800 μ of axon can be estimated to be 3.38×10^{-4} μl/hr ($\sigma = 1.7$; confidence interval for $p = .05$, 2.86 to 3.90). In the presence of glucose (10 to 100 mg/100 ml) a very substantial increase in respiration (up to 1.5-fold) was observed (*7*).

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References and Notes

1. C. A. Terzuolo and Y. Washizu, *J. Neurophysiol.* **25**, 56 (1962).
2. C. A. G. Wiersma, E. Furshpan, E. Florey, *J. Exptl. Biol.* **30**, 136 (1953); C. Edwards, C. A. Terzuolo, Y. Washizu, in preparation.
3. K. Linderstrom-Lang, *Nature* **140**, 108 (1937).
4. E. Zeuthen, *J. Embryol. Exptl. Morphol.* **1**, 239 (1953); E. Giacobini and J. Zajicek, *Nature* **177**, 185 (1956).
5. E. Zeuthen, in *General Cytochemical Methods*, J. F. Danielli, Ed. (Academic Press, London, 1961), vol. 2, p. 144; E. Giacobini, *J. Neurochem.* **2**, 169 (1962).
6. O. Lowry, *J. Biol. Chem.* **140**, 183 (1941).
7. Preparations which do not include the axon belonging to the fast-adapting receptor have been obtained only recently by G. Bonewell. Dr. R. E. McCaman from Indiana University constructed and calibrated the quartz microbalances. Work supported by U.S. Public Health Service grants B-2527 and 2G-572.

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Ion Exchange at Edge and Interlayer in Montmorillonites Differing in Size

Abstract. *Titrations of hydrogen-saturated montmorillonite with sodium hydroxide indicate that two kinds of hydrogen are exchanged on the surface of the clay. At low* pH *there is more titratable hydrogen in a fine-size fraction of the clay than there is in a coarse fraction. This indicates neutralization of hydrogen adsorbed at the crystallite edge. The hydrogen adsorbed on the interlayer flake surface is neutralized at a higher* pH, *and there are more titratable hydrogen ions for the coarse fraction than for the fine. This suggests that surface-charge density is a function of crystallite size, a proposal already made on the basis of other properties of clay.*

The technique described by Pommer and Carroll (*1*) for titrating separately the cation-exchange capacity (CEC) of the edge and surface of hydrogen-saturated montmorillonite offers exciting possibilities for further experimentation. Measuring the ion exchanged on the flake surface alone allows information to be gathered on the isomorphous substitutions on which this exchange is assumed to depend. Heretofore only measurement of the total exchange was possible; this included an unknown contribution from the exchange at the crystal edges. The method (*1*) is applied here to two fractions of montmorillonite, differing in particle size, in an effort to determine whether variations in the two cation exchange capacities are related to the size of the clay crystallite. The first equivalence point in the titration curve corresponds to a cation exchange capacity that increases as the size of the particles decreases. The exchange capacity corresponding to the second reaction tends to decrease with decreasing size of the particles.

As a result of careful and laborious supercentrifugation (*2*) a set of size fractions for several bentonitic montmorillonites was produced. For the Wyoming bentonite (commercial Aquagel) used here, the finest suitable fraction was 15N with ESD (equivalent spherical diameter) range of 0.05 to 0.02 μ. The coarse fraction was 15H with ESD ranges of 0.4 to 0.3 μ.

The fractionated clay samples were stored as dry powders. These were suspended by mixing with demineralized water in a blender. Dowex-50 resin in the hydrogen form (H-resin) was added to the suspension so that the exchange capacity was twice that of the clay. After the mixture had been stirred for 3 hours, it was separated by sieving and treated twice again with fresh H-resin.

A stock suspension of the hydrogen-saturated clay was prepared for each fraction. Samples of 10 ml were pipetted into covered polyethylene bottles. Varying quantities of 0.1*N* NaOH were added with enough water to bring the total volume to 15 ml. After 1 week the clay was separated by centrifugation, and the solutions were returned to the cleaned bottles. Measurements of *p*H were made on these solutions. The weight of clay used in the bottles for the titration was determined separately for each fraction by weighing samples of the stock suspension.

