyrimidines cytosine, thymine, and uracil, and the nucleosides adenosine, guanosine, inosine, cytidine, thymidine, and uridine by Li-, Na-, Mg-, and Ca-montmorillonite have been studied in aqueous solutions over a range of pH values 2-12. The initial organic concentrations were between 0.8 and 1.4 m.molar. The ratio clay to organic compounds was such that only up to 30% of the exchange capacity could be saturated by organic cations, but, depending on conditions, up to 100% of the available organic material was absorbed.

The results show that absorption occurs primarily as a cation exchange reaction. Thymine, uracil, and their nucleosides are not absorbed under the experimental conditions. The absorption of the other compounds at various pH's depends on their basicity, their aromatic or non-aromatic character and the possible extent of the van der Waals interaction with the silicate layers. This is illustrated by comparison of the absorption behavior of pyridine, 5-amino-6-methyl-uracil, and caffeine with that of the above named compounds. Nucleosides are generally less strongly absorbed than their purines or pyrimidines since their non-planar structure allows less van der Waals interaction; their absorption is strongly influenced by the differences in swelling behavior of montmorillonite with mono- and divalent cations.

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- INTRODUCTION

In this and the following paper, results are reported for the absorption of a number of biochemically important molecules from aqueous solutions by montmorillonite saturated with various cations. The work was undertaken to test and possibly to amplify earlier suggestions (Bernal, 1951) that organic absorption on clay minerals may have played an important part in the initial development of living forms by synthesizing and concentrating the necessary organic molecules. Under the stimulus of ultraviolet light, simple molecules such as H_2O , CO_2 , NH_3 , . . . present in the primitive earth, could have given rise to simple organic molecules. As Bernal (1951, p. 33) stated: "Condensations and dehydrogenations are bound to lead to increasingly unsaturated substances and ultimately to simple and possibly even to condensed ring structures, almost certainly containing nitrogen, such as the pyrimidines and purines." A further important consideration is that crystalline materials, especially clay minerals, might have been important in concentrating the simple organic molecules in an ordered manner and bringing them into a state where further polymerisation and synthesis would be possible. Bernal's ideas have been discussed by other authors (Oparin, 1957; Hofmann, 1961; Cairns-Smith, 1966) but the only experimental work known to the present authors has been by G. Steinman (1966 and private communication) who obtained very promising results on the polymerisation of amino acids in the presence of clay minerals.

EXPERIMENTAL

The absorption isotherms of various pyrimidines, purines, and nucleosides have been determined in relation to pH and the exchangeable cations Li^+ , Na^+ , Mg^{2+} , and Ca^{2+} , and, in the following paper, Fe^{3+} , Co^{2+} , Ni^{2+} , and Cu^{2+} .

Preparation of the various montmorillonites

A refined white bentonite from Texas, supplied by the Georgia Kaolin Co., was fractionated to give $<1\mu$ particle size material. Li- and Na- saturated forms were prepared by treating the clay two times for 24 hrs. with 2N solutions of the chlorides and 0.05 molar solution of tartaric acid with approximately 30 g clay per liter. The tartaric acid was added to keep exchangeable Al^{3+} in solution. After the second treatment, the clay was washed Cl-free by centrifugation and then by dialysis. The Na- or Li-montmorillonite was then treated three times for 24 hrs. with 1N chloride solutions of the above named cations. All clays except Fe-montmorillonite were then dialyzed at 60°C since at elevated temperatures the dialysis goes much more rapidly. After they were Cl-free the water was changed five times more. Fe-montmorillonite was washed by centrifuging at room temperature.

To determine the concentration of the montmorillonites in suspension an aliquot of each suspension was evaporated and the clay heated to 150°C in vacuum over liquid N_2 for 24 hrs.

The exchangeable cations in the various clays were determined after exchange with 0.7N BaCl_2 solution (for the Fe-exchange, tartaric acid was added) by means of emission spectroscopy and

flamephotometry:

Li- and Na-montmorillonite: 95 meq/100 g Li^+ or Na^+ , 1 meq/100 g
 $\text{Mg}^{2+} + \text{Ca}^{2+}$.

Mg-, Ca-, Co-, Ni-, and Cu-montmorillonite: \sim 100 meq/100 g of the
 main cation

< 5 meq/100 g of other
 cations.

Fe-montmorillonite: 107 meq/100 g Fe^{3+} , no other cations.

The supernatant of the Fe-montmorillonite suspension had pH 4.8.

Table 1

The organic compounds studied are listed in Table 1 with their source of supply and other data.

Absorption experiments

To 5 ml of 6.5 millimolar aqueous solutions of the organic compounds were added various amounts of HCl or NaOH and H_2O to give 20 ml, and finally 5 ml of a clay suspension (27 g/liter). The initial concentration of the organic compounds was therefore usually 1.3 millimolar (for guanosine only 0.8 and for hypoxanthine 1.0 millimolar because of their small solubilities). The ratio of organic compounds to montmorillonite was such that only approximately 25% of the exchange capacity could be saturated by organic cations if all organic molecules available in solution were absorbed. Under these conditions, equilibrium was achieved within 10 hours whereas with twice the amount of organic even after two weeks of reaction further absorption was observed.

After approximately 15 hrs. at room temperature in sealed bottles, the suspensions were centrifuged for 45 min. at 14000 rpm. with a Sorvall SS-1 highspeed angle centrifuge. Aliquots of the supernatant solutions were diluted with buffer solutions and the concentration of the remaining organic molecules determined with a Beckman DU ultraviolet spectrophotometer. The pHs of the supernatant solutions were determined with a Beckman Expandomatic pH-meter.

