acetate seems admirably suited for this purpose. It is neutral and buffers gastric acidity to pH 4 to 4.5. While this work was in progress McDermott *et al.*[6] successfully employed magnesium trisilicate in this manner.

Clinical work is now in progress using the combination in infections known to be susceptible to parenteral penicillin therapy.

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CORNEAL VASCULARIZATION IN RATS ON A TRYPTOPHANE DEFICIENT DIET[1]

IN a previous publication[2] we have reported that both weanling and adult rats developed vascularization of the cornea within two to three weeks on a tryptophane deficient diet. Inasmuch as corneal vascularization has also been repeatedly reported to be a manifestation of riboflavin deficiency in the rat and man,[3] the need for an inquiry into the apparently dual etiology of the symptom was obvious. Since the animals maintained on the tryptophane-supplemented tryptophane deficient diet grew normally and failed to show ocular lesions, it was felt that this diet contained adequate quantities of riboflavin and the possibility that the vascularization might arise from increased riboflavin requirement which was not met by the tryptophane deficient diet needed investigation.

To test this possibility, 18 normal weanling male and female rats (50–60 gm) from a hybrid albino and hooded Norwegian rat colony were divided into two groups of 9, which were fed on the tryptophane deficient diet.[4] One of these groups was given daily a freshly prepared riboflavin solution (30 γ per cc) to drink from amber-colored, glass-graduated drinking tubes, and the other group, serving as a control, received tap water instead. Despite an average daily intake of 120 γ of riboflavin, the rats of the riboflavin supplemented group developed corneal vascularization and cataracts[5] with a rapidity and incidence equal to that of the control group. Moreover, the weight losses and general poor appearance of the animals in both groups were similar to those previously reported. After 5 weeks, the riboflavin supplementation for the first group was discontinued and both groups of animals were fed the tryptophane-supplemented tryptophane deficient diet.[4] On this regimen, the animals gained weight rapidly, and complete recovery from the corneal lesions with partial disappearance of the cataracts was noted.

The results of this experiment suggest that tryptophane deficiency exerts some direct effect upon the cornea, rather than inducing a deficiency of riboflavin. The recent reports of corneal vascularization in man which failed to respond to riboflavin therapy[6, 7] suggest the possibility of an exogenous or endogenous deficiency of tryptophane and the employment of tryptophane as a therapeutic measure.

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THE SOLUTION OF SOIL MINERALS IN DILUTE ACIDS

MANY soil minerals, particularly those of the montmorillonite group, are known to dissolve or disperse readily in water solutions containing 0.01 to 0.4 per cent. acid.[1] The evaporated clear sol has approximately the composition of the residue and of the mineral from which it was derived, going from sol to gel to amorphous solid as evaporation proceeds. Solubilities as high as 60 per cent. of the clay have been found by the writer for halloysite and montmorillonite. High solubility requires sufficient acid to take the bases and sufficient water to dissolve the silica set free by removal of the bases. The solubility of an ordinary salt does not depend upon the amount of salt present, but that of a clay may vary by a factor of 4, as clay alone is varied.

The problem is to find definite relations between the amount of sol formed and the amounts of acid, clay and water present. Analyses of residues, of the dried sols and of the water-soluble portion of the latter indicate the nature of the attack and of the preferred recombination. Any exact relations found supply a foundation for theoretical generalizations.

Such an investigation of the simple mineral halloysite has been reported by the writer[1] and formed the basis of the present work on montmorillonite by the same method. Starting with a pure halloysite analyzing $Al_2O_3 \cdot 2SiO_2 \cdot 3.4\ H_2O$ under room conditions after grinding to 0.1 mm, the washed sol after drying at 160° C was found to be $2Al_2O_3 \cdot 3SiO_2 \cdot 7H_2O$

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[6] W. McDermott *et al.*, SCIENCE, 101: 228, 1945.

[1] This investigation was aided by grants from the Rockefeller Foundation and Nutrition Foundation, Inc.

[2] A. A. Albanese and W. Buschke, SCIENCE, 95: 584, 1942.

[3] O. A. Bessey and S. B. Wolbach, *Jour. Exp. Med.*, 69: 1, 1939; V. P. Sydenstricker, W. H. Sebrell, H. M. Cleckley and H. D. Kruse, *Jour. Am. Med. Asn.*, 114: 2437, 1940.

[4] A. A. Albanese, L. E. Holt, C. Kajdi, J. E. Frankston, *Jour. Biol. Chem.*, 148: 299, 1943.

[5] We are indebted to Dr. Wilhelm Buschke for some of these examinations.

[6] H. Scarborough, *Brit. Med. Jour.* 11: 601, 1942.

[7] T. E. Machella and P. R. McDonald, *Am. Jour. Med. Sci.*, 205: 214, 1943.

