TABLE I

HEAT OF WETTING, AMMONIA ADSORPTION AND $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ RATIO OF COLLOIDAL MATERIALS ISOLATED FROM DIFFERENT SOILS

Source of colloidal material	Molecular ratio $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$	Heat of wetting per gram of colloid	NH_3 adsorbed per gram of colloid	Relative values		
				$\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$	Heat of wetting	NH_3 adsorbed
		Calories	Grams			
Cecil subsoil	1.20	4.5	0.0192	0	0	3
Cecil soil	1.34	6.2	0.0230	7	13	12
Chester soil	1.77	7.2	0.0293	28	21	26
Norfolk subsoil	1.84	6.0	0.0295	32	11	27
Huntington soil	1.86	8.3	0.0319	33	29	32
Sassafras subsoil	1.89	9.8	0.0340	34	40	37
Hagerstown subsoil	1.89	7.9	0.0299	34	26	28
Susquehanna subsoil	1.98	5.3	0.0177	40	6	0
Miami subsoil	2.66	11.8	0.0358	72	56	41
Marshall soil	2.82	14.2	0.0536	80	74	82
Stockton soil	2.85	16.3	0.0617	81	90	100
Wabash soil	3.16	17.6	0.0614	97	100	99
Sharkey soil	3.23	16.3	0.0609	100	90	98

some marked exceptions. In the case of the Susquehanna subsoil colloid there is poor agreement between the heat of wetting and the silica ratio, although the agreement between heat of wetting and ammonia adsorption is very good. A correlation of the silica ratio with the heat of wetting or adsorption would not be expected to hold for colloids from peat soils, which are composed chiefly of organic matter and contain comparatively little silica, alumina and iron. Furthermore, colloids exceptionally low in silica and high in alumina and iron may as a class be exceptions to this correlation. For instance, a colloid containing only 15 per cent. of silica with a $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio of 0.55 has recently been isolated from a deep tropical subsoil. The heat of wetting of this sample is 8 calories, or approximately the magnitude usually given by colloids having a silica ratio of about 1.9.

The $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio is not the only expression of chemical composition that shows a parallelism to the properties of the colloid. It was pointed out in a recent bulletin of this Bureau[5] that the colloids high in silica were usually low in alumina, high in monovalent and divalent bases and low in combined water. A fairly good correlation obtained between the ratio, $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$, and the ratio $\frac{SiO_2}{CaO + Na_2O}$ for a series of soils. In view of these relationships, it follows that other chemical constituents beside the silica ratio correlate with the properties of the colloid. For instance, some properties appear to be closely related to the percentage of calcium or to the total exchangeable monovalent and divalent bases. Joseph and Hancock[6] have suggested that the properties of colloids from soils and clays probably bear a general relationship to the $\frac{SiO_2}{Al_2O_3}$ ratio, the more plastic clays having the higher ratios.

A knowledge of the interrelationships of the properties of soil colloids and of the correlation between chemical composition and properties is of practical value in supplying a basis for predicting the general behavior of the colloids without extensive physical tests or complete chemical analyses. Similar relationships between properties and chemical composition doubtless obtain for the colloidal materials of ceramic clays and the recognition of these relations should be important in the ceramic industries.

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[5] Robinson, W. O., and Holmes, R. S., p. 16, *l. c.*

CONTINUOUS REPRODUCTION OF MICRO-ORGANISMS IN SYNTHETIC MEDIA

THE controversies and work of Pasteur, von Liebig, Mayer and Nageli suggest that yeast may fail of

[6] Joseph, H. F., and Hancock, J. S., "The composition and properties of clay," Trans. Chem. Soc. 125: 1888–1895 (1924).

continuous cultivation in media affording an only source of nitrogen in ammonium salts. Growth and reproduction in a medium wholly synthetic has been generally considered impossible. Vitamine B or substances of the nature of "bios" are held necessary by various investigators for the continuous growth and reproduction of *Saccharomyces cerevisiae.*

Fulmer, Nelson and coworkers[1, 2, 3, 4] have reported continuous growth and reproduction in their medium E, composed of inorganic salts and cane sugar, and that yeast was subcultured when a synthetic product, methose, was substituted for cane sugar. Relatively large inoculations were used and single cell cultivation was not attempted. MacDonald and McCollum[5] question the dependence of yeast on vitamine B or substances of the nature of "bios."