No corrections were made for the clay volume and the so-called negative absorption for reasons which will be discussed with the results of the X-ray investigations.

X-ray investigations

The nature of the expanded state of the montmorillonite under the actual conditions of the absorption experiments has been examined by X-ray diffraction with the clay in equilibrium with the organic solution. The clay suspensions were brought into glass capillaries (0.5 mm diameter, 0.01 mm wall thickness; supplied by Unimex-Caine Corp., Chicago), the capillaries sealed with Picein wax and X-rayed in a Norelco powder camera, dia. 11.46 cm. The primary beam trap of this camera was modified to allow the observation of reflections of basal spacings up to 40 \AA . Some dried oriented samples were examined with the X-ray diffractometer to obtain more exact basal spacings.

RESULTS

Adsorption isotherms

The absorption experiments were made with relatively small proportions of organic compounds in order to achieve equilibrium

quickly but also to have conditions approximating more closely to those which might have existed on a primitive earth. An important consequence of these working conditions is that only 25% of the exchange capacity was saturated with organic cations when all the available molecules were absorbed in the cationic form. Therefore, the results are expressed in the form of percentage organic material absorbed against the equilibrium pH. A value of 100% means that no measurable organic material remained in solution.

The accuracy in the measurement of concentration is better than $\pm 1\%$ with Mg- and Ca-montmorillonite, and in the acidic range with Li- and Na-montmorillonite also. In the neutral and alkaline pH ranges, the Li- and Na-montmorillonite are not completely removed from the solutions even after long periods of high-speed centrifugation and the recorded values of "% absorbed" are possibly a little too small. The accuracy of the pH measurements was about ± 0.05 below pH5, and above pH5 about ± 0.1 .

Figures 1-3 show the absorption isotherms except those for uracil, thymine, thymidine, and uridine which under the given conditions were not absorbed in the range from pH 1.7 to pH 11.

Figure 1 a) b)

Figure 1 c) d)

It is requested that 1 a) b) and 1 c) d) be placed on facing pages.

Figure 2 a) b)

Figure 2 c) d)

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Figure 3

Similar results for some of the investigated compounds have been reported by Durand (1964), but results reported by Shaw (1965) differ particularly with regard to the pH range in which absorption takes place; the differences are probably due to the fact that 15 minutes of centrifugation with 1000 g are insufficient to remove the clay completely from the solution. In the present experiments, Li- and Na-montmorillonite were not completely removed even after 45 minutes of centrifugation with 2400 g.

In Figure 4 are compared the milliequivalents (meq) of measurable free Na^+ and Ca^{2+} ions with the meq of absorbed adenine cations. The differences between absorbed adenine and free Na^+ and Ca^{2+} , respectively, must be attributed to protons and possibly also to Al^{3+} ions extracted from the silicate lattice by proton attack. To check the extent of this attack, Na-montmorillonite (6 g/l) was shaken during 24 hrs. with HCl and then treated for cation exchange with a 0.6N BaCl_2 and 0.05 molar tartaric acid solution. The increase of exchangeable Al^{3+} due to the HCl treatment was at pH 4, 0.3 meq/100 g, at pH 3, 2.0 meq/100 g, and at pH 2, 7.3 meq/100 g.

Figure 4 a) b) ~~the~~ ~~the~~

These ^{two} ~~two~~ parts should be placed on one page.

X-ray data

Adenine, guanine, and cytosine complexes: The basal spacings of complexes formed with these molecules as cations are $12.5 \pm 0.2 \text{ \AA}$. The same values were reported by Hendricks (1941). More interesting

results were obtained by X-raying the equilibrated suspensions of Na- and Ca-montmorillonite after absorption of some of the organic compounds.

Na-montmorillonite itself equilibrated with various amounts of HCl (equilibrium pHs 2 to 8) gave no basal reflection or, at higher concentrations, a diffuse reflection $> 40 \text{ \AA}$. After absorption of 0.2 molecules adenine per unit cell ($= 27 \text{ meq/100 g}$) at pH 2 a medium strong 12.5 \AA reflection appeared. The same reflection, but stronger, appeared at pH 4 to pH 7. In the range of pH 3 to pH 4, no well-defined basal reflection was observed, but a weak diffuse darkening of the film appeared from 12.5 to 40 \AA and from 40 \AA on to higher spacings the intensity increased rapidly. A similar behavior of the Na-montmorillonite occurred after absorption of cytosine or pyridine. With an equal number of absorbed molecules per unit cell, the intensity of the 12.5 \AA reflection decreased in the order adenine $>$ cytosine $>$ pyridine. Figure 5 gives the general features of the Na-montmorillonite in relation to the measurable free Na^+ ions and the absorbed adenine cations. A similar behavior occurs with Li-montmorillonite.

Figure 5

Ca-montmorillonite without absorbed organic molecules has in suspension in the range from pH 2 to pH 7 a basal spacing between 18 and 19.5 \AA . At pH 2, there appeared after absorption of 0.19 molecules adenine per unit cell a medium strong 12.5 \AA reflection in addition to the strong 19 \AA reflection. With increasing amounts of absorbed adenine, the intensity of the 12.5 \AA reflection increased

in the same manner as the 19 \AA reflection decreased, until the latter disappeared. At this point about 0.4 molecules adenine were absorbed per unit cell (= 54 meq/100 g). The same amount of absorbed pyridine gave only a relatively weak 12.5 \AA reflection. The higher the pH and at the same time the more Ca^{2+} ions remained in the montmorillonite, the weaker became the 12.5 \AA reflection.

