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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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

Table 1. Effect of various dietary supplements on rats receiving a diet that produced liver necrosis.

Rats (No.)	Urinary excretion of methylmalonate (μg)	Death (days)	Liver necrosis	Weight gain per week (g)
		No supplement		
16	60 to 180	26 to 51	4+	7 ± 2
		Vitamin B_{12}		
15	0	28 to 48	4+	7 ± 2
		Selenite		
4	60 to 180	28 to 48	0	5 to 8
		Folic acid		
4	60 to 180	31 to 37	4+	4 to 8
		Vitamin E		
16	60 to 180	31 to 37	0	6 ± 2

urine of one rat in 1 day, of another in 2 days, and in the remaining animals by the 7th day. When 10μg were given in one oral dose, methylmalonate disappeared from the urine of all rats within 5 days. A single intraperitoneal dose of 10 μg was followed by disappearance of methylmalonate from the urine of four rats on the second day.

The rats that received a vitamin B_{12} supplement grew at the same rate as those on the basal diet. They developed liver necrosis at the same time as the control rats.

Methylmalonate did not reappear in the urine of any of the treated rats for as long as they survived. Survival in this group of rats averaged 37 days after the diet was begun.

The organic acids which appear in most of the rats on this diet include α ketoglutaric, citric, succinic, and hippuric, as well as methylmalonic acids. Lactic, cis-aconitic, pyrollidone carboxylic, and malic acids appear not infrequently. Approximately 1 to 3 meq of each acid are excreted daily. On the day of the maximum decrease of methylmalonate excretion, a consistent increase in succinate excretion was noted.

Sodium selenite was added to the diet of four rats at 0.2 mg per kilogram of diet. The rats lived and did not develop liver necrosis. No effect of added selenite on methylmalonate excretion was noted during 1 month.

Folic acid deficiency results in a macrocytic anemia which may be confused with vitamin B_{12} deficiency. The diet of four rats was supplemented with folic acid, 1 mg per day. This did not affect the development of liver necrosis or the excretion of methylmalonate.

Methylmalonate excretion was found to decrease in some older rats weighing 50 to 55 g at the start of the diet that produced liver necrosis. This decrease appeared related to a general decrease in organic acid excretion in the older rats.

Sixteen rats were given vitamin E, 1 mg per day. No change in methylmalonate excretion was noted, though these rats did not develop liver necrosis (*6*).

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Aeolian Zone

Abstract. *It is postulated that a widespread biotic zone, based on wind-blown organic materials such as pollen and oceanic albuminoids, exists in mountainous and polar regions. The aeolian zone or biome, distinct from alpine and arctic tundra, may be divided into terrestrial, nival, and aquatic divisions.*

The alpine life zone may be defined as a biome existing above timber line in mountainous areas. The upper limits of the zone have not been clearly delineated but, in general, the alpine area has been assumed to extend to the upper limit of green plants. In addition, alpine communities have been considered to be based upon energy derived from resident photosynthetic vegetation. In reports on the ecology of the high altitudes of the Himalayas (*1*), I have referred briefly to a new ecological entity, the aeolian zone, which seems to be discrete and, although at times overlapping the altitudinal range of alpine life, does not appear to be truly alpine in nature. The discovery of animals such as collembolan and thysanuran insects, which feed on wind-blown organic debris or its decay products and live permanently in areas where there are no visible green plants, suggests that there is a distinct ecological category for life supported by wind-transported food.

The presence of salticid spiders on Mt. Everest at an altitude of 6700 m (*2*) extends the known range of animals far above the level of the highest recorded green plants. Salticid spiders, observed between 5200 m and 6100 m on Makalu, near Mt. Everest, feed on mycetophagous anthomyid flies and omnivorous collembolans. In the eastern Himalayas, therefore, it appears that a terrestrial division of the aeolian zone may include these organisms, as well as scavenger and debris-feeding animals such as mites and machilid thysanurans.

The more obvious aeolian food materials consist of pollen, seeds, spores, dead insects, and miscellaneous fragments of plants. This wind-blown debris may have a fairly local origin or it may be carried in the atmosphere from distant sources. Wind-blown albuminoid may also be present. Wilson (*3*) found that albuminoid is an abundant and widespread component of fresh snow in the mountains of New Zealand. The concentration in snow ranges from 0.02 to 0.2 parts per million, a fraction which may amount to several pounds per acre. The source of this albuminoid is sea foam, which contains a high concentration of products of plankton decay. In addition to these aeolian organic materials there are also inorganic nitrogenous substances (ammonia and nitrates) in snow and rain, which may contribute to the nutrition of various organisms.

Nival organisms are primarily dependent upon nutrients obtained by atmospheric transport. Except in special cases (such as the contamination of snow by the excreta of animals), organic and inorganic nitrogenous ma-