The data are plotted, according to the method of Pommer and Carroll (*1*), in Fig. 1. The first straight portion of the graph represents neutralization of one of the two acids, either hydrogen ions exchanged at the crystallite edge or hydrogen ions exchanged at the crystallite flat surface. The second straight portion of the graph represents neutralization of the other hydrogen-bearing exchange site. Carroll (*3*) has discussed the advantages and possible difficulties in this experimental technique for determining the relative amounts of exchange at each site.

Table 1. Cation exchange capacities for two montmorillonite fractions, differing in size. The sample weight for 15H was 0.0461 g and for 15N was 0.0469 g.

Acid titration	Meq/100 g	
	15H	15N
1st	30	41
1st and 2nd	152	139
2nd only	122	98

Because the concentrations of the two stock suspensions were slightly different, the weights of clay titrated for the two graphs were different, and the two curves are therefore not directly comparable. The significant points on the curves are the two points of intersection of the three lines, and these can be adjusted to represent exchange capacity in terms of 100 g of clay.

Table 1 shows the cation exchange capacity for the two fractions. The first reaction is completed after NaOH is added in the amount of 30 meq/100 g of clay for the coarse fraction (15H). The fine fraction shows the same reaction to be completed at 41 meq/100 g of clay. If the same reaction were titrated for both fractions, it would be necessary for sample 15H to contain almost one-third inert diluent in order to explain its difference from the measured cation exchange capacity of sample 15N. It is reasonable to conclude that the exchange capacity of the first reaction increases with de-

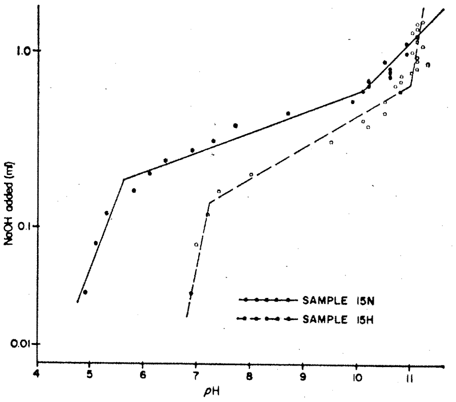

Fig. 1. Titration of H-montmorillonite with NaOH: two distinct reactions.

crease in crystallite size of the montmorillonite.

The second reaction is complete after the total equivalent addition of NaOH in the amounts of 152 meq/100 g of clay for the coarse fraction and 139 meq/100 g of clay for the fine fraction. The corresponding exchange capacity attributed to the second acid neutralization is 122 meq/100 g for the coarse fraction and 98 meq for the fine. Any error caused by dilution of the clay would be expected to be greater for the coarse fraction and would amplify the difference between the capacities of the coarse and fine fractions.

The first titration reaction was considered by Garrels and Christ (*4*) to be neutralizing surface-exchange sites, whereas the second reaction neutralized edge sites. Blackmon (*5*) showed that $-\log K$ for the first or low pH exchange was always a small number of the order of 2 to 3. This is to be contrasted with the larger $-\log K$, ranging from 5.7 to 8.3, for the second exchange. The exchange capacity of the first exchange was consistently higher than that of the second except for ammonium ions on bentonite. Pommer and Carroll (*1*), using a different method, obtained more nearly equal exchange values for the two sites, namely 50 meq/100 g for the first acid and 40 meq/100 g for the second. Also, the two values of $-\log$ K were more nearly equal, 6.4 and 8.3 for the first and second reactions, respectively.

The exchange capacities measured in the present experiment are somewhat lower than those of Pommer and Carroll for the first acid. They may not be directly comparable with Pommer and Carroll's measurement because the clay and size fractions were different, but their true significance lies in the comparison between the capacities measured for the first acid for the fine and coarse fractions. If one of the acid neutralizations were to be attributed to edge-exchange sites, it would be expected to increase with decreasing crystallite size. Because the first acid behaves in this manner, it may well represent the edge exchange rather than the surface exchange as suggested by Garrels and Christ.

In addition, that part of the total exchange attributed to the second neutralization reaction is less for the fine fraction than for the coarse. It would be doubly difficult to account for a decrease in this exchange with a decreasing crystallite size if the second reaction were to be attributed to edge exchange. The second neutralization reaction, therefore, does not behave in a way expected for the edge exchange, and therefore it must be attributed to the surface exchange.