Because the clay suspension was dropped into the adenine solution the formation of the two phases could have been due to the decrease of the adenine concentration during the addition of the clay. Therefore a sample was prepared at pH 2 by dropping adenine solution and Ca-montmorillonite suspension with equal rates into stirred water. The same result was obtained.

5-Amino-6-methyl-uracil (AMU): This molecule is comparable in size with adenine. At pH 2 it formed a complex with Ca-montmorillonite, with 0.25 molecules of AMU per unit cell and with a single reflection corresponding to a 19 \AA basal spacing, but with 0.36 molecules per unit cell, a diffuse reflection was obtained extending from 12.5 to 19 \AA . A basal spacing of $12.8 \pm 0.2 \text{ \AA}$ was measured with the X-ray diffractometer on a dried sample which was saturated with AMU at pH 2.

Caffeine complexes: After absorption of caffeine, two different spacings were measured by X-ray diffractometry on samples dried over P_2O_5 . In the acidic pH range, Ca-montmorillonite saturated with caffeine gave a basal spacing of $12.75 \pm 0.1 \text{ \AA}$. The same spacing was observed on neutral samples with less than 0.24 molecules caffeine per unit cell (= 33 meq/100 g). Higher amounts gave unsymmetric diffraction peaks; saturated samples gave a very good pattern of a homogeneous phase with $15.86 \pm 0.1 \text{ \AA}$ basal spacing.

Suspensions of Na-montmorillonite with 0.16 molecules caffeine per unit cell at pH 2.1, and with 0.09 molecules per unit cell at pH 5.7, gave a broad reflection of about 12.8 \AA . At pH 9.7 and with 0.04 molecules caffeine per unit cell a very weak 16 \AA reflection was obtained. The same reflection, but medium-strong, was observed with 0.1 molecules per unit cell.

Suspensions of Ca-montmorillonite with 0.18 molecules caffeine per unit cell gave a strong reflection of 15.8 to 18.8 \AA and weak (003)-reflections corresponding to 15.8 \AA and 19.2 \AA at pH 6.8 and pH 9.5. With 0.23 molecules per unit cell, a single strong 16.0 \AA reflection was observed at pH 6.8.

Adenosine complexes: With 0.16 molecules per unit cell absorbed by Na-montmorillonite in suspension at pH 2 and at pH 4, a very weak reflection of about 16.5 \AA was observed. The same amount of adenosine absorbed by Ca-montmorillonite at pH 2 gave an 18.4 \AA reflection, whereas with 0.28 molecules per unit cell only a 16.2 \AA reflection was observed.

In agreement with Hendricks (1941), a $13.35 \pm 0.1 \text{ \AA}$ basal spacing was observed on oriented samples (dried over P_2O_5) as long as less than 0.33 molecules adenosine per unit cell (= 45meq/100 g) were absorbed. With larger amounts of absorbed adenosine the spacing increased to $16.3 \pm 0.3 \text{ \AA}$ obtained with 0.64 molecules adenosine per unit cell (= 87.5 meq/100 g).

DISCUSSION

Reaction mechanisms

A considerable variety of mechanisms are involved in the formation of complexes by the organic materials studied. For

convenience, the equations describing these mechanisms are tabulated together at the end of this section.

The compounds investigated have a more-or-less basic character and therefore are able to form cations according to equation [2] (see p.). A measure of the basicity is, by definition,

$$pK_a = pH - \log [\text{base}]/[\text{acid}] \dots \dots \dots [1]$$

The most important absorption mechanism by non-transition metal montmorillonite is the cation exchange reaction, equation [3], when absorption takes place from dilute aqueous solutions with insufficient organic material to saturate the montmorillonite.

The amount of exchanged inorganic cations under these conditions is always larger than the amount of absorbed organic cations, as illustrated in Fig. 4. The difference between absorbed organic cations and free metal ions must be compensated by protons (equation [4]) and by Al^{3+} ions extracted from the crystal structure by proton attack.

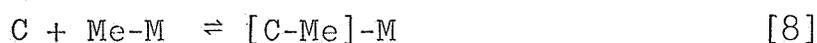
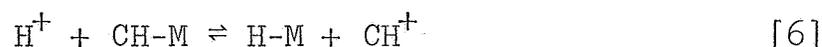
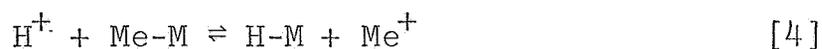
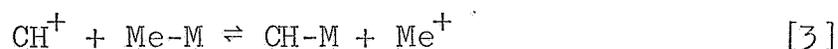
From equation [1], it is seen that only 1/100 of the organic molecules are in the cationic form when the pH of the solution is 2 units larger than the pK_a of the organic compound. But strong absorption takes place to much higher pHs (Fig. 1 and Fig. 2). This is possibly due to the proton concentration in the interlayer space of Mg- and Ca-montmorillonite and close to the dispersed layers of Li- and Na-montmorillonite being much higher than the measured proton concentration of the supernatant solution after centrifugation. The interlayer proton concentrations in Ca-montmorillonite were calculated, using the data from Fig. 4, to be 1.5N at pH 2, and 0.3N at pH 7. The thickness of the water layer between the silicate sheets was assumed to be 10 Å. It

seems reasonable, therefore, to assume that the absorption of neutral organic molecules by H-montmorillonite is important at pHs higher than the pK_a s of the compounds. Protonation would then take place in the interlayer space (equation [5]).

On the other hand the relatively high concentrations of H^+ ions below pH 3 compete with the organic cations for the exchange sites on the clay, (equation [6]).

