that the exposures to the air had not interfered with the purity of the solvent. These facts do not belittle the care which should be taken to keep away moisture, for slow hydrolysis takes place when formamide is exposed to water, the products of hydrolysis being ammonia and formic acid, substances which do materially lower the freezing point, as pointed out by Freer and Sherman.[1]

Summary.

The important facts upon which this paper wishes to lay emphasis are the following:

1. The freezing point of formamide has been found to be 2.25°, which is the highest freezing point so far obtained for it, and which is probably the true freezing point of the pure substance.
2. Formamide can be obtained pure and is best freed from its products of decomposition and hydrolysis by fractional distillation.
3. Although formamide is hygroscopic in nature, when reasonable care is exercized regarding its exposure to the air, moisture is not taken up in quantities great enough to interfere with its purity as determined by its freezing point.

MADISON, WISCONSIN.

[CONTRIBUTION FROM THE LABORATORIES OF THE OFFICE OF SOIL FERTILITY, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE.]

THE ACTION OF NEUTRAL SALTS ON HUMUS AND OTHER EXPERIMENTS ON SOIL ACIDITY.

BY LOUIS J. GILLESPIE AND LOUIS E. WISE.

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Introduction.

The action of neutral salts on soils is an interesting one, and has been the subject of numerous investigations, the aim of which in many cases was to measure the quantity of acid substance in soils and to determine the nature of soil acidity. There are undoubtedly many soils of so-called acid character, from which very little acid can be extracted with water, in many cases so little that the water extracts are not observed to turn litmus paper. The acidic nature is manifested, however, in other ways. The soil may (1) affect vegetation in certain characteristic ways, (2) give a water extract which does not react alkaline to phenolphthalein when the carbon dioxide is removed by boiling, even after small quantities of lime have been added to the soil, (3) act on neutral salts with the development of appreciable quantities of acid, and (4) act readily on litmus paper if the paper is brought into intimate contact with the moist soil. These manifestations are not always perfectly correlated, but whenever evident, they may be caused to disappear by adding sufficient lime or other basic material.

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[1] *Am. Chem. J.*, 20, 223 (1898).

At first sight there would seem to be no doubt that such soils, which can at least be described as acidic in a general sense, actually contain acid substances. Closer scrutiny of some of the phenomena, especially the action of salts on soils and the litmus test, has led to the conclusion that such manifestations do not prove the presence of "true acids," and some workers have gone further and have concluded that "truly acid" soils do not exist, save in the few cases where the water extract turns litmus paper.

Now the characteristic effects of acid solutions are due mainly to the preponderance of hydrogen ions over hydroxyl ions. This preponderance is regulated entirely by the hydrogen-ion concentration at any given temperature and can not be found by measuring the titratable acidity in complicated mixtures such as soils, even if the titer could be accurately determined. One of us[1] showed, however, that when soils of various type and origin are treated with water, extracts can be obtained which possess experimentally definite hydrogen-ion concentrations. A centrifuge was used, and filtering avoided. Such extracts, though they are not usually observed to turn litmus paper (without special precautions), turn more brilliant indicators when these are used in solution rather than on paper. By the use of these indicators it was found possible to measure the hydrogen-ion concentration of each soil extract, generally with two different indicators. The results so obtained agreed well with each other and with hydrogen-electrode measurements of the soils in suspension.

Some of the hydrogen-ion concