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

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

breaking its surface. We find that with the brand of agar recommended[2] a concentration of 0.3 per cent. gives satisfactory results. Another brand tried required higher concentration, resulting in diminished diffusion of the active substance. The addition of glucose for staphylococci and of sodium nitrate for *B. coli* insures good growth in the depth of the medium. The medium is sterilized in bulk at 115° C (10 lbs. pressure) for 20 minutes and cooled down to 40°–42° C. One agar slant of a 24-hours old culture of the test organism is washed with 5 ml of saline, and 0.2 ml of this emulsion is added to 1 litre of the medium, which is then distributed into tubes to a height of approximately 5 cm, the diameter of the tube being of no importance. The tubes are placed immediately in the refrigerator and are ready for use after two hours, but can be kept for at least one week.

The solutions to be tested, which need not be sterile, should not be excessively acid or alkaline. They can be diluted with water or any suitable buffer between pH 5 and 8. They must not contain ether or chloroform and not more than 10 per cent. alcohol or acetone. Approximately 0.5 ml of each solution (the exact volume does not matter) is pipetted on top of the agar stab. Standard solutions are treated in the same way. After overnight incubation, the degree of inhibition can be measured by placing a transparent millimeter scale on the wall of the tube or by the use of a pointed caliper.

The assay of penicillin is carried out in the same way as described for the cylinder method, *i.e.*, several dilutions of a standard are set up simultaneously with the unknown, and a graph is constructed by plotting the zone of inhibition in mm against the number of units per ml. Using amounts of penicillin between 0.2 and 2 units/ml a curve similar in shape to that given by Heatley is obtained.

Being based on the same principle as the cup assay, the method is subject to the same limitations, *i.e.*, the diffusibility of the inhibiting substance and a number of other factors are apt to influence the results.

During the past year the method has been successfully applied to the study of a number of other antibiotics derived from moulds and has proved particularly useful in the survey of large numbers of mould cultures and in the follow-up of the developing antibiotic activity.

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[2] Agar, powdered, natural. Agar Products Company, 616–618 North Robertson Blvd., Los Angeles 46, Calif.

SEPARATORY FUNNELS AS EXPERIMENTAL CHAMBERS IN STUDIES OF INSECT PHYSIOLOGY

IN studies of insect physiology an array of experimental chambers is frequently required within which the animals can be exposed to various gases or vapors. Preferably, such chambers should be not only durable and inexpensive, but also transparent, air-tight and capable of being easily washed out with and sealed to contain the gas mixtures.

I wish to call attention to the fact that separatory funnels fulfil these requirements in every respect, although they have apparently not been previously used for this purpose. The gas mixtures are readily introduced through the stem of the funnel, the chamber, by virtue of its smooth contours, easily washed out, and, most important, the experimental conditions then maintained by lubricated ground-glass seals at both ends. The further fact that separatory funnels in a variety of sizes and shapes are already at hand in most laboratories is not the least advantage at the present time.

In experiments in which one desires to test a large number of insects within a single chamber, it may prove convenient to enclose each animal within a gelatin capsule of suitable size. In order to insure continuous equilibration with the gas mixture in the funnel, numerous perforations should previously be made with a hot needle in both ends of each capsule. The capsules can then be numbered with ink and, if so desired, arranged in a continuous strip by placing them transversely between two longitudinal lengths of Scotch tape.

In prolonged experiments the atmosphere in the funnels can be washed out and renewed at suitable intervals. In studies not involving gas mixtures containing carbon dioxide, a loosely plugged glass vial containing soda lime should always be enclosed in the chamber.

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SCIENTIFIC BOOKS

IMMUNO-CATALYSIS

Immuno-Catalysis. By M. G. SEVAG. Pp. xv + 272. Illustrated. Springfield (Ill.) and Baltimore: Charles C Thomas. 1945. $4.50.

THE scope of this book is well stated in the preface by Dr. Stuart Mudd: "The fullness of the integration possible between the fields of enzyme chemistry, immunochemistry and the mechanisms of infectious disease, has, indeed, in the writer's belief, been indicated for the first time in this volume . . ." As stated, the