Physical absorption due to van der Waals attraction between the organic molecules and the silicate layers was observed only with the large molecules caffeine, 7- and 9-methyladenine in the alkaline pH range, (equation [7]).

The absorption of caffeine in the case of Na-montmorillonite is probably due to van der Waals attraction alone. With Li-, Mg-, and Ca-montmorillonite, it certainly contributes to complex formation with the interlayer cations (equation [8]). Complex formation of caffeine with these cations causes an increase of the solubility of caffeine whereas Na^+ ions decrease the solubility (Gusyakov ~~and~~ and Brazhnikova, 1961). A similar absorption due to complex formation occurs with hypoxanthine. Because of the formation of anions ($pK_a = 8.94$) the absorption isotherms drop down between pH 8 and pH 9.



where: C = organic compound; CH^+ = organic cation; Me-M represents Li-, Na-, Mg-, and Ca-montmorillonite respectively; Me^+ represents Li^+ , Na^+ , $1/2 \text{Mg}^{2+}$, and $1/2 \text{Ca}^{2+}$ respectively; CH-M = montmorillonite with absorbed organic cations; [Me-M-C] represents montmorillonite with physically absorbed organic molecules; [C-Me]-M represents montmorillonite with absorbed complexes.

At each pH level, probably more than one of the reactions expressed by equations [3] - [8] are involved. Most of these equations have been discussed by Weber (1966) who observed similar absorption characteristics with s-triazine compounds.

Influence of the exchangeable cations

The main difference between montmorillonite containing the monovalent cations Li^+ and Na^+ , and the divalent cations Mg^{2+} and Ca^{2+} , is that the monovalent are more easily exchanged by the organic cations and by protons. This influences the equilibrium constants of the reactions expressed by equations [3] and [4]. The absorption of Li- and Na-montmorillonite therefore takes place at higher pHs than with Mg- and Ca-montmorillonite and the equilibrium concentrations of the organic compounds also are smaller (Figs. 1-3)

A comparison of the measured exchanged Na^+ and Ca^{2+} ions after absorption of adenine (Fig. 4) suggests rather that Ca^{2+} is easier to exchange than Na^+ . This discrepancy with the general experience is probably due to the different swelling behavior of montmorillonite with mono- and divalent interlayer cations. Hydrated Ca-montmorillonite has a basal spacing of about 19 Å whereas Na-montmorillonite may expand to more than 100 Å (Norrish, 1963). In dispersed Na-montmorillonite, therefore, the "external" surface with its diffuse electric double layer (Olphen, 1963) is many times larger than in Ca-montmorillonite. The difference between the Na^+

concentration in the diffuse double layer and the measurable Na^+ concentration of the solution depends on the pH since Na^+ and H^+ in the diffuse double layer are in equilibrium with each other. At higher pHs, a face-to-face flocculation takes place during the long high speed centrifuge runs and the "external" surface area with its diffuse double layer is diminished.

In the case of the nucleosides, the difference in swelling behavior of the montmorillonite with monovalent and divalent cations influences the amount absorbed significantly (Figure 3). A similar stronger absorption of sugars by montmorillonite with monovalent cations was shown by Greenland (1956).

Effects of the molecular structure

The effects of the various molecular structural properties on the absorption cannot be discussed separately. Basicity, size and steric structure of the molecules, aromatic character and the possibility of resonance structures of the organic cations may all influence the absorption at the same time. Fig. 6 illustrates schematically the wide range of properties of the investigated compounds.

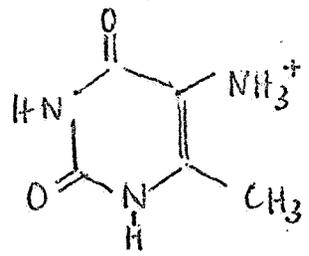
Figure 6

In the following an attempt will be made to show for groups of these compounds the influence of the various properties.

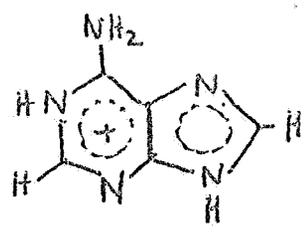
According to equations [2] and [3] the sharp decrease of the absorption isotherms should appear at higher pHs the more basic the compounds, if the other features of the molecules are comparable. This is the case in the sequences 6-chloropurine-purine-adenine (Fig. 1) and inosine-guanosine-adenosine (Fig. 3).

The importance of the aromatic character and of the possible formation of several resonance structures of the cations is

illustrated in Fig. 2d) by the absorption isotherms of the compounds hypoxanthine - 5-amino-6-methyl-uracil (AMU) - adenine, which have comparable molecule sizes. AMU, both as a neutral molecule and as a cation in the protonated form, is non-aromatic whereas hypoxanthine and adenine in both states are aromatic.



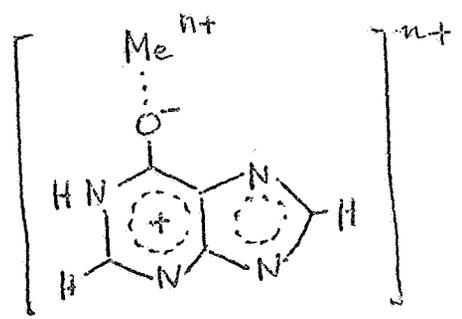
5-amino-6-methyluracil cation



adenine cation
(Cochran, 1951; Kleinwaechter et al., 1967)

In parallel with the stronger absorption of the aromatic cations goes the formation of the organophilic 12.5 Å phase which appears with small amounts of absorbed adenine but only imperfectly with large amounts of absorbed AMU.