In recent articles Robertson and Davis[6] and Robertson[7] conclude that yeast is incapable of synthesizing its own growth-stimulating substance or substances in their media, and that yeast cells to a large extent possess the power of "taking over" these essential food substances, and that growth in synthetic media is roughly proportional to the number of cells used for the original inoculation. They failed to obtain reproduction beyond the 12th or 15th generation (transfer). Willaman and Olsen[8] stress the possibility of retention by the sugar of unanalyzable traces of "bios." Williams[9] maintains that vitamine B is necessary for the continuous growth of yeast.

We have employed the medium E base of Fulmer and Nelson, Robertson and Davis medium and Nageli solution. Special emphasis was laid on the purity of the chemicals and cleanliness of the glassware and precautions taken to destroy any vitamine B that might be adsorbed. For energy sources we have employed synthetic methose and succinic acid and also distilled glycerol (280° C.) and 7-day hot 95 per cent. alcohol continuously extracted dextrose. Methose, first synthesized by Loew,[10] was prepared from formaldehyde by the catalytic action of lead, MgO and $MgSO_4$ using CO_2 and K_2HPO_4 to precipitate the salts.

Single cell cultivation was carried out in the following manner: Direct microscopic examination was made of nutrient silicic acid gel plates smeared with a culture that had grown in the liquid synthetic medium for several weeks; single cells were marked, and after growth, transfers were made into the liquid medium. Since then cultures have been transferred on the average every 2 or 3 days for longer than 5 months. The use of Nageli solution was abandoned in favor of medium E. Robertson and Davis medium in our hands has not afforded continuous reproduction of our cultures of *Saccharomyces cerevisiae* when used at 30° C.

Medium E base at 30° C. $\pm$ 1° and succinic acid, methose, glycerol or dextrose have afforded continuous growth and reproduction of the following pure cultures: 14 races of *Saccharomyces cerevisiae,* 1 *Torula rosea,* 1 *Torula liquefaciens,* 1 *Oospora lactis,* 1 *Saccharomyces ellipsoideus.* Two cultures of cerevisiae failed to adapt themselves and were lost. Aeration greatly assisted liquid culturing. Heretofore results, apparently, have depended upon the use of 1 race of *Saccharomyces cerevisiae,* or 1 species of yeast giving rise to discrepancies and to results not applicable to generalization.

The same four energy sources in a base of K_2HPO_4 2 gm, $MgSO_4$ 0.1 gm, Fe_2Cl_6 trace, $(NH_4)_2\ SO_4$ 2gm (omitted in growth of nitrogen-fixing bacteria), $CaCl_2$ 0.1 gm and water 1 liter, have been employed in a study of bacterial vitamine requirements. The following organisms, subcultured by using an inoculum equivalent to the amount of a straight needle transfer from liquid medium, have been continuously cultured in this liquid medium base, utilizing at least three of the four energy sources: *Escherichia coli, Aerobacter aerogenes, Proteus vulgaris, Pseudomonas fluorescens, Encapsulatus pfeifferi, Pseudomonas cyanogena, Bacillus megatherium, Bacillus mycoides, Bacillus subtilis, Serratia marcescens, Azotobacter chroococcum, Rhizobium leguminosarum.*

The essential presence of vitamine B or substances of the nature of "bios" in the medium for growth and reproduction of certain yeasts and bacteria, notably *Azotobacter chroococcum,* has not manifested itself in our work unless such substance or substances were synthesized during the synthesis of methose and succinic acid, two entirely different processes; and failed to be extracted from the dextrose and did distil over with the glycerol. It appears reasonable to conclude in the light of our present knowledge that certain yeasts, torulae and bacteria may be continuously cultured in a medium wholly synthetic and that no addition of vitamine B or substances of the nature of "bios" is necessary. If such substances are necessary for the growth and reproduction of these organisms, they are metabolized.

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[1] *Jour. Am. Chem. Soc.,* 1921, *43,* p. 186.
[2] *Jour. Biol. Chem.,* 1922, *51,* p. 77.
[3] *Jour. Infect. Dis.,* 1923, *33,* p. 130.
[4] *Jour. Biol. Chem.,* 1923, *57,* p. 397.
[5] *Jour. Biol. Chem.,* 1921, *46,* p. 77.
[6] *Jour. Infect. Dis.,* 1923, *32,* p. 153.
[7] *Jour. Infect. Dis.,* 1924, *35,* p. 311.
[8] *Jour. Biol. Chem.,* 1923, *55,* p. 815.
[9] *Jour. Infect. Dis.,* 1919, *38,* p. 465.
[10] *Ber. Chem. Ges.,* 1889, *22,* p. 470.