It is justifiable to ask how to account for the reduction in cation exchange capacity for the second neutralization reaction with decreasing crystallite size if it is interlayer surface exchange. The distribution of negative charge on the clay crystallite flat surface is not uniform (*6*). It is high near the center and diminishes outward toward the crystallite terminations. This reduction in charge density across the flake surface is caused by the selective removal by solution at the edge of the flake of tetrahedral aluminum ions with respect to silicon ions and of octahedral magnesium ions with respect to octahedral aluminum ions. The more ionic character of the aluminum-oxygen bond causes it to be more readily ruptured in an aqueous environment than the silicon-oxygen bond. The extent of this reduction of charge at the interlayer surface terminations is, of course, dependent upon the ratio of edge to interior. Micaceous clay crystallites whose particle size is large and whose flakes have large, unterminated surface areas facing the interlayer space will have an *average* surface charge density nearly like the surface-charge density at the center of the flake. On the other hand, smaller crystallites with smaller unterminated surface areas have *average* surface-charge densities considerably smaller than the surface charge density at the center of the flake. The exchange capacity of the second reaction is a measure of the average surface-charge density. Size fraction 15N with its smaller crystallite size would be expected under these circumstances to have a lower second reaction exchange capacity because its average surface-charge density would be smaller than that for fractions containing larger crystallites such as fraction 15H.

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References

1. A. M. Pommer and D. Carroll, *Nature* **185**, 595 (1960).
2. N. Mungan, unpublished dissertation, University of Texas (1962).
3. D. Carroll, *U.S. Geol. Surv. Profess. Papers 400-B* (1960), p. B 436.
4. R. M. Garrels and C. L. Christ, *Am. J. Sci.* **254**, 373 (1956).
5. P. D. Blackmon, *ibid.* **256**, 733 (1958).
6. E. C. Jonas, *Intern. Geol. Congr.* 21st session, part 24, (1960), pp. 7–16.

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Methylmalonate Excretion in Vitamin B_{12} Deficiency

Abstract. *Urinary methylmalonate excretion is increased in rats with an insufficiency of vitamin* B_{12}. *Excretion of methylmalonate is not affected by folic acid, vitamin E, or selenium, but is markedly decreased by small amounts of vitamin* B_{12} *added to the diet.*

Methylmalonate was found in the urine of rats developing dietary liver necrosis (*1*). Excretion of methylmalonate was not related to vitamin E in the diet, even though vitamin E protected the rats against liver necrosis. Addition of certain antibiotics (aureomycin, penicillin) decreased excretion of methylmalonate and other ether-soluble acids, but methylmalonate was still detectable in the urine. Previously it had been found in the urine of normal rats or in rats receiving anthracene (*2*).

Attempt has been made further to modify excretion of methylmalonate. Recently, it has been noted that patients with pernicious anemia excreted large quantities of methylmalonate (*3*). It is believed that this occurs because the reaction

$$\text{Methylmalonylcoenzyme A} \rightleftharpoons \text{succinylcoenzyme A}$$

requires a coenzyme which is derived from vitamin B_{12} (*4*). The effects on mortality and methylmalonate excretion of vitamin B_{12}, folic acid, selenium, and vitamin E were therefore studied. Folic acid was added because of its effects on macrocytic anemia, and selenium because of its protective effects in dietary liver necrosis (*5*).

A liver necrosis-producing diet, consisting of cornstarch 79 percent, torula yeast 18 percent, USP No. XIV salt mix 3 percent, cod liver oil 1.4 ml, and stripped lard D.P.I. 6.3 cc, was fed to male weanling Sprague-Dawley rats. A daily supplement containing thiamine 20 μg, calcium pantothenate 100 μg, pyridoxine 20 μg, riboflavin 25 μg, and vitamin K 20 μg was given. This diet was not known to be deficient in any vitamin other than vitamin E. Rats were kept in individual cages. Urine was collected daily under toluene; output was measured. An ether extract of urine was prepared and chromatographed as previously described (*3*). Results are presented in Table 1.

One microgram of vitamin B_{12} was added in the daily diet of nine rats. Methylmalonate disappeared from the