Whether the possible interaction between the π -electrons of the aromatic cations with the oxygen layer of the silicate lattice, as discussed by Haxaire (1956), is more important or the distribution of the positive charge over a large area, cannot be decided from the reported results. However, the increased absorption of hypoxanthine in the range of pH 7-9 (equation [8]) could result from the formation of a complex of the form



(Me^{n+} = metal cation)

which would provide a distribution of the positive charge over a larger area.

It is now predictable that the non-aromatic molecules uracil and thymine will not be absorbed at all since they have a very small basicity, ($pK_a \sim 0.5$ and $pK_a \sim 0$, respectively).

A most important contribution to the absorption process is provided by the attractions due to van der Waals-London forces. The strength of these forces is related ^{mainly} to molecular size and steric structure or, in other words, to the area of close contact between the organic molecule and the oxygen plane of the silicate layers. These forces have been computed by Pullman et al. (1965) for the neutral molecules of caffeine and some of the purines and pyrimidines. The outstandingly strong absorption of caffeine (Fig. 1) is in agreement with their results. Caffeine has a very low pK_a , non-aromatic character and therefore a non-planar molecular structure. Due to the latter, a basal spacing of $12.75 \pm 0.1 \text{ \AA}$ has been observed under conditions where a single sheet of molecules was absorbed. (It is of interest that Hendricks (1941) found a basal spacing of 12.8 \AA after absorption of the non-aromatic compound 3-methylcytosine. Montmorillonite saturated with 5-amino-6-methyl-uracil cations has also a basal spacing of 12.8 \AA .)

Strong attraction certainly occurs between the caffeine molecules themselves since after absorption of neutral caffeine molecules a phase with $15.86 \pm 0.1 \text{ \AA}$ basal spacing is observed which may represent a double sheet of absorbed molecules, or molecules standing ~~parallel~~ ^{inclined} to (001). In aqueous suspension, this spacing occurs even with small amounts of absorbed caffeine. During drying the caffeine molecules probably slide under the pressure of the silicate layers and form a compact single sheet. It is interesting that after absorption by Na-montmorillonite under acidic conditions only a single sheet complex is observed which indicates that most of the caffeine molecules

become protonated in the-interlayer space according to equation [5].

The absorption isotherms in Fig. 2c) d) show that van der Waals attraction has a much larger influence on the absorption than the basicity of the molecules.

From these considerations it is to be expected that the nucleosides will be absorbed to a much lesser extent than the planar purines and pyrimidines. This is confirmed by the experiments (Fig. 3). In the expanded montmorillonite, the heterocyclic ring system probably is attached to only one silicate layer and only a few atoms of the sugar ring may come into close contact with the opposite silicate layer. The van der Waals energy due to this interaction is probably only sufficient to compensate the hydration energy when large amounts of the nucleosides are absorbed. Then the basal spacing diminishes to about 16.5 Å.

The basal spacing of 13.35 Å which was observed with small amounts of absorbed adenosine after drying over P_2O_5 is energetically not favorable since a considerable stress must be put on the glycosidic bond to bring the molecule into such a flat configuration.

The difference between the absorption isotherms of cytidine and adenosine (Fig. 3c) d)) is much more significant than in the case of cytosine and adenine (Fig. 2c) d)). This may be due to the fact that only the non-aromatic form of the cytosine ring system exists in cytidine.

SUMMARY AND CONCLUSIONS

These studies have shown that absorption of the nucleic acid-related purines, pyrimidines and nucleosides by montmorillonite

from dilute aqueous solutions is possible in the acidic pH range. Under the experimental conditions, thymine, uracil, and their nucleosides are not absorbed. The absorption of the other compounds is due mainly to cation exchange. Van der Waals attraction and the aromatic character of the molecules influence the extent to which these compounds are absorbed.

The interlayer cations influence the absorption insofar as the monovalent are more easily exchanged than the divalent, and as only montmorillonite with monovalent interlayer cations disperses completely in the dilute suspensions applied.

Due to the van der Waals attraction between the organic cations and the silicate layers under various conditions two phases are formed during the absorption. Since the phase with the absorbed purines or pyrimidines has a basal spacing of only 12.5 Å, experiments to synthesize nucleosides from ribose and these absorbed species will be limited to those conditions where the montmorillonite remains expanded after absorption took place.

However, it has been shown that living forms persist under the experimental conditions (17°C to 35°C) since all three authors are still alive!

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Table 1. - Organic compounds used

Compound ⁽¹⁾	M.W.	pK _a ⁽²⁾	origin ⁽³⁾ and quality
purine	120.12	2.39; 8.93	CCC, grade 1
adenine	135.13	4.22; 9.8	NBCo; CBC, A grade
hypoxanthine	136.12	1.98; 8.94	CBC, A grade
6-chloropurine	154.56	~0.8; ⁽⁴⁾ 7.75 ⁽⁵⁾	CCC, grade 1
7-methyladenine	149.16	4.17 ⁽⁵⁾	CCC, grade 1
9-methyladenine	149.16	4.00 ⁽⁵⁾	CCC, grade 1
caffeine	194.2	0.61 ⁽⁶⁾	CBC, U.S.P. C grade
cytosine	111.10	4.60; 12.16	NBCo
thymine	126.11	~0 ; 9.82	NBCo
uracil	112.09	~0.5 ; 9.45	NBCo
5-amino-6-methyl-uracil	141.13	3.28 ⁽⁵⁾	Fluka, puriss
pyridine	79.10	5.23 ⁽⁶⁾	Fisher, reagent grade
adenosine	267.24	3.45	NBCo
guanosine	283.26	1.6 ; 9.16	NBCo
inosine	268.24	1.2 ; 8.75	CBC, A grade
cytidine	243.23	4.22	NBCo
uridine	244.20	9.17	NBCo
thymidine	242.23	9.8	NBCo

(1) The formulas of these compounds are shown in the appendix.