[1] The formation of colloid from halloysite in dilute acid solutions, *Jour. Wash. Acad. Sci.*, April 15, 1944, contains references to previous work.

through a wide (16:1) range of concentrations in the 27 combinations investigated. The sol from solutions less than 0.01 per cent. acid runs higher in SiO_2, that from solutions above 0.3 per cent. acid higher in Al_2O_3. Test runs with kaolinite and muscovite instead of halloysite gave a sol of the same composition, $2Al_2O_3 \cdot 3SiO_2 \cdot 7H_2O$. These results indicate that the sol formed is not merely a peptized dispersion of the original mineral and that there is a preferred composition. That it is not merely a coprecipitation of alumina and silica is indicated both by the constancy in composition and by the low pH (1.5 to 2.5) of the solutions formed.

The quantitative relation found for halloysite between sol (S) formed and the acid (A, HCl), clay (C) and water (W) reacted was

$$(1)\ \text{Log } S = \log A + 0.322 \log C - 0.318 \log W - 1.421$$

using natural logarithms. S and C are in grams, the unit for A is 0.75 gram and for W is one liter. This may be written simply

$$(2)\ 4.14 \frac{S}{A} = \left(\frac{C}{W}\right)^{.32}$$

or the ratio of sol to acid is proportional to about the cube root of the clay concentration in grams per liter.

The chemical processes involved appear to be very simple. After the clay mineral has adsorbed sufficient anions, it is attacked by them. Free silica liberated and chlorides formed go into solution, the chlorides are hydrolyzed, the alumina set free combining with the free silica to form hydrosol, while the acid set free by hydrolysis returns to the clay to form more chlorides. Action ceases in each run when the potential of the accumulated sol becomes equal to that of the residual mineral. The mass action "constant" varies with conditions. For C and W each = 1, $S = 0.2415A$. The writer has not yet been able to break down (1) into a set of general laws even with the help of the Gibbs condition that each component has the same chemical potential in all its phases. Attempts to form allophane from $AlCl_3$ and silica solution in proper proportion, with water and pH adjusted to the proper values, gave no recombination. From this it is concluded that only *nascent* ions of silica and alumina are able to recombine. While the sol formed is within the range of allophanes in composition it is slightly lower in silica than the average.

Working with montmorillonite instead of halloysite as soluble mineral, the center of interest is still sufficient acid (HCl) to dissolve the bases with sufficient water to dissolve the silica set free or one gram of acid and one liter of water to each gram of clay. One half, 1 and 2 times these amounts were run in their 27 combinations at 96–98° C for 72 hours. Of these 27 combinations, 8 are duplicates except for overall amount (such as 1:2:1 and 1/2:1:1/2), these serving to check results.

After reaching equilibrium, the final 16 hours without stirring, the clear solution was removed with a suction flask, evaporated and dried at 160° C and weighed. This residue was composed of the sol (S) and water-soluble chlorides which were separated by a ten-minute treatment in 150 cc of hot distilled water. Analysis of the chlorides, the sol and the residue of the original clay completed each run. The amount of chloride-free sol ranged from 7.4 to 63 per cent. of the clay used.

The montmorillonite used was the very pure oligocene bentonite from the Husband farm in northwestern Smith County, Miss., composed largely of shards and containing little or no alien sedimentary or redeposited material. Analyses by R. E. Stevens gave:

SiO_2	50.38	CaO	1.26
Al_2O_3	17.24	K_2O	0.51
Fe_2O_3	2.76	H_2O-	12.30
MgO	4.58	H_2O+	10.52

with traces of FeO and Na_2O. A few hours in hot 20 per cent. HCl removes the bases from this clay, leaving water-soluble pure silica. Dilute acid comes to equilibrium with it.

The sol formed showed a nearly constant composition averaging $Al_2O_3 \cdot 4.1\ SiO_2 \cdot 1.5\ H_2O$ for the 27 experiments with no systematic variation as clay, acid and water were independently varied in the range 1/2, 1 and 2 units, indicating the formation of a definite compound like an amorphous pyrophyllite. If it were an adsorption effect, its composition would have varied with the concentration of either silica or alumina (or both) remaining in solution. Only traces of either were found in the water-soluble portion separated from the sol. The molar ratios $SiO_2 : Al_2O_3$ found ranged from 3.8 to 4.5 (averaging 4.1), apparently due to accidental variations in stirring or rate of heating but not to the large variations in the clay: acid: water ratios. All Ca and Mg removed from the original clay remained as chlorides in the solution. None of either recombined with the aluminosilicate sol. The Fe behaved like the Al. The pH of the solution ranged from 2.5 to 3.5 and dropped as it evaporated until free acid was gone so that coprecipitation (of alumina with silica) seemed very unlikely.