(2) pK_a-values, except those marked with footnotes, are from the table, "Properties of the nucleic acid derivatives," presented by Calbiochem.

(3) CBC = Calbiochem, Los Angeles, Calif.
 CCC = Cyclo Chemical Corp., Los Angeles, Calif.
 Fisher = Fisher Scientific Co.
 Fluka = Fluka, supplied through ICN, City of Industry, Calif.
 NBCo = Nutritional Biochemical Corp., Cleveland, Ohio

(4) Determined by spectrophotometric titration according to Rosenblatt (1954).

(5) Determined by titration according to Dixon, Woodberry, and Costa (1947).

(6) Handbook of Chemistry and Physics, 47th Edition, 1966 - 1967, p. D-85.

Captions to Figures

Absorption of Pyrimidines, . . . by Li-, Na-, Mg- and Ca-Montmorillonite (Clay-Organic Studies XII)

by G. E. Lailach, T. D. Thompson, and G. W. Brindley

- Figure 1. Absorption by (a) Li-; (b) Na-; (c) Mg-; (d) Ca-montmorillonite. Absorbed compounds: adenine \odot ; hypoxanthine \circ ; purine \square ; 6-chloropurine \blacksquare ; 5-amino-6-methyl-uracyl \blacksquare ; caffeine \blacksquare .
- Figure 2. Absorption by (a) Li-; (b) Na-; (c) Mg-; (d) Ca-montmorillonite. Absorbed compounds: adenine \odot ; 7-methyladenine \triangle ; 9-methyladenine \blacktriangle ; cytosine \ominus ; pyridine \oplus .
- Figure 3. Absorption of nucleosides by (a) Li-; (b) Na-; (c) Mg-; (d) Ca-montmorillonite. Adenosine \odot ; inosine \circ ; guanosine \oplus ; cytidine \ominus .
- Figure 4. Adenine absorbed and (a) Na^+ , (b) Ca^{2+} liberated in meq/100 g of respectively Na-, Ca-montmorillonite, versus equilibrium pH.
- Figure 5. Characteristics of adenine absorption by Na-montmorillonite in relation to equilibrium pH.
(a) adenine absorbed in molecules/unit cell and Na^+ ions liberated per unit cell.
(b) schematic representation of flocculation type and color.
(c) basal spacings of the clay in the equilibrium solutions, (d = diffuse, br = broad, sh = sharp, st = strong, vw = very weak) and relative clay volumes after short period of centrifugation.

Figure 6. Molecular size, represented by sum of carbon, nitrogen, and oxygen atoms, and pK_a values of compounds studied: A = adenine, AMU = 5-amino-6-methyl-uracil, C = cytosine, Ca = caffeine, 6-ClP = 6-chloropurine, H = hypoxanthine, 7MA = 7-methyladenine, 9MA = 9-methyladenine, P = purine, Py = pyridine, T = thymine, U = uracil, \odot = mostly aromatic character, \circ = mostly non-aromatic character.

Appendix: Formulae, pK_a -values, and molecular weights of the organic compounds used.

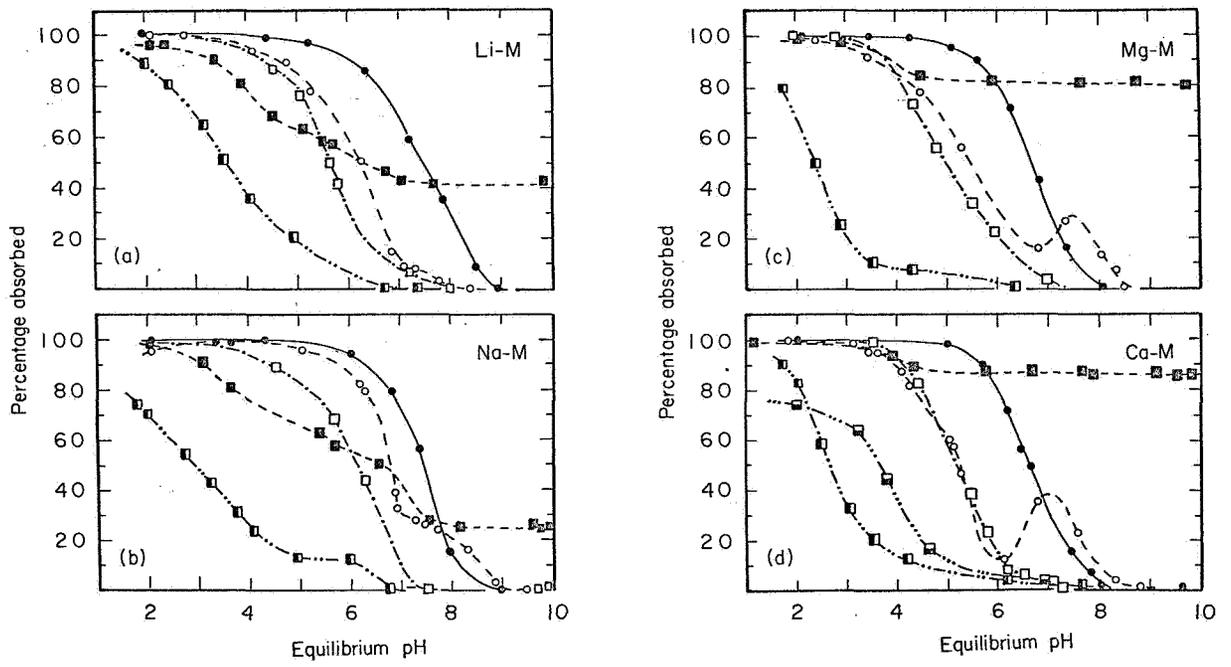


Figure 1

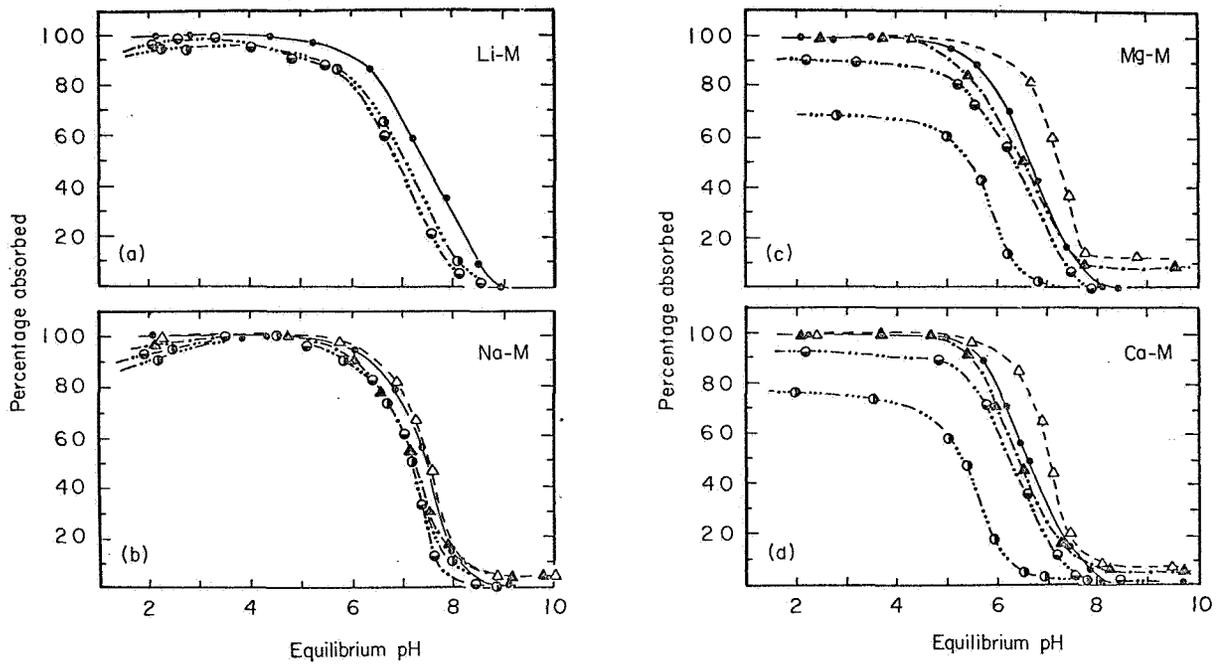


Figure 2

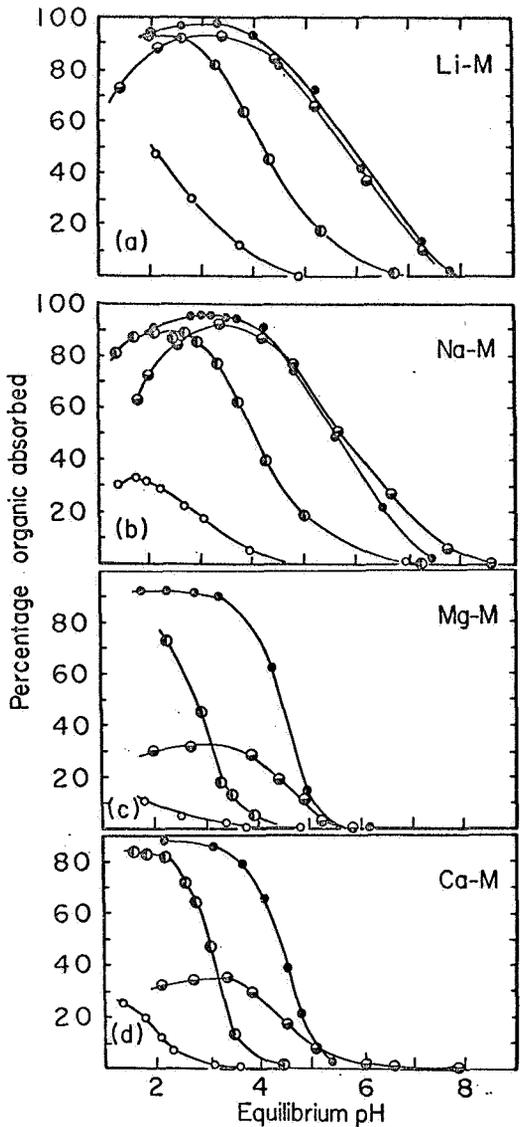


Figure 3

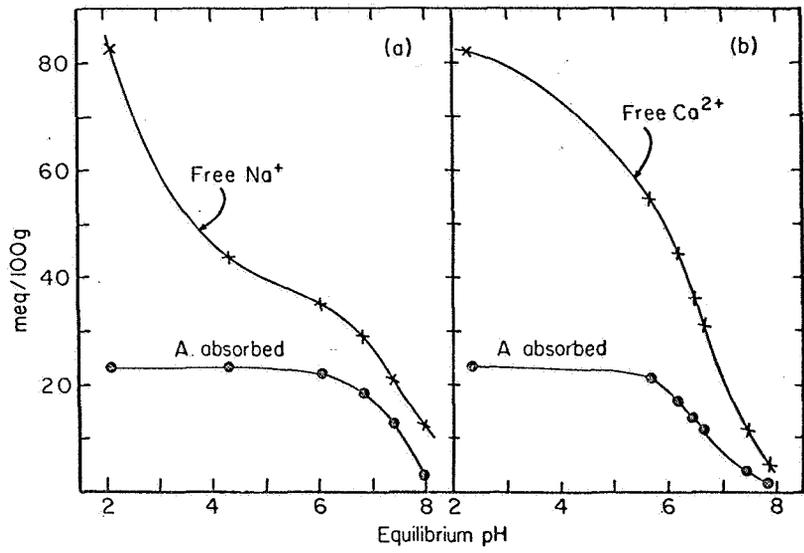


Figure 4

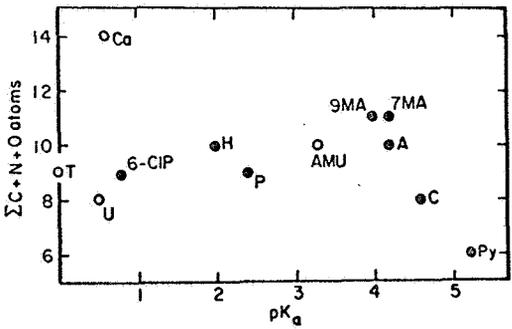


Figure 6

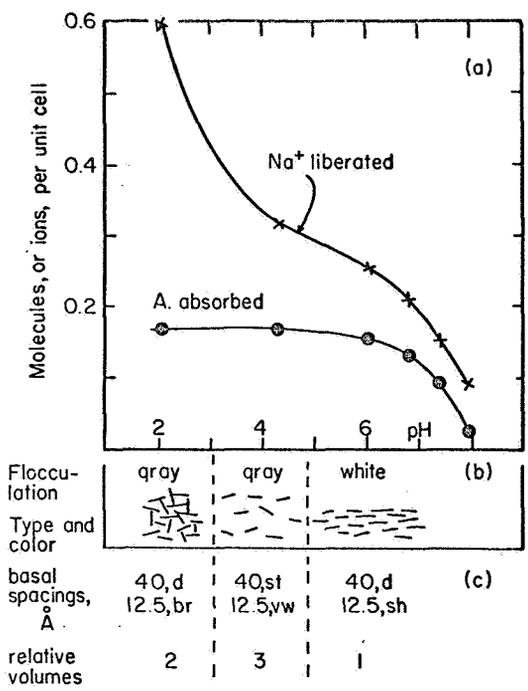
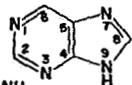
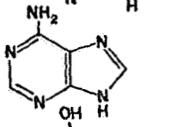
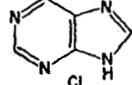
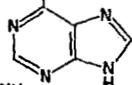
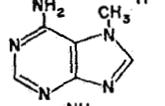
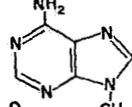
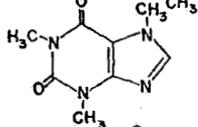
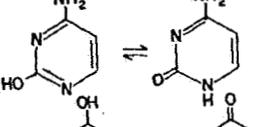
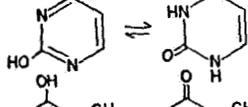
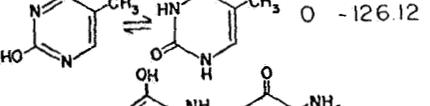
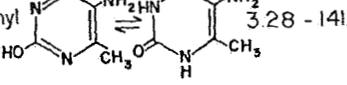