The quantitative relation between sol (S), clay (C), acid (A) and water (W) found for montmorillonite clay may be summarized in the relation

$$(3)\ \text{Log } S = .756 \log A + .424 \log C - .156 \log W - 1.55$$

in natural logarithms; sol, acid and clay are in grams, water in liters. With C = 1 and W = 1

$$(4)\ S = 0.212\ A^{0.756}$$

These relations differ essentially from those (1) and (2) for sols from halloysite. In (1) the ratio log

S: log A averaged 1.015 (essentially unity) and the coefficients of log C and log W are very nearly the same. With montmorillonite, these conditions do not obtain, perhaps because the Cl ions are in four combinations instead of only two (Al and water). It is not that Ca and Mg take part of the acid, for all those elements in one gram of this clay would take only 0.1 gram of HCl. The effect is in the nature of a lowering of the chemical potential of the acid in a fixed ratio.

The complete replacement of shells by montmorillonite (Pontotoc, Miss.) indicates that aluminosilicate sols may be deposited under conditions that suffice to completely remove other bases present in the shells. Very slowly percolating ground waters, saturated with CO_2 (about 0.03 per cent.) from the air, could readily take up aluminosilicates and redeposit them later on contacting alkaline earth or on reaching an exposure. On the other hand, drainage waters contain chiefly soluble salts, very little alumina or aluminosilicate and SiO_2 is low except when alkalies are present.

The writer in 1932 made solutions of 22 different clay minerals in 0.2 per cent. HCl. The yield of washed residue from a liter of evaporated solution was from one to two grams in every case except for pyrophyllite, sericite and bauxite, which gave less than half a gram. In every case the recovered material closely resembled montmorillonite in composition but was low in Ca, Mg and alkalies. Even soil, glauconite and the fines associated with decomposed (D.C.) granite yield readily to dilute acid, but more slowly than the bentonites. Rain water, saturated with the CO_2 of the atmosphere, would have a concentration of about .03 per cent., well within the range investigated. Given sufficient water to dissolve the silica set free (about five liters per gram at earth temperatures), ground waters should maintain a steady supply of colloids in all ordinary soils.

SUMMARY

Montmorillonite goes readily into solution in slightly acid water. In strong acids only bases, in pure water only free silica is removed.

In the range from 0.02 to 0.5 per cent. acid, both bases and silica go into solution and recombine as an aluminosilicate sol and as highly soluble salts.

The sol recovered by evaporation, washing and drying is an amorphous colloid near pyrophyllite in composition, $Al_2O_3 \cdot mSiO_2 \cdot nH_2O$, with m averaging 4.1 (3.9 to 4.5) and n about 1.5. The slight variation in m is unrelated to either sol, acid or clay concentration.

A general characteristic relation between the amount of sol formed and the amounts of clay, acid and water used has been found for montmorillonite clays. This relation differs in two essential properties from that previously deduced for halloysite-allophane solutions. The variation with temperature of the constants of these equations remains to be investigated.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLIFIED METHOD FOR THE ASSAY OF ANTIBIOTICS

AMONG the various methods used for the assay of penicillin and other antibiotics, Heatley's[1] cylinder method and its modifications is given preference by many investigators. While it has been found reliable and accurate in the hands of a team of skilled workers, the somewhat tedious and time-consuming preparatory procedure has proved a handicap for smaller laboratories who lack a sufficient number of highly trained personnel to carry out the preliminary work. The method described here has, we believe, the advantage of greater simplicity.

Principle: Soft nutrient agar (about 0.3 per cent.) is inoculated in bulk with the test organism, distributed into tubes and left to solidify in an upright position. The solution to be tested is added on top of the stab. The intensity of antibiotic activity is determined by the depth of the clear zone of growth inhibition extending downwards from the area of contact between the agar and the solution.

Details of the method: The composition of the nutrient medium is of no importance provided that it allows abundant growth of the test organism and that a standard of known activity is used with every test. In our experience the following composition proved to be satisfactory:

(a) For staphylococci:	Bacto-peptone No. 3	20	grams
	Sod. chloride	8.5	"
	Glucose	10	"
	Agar (Agar Products Co.[2])	3	"
	Dist. water	1,000	ml
(b) For *B. coli:*	Bacto-peptone No. 3	20	grams
	Sod. chloride	8.5	"
	Sod. nitrate	1.5	"
	Agar (Agar Products Co.[2])	3	"
	Dist. water	1,000	ml

The reaction of both media is adjusted to pH 6.5–6.6.

The concentration and the brand of agar influences the degree of diffusion of the active substance. In composing the medium the following rule is suggested: the optimal quantity of agar is the least amount which, during the performance of the test, allows the addition of fluid on the top of the agar without

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[1] N. G. Heatley, *Biochem. Jour.*, 38: 61, 1944.