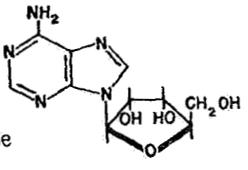
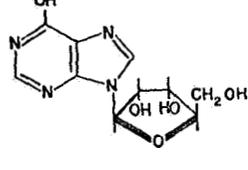
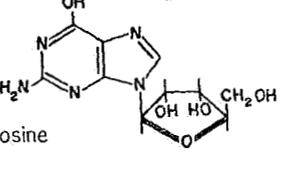
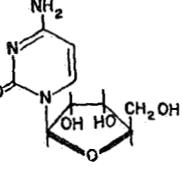
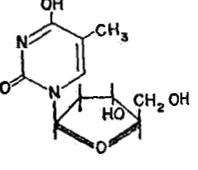
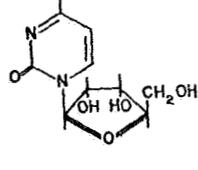


Figure 5

Appendix

		pK _a	MW.			pK _a	MW.
Pyridine		5.23	79.10				
Purine		2.39	120.12				
Adenine		4.22	135.13				
Hypoxanthine		1.98	136.12				
6-Chloropurine		0.8	154.56				
7-Methyladenine		4.17	149.16				
9-Methyladenine		4.00	149.16				
Caffeine		0.61	194.2				
Pyrimidine			80.09				
Cytosine		4.60	111.1				
Uracil		0.5	112.9				
Thymine		0	126.12				
5-Amino-6-methyl-uracil		3.28	141.13				
Adenosine		3.45	267.2				
Inosine		1.2	268.2				
Guanosine		1.6	283.2				
Cytidine		4.22	243.2				
Thymidine			242.2				
Uridine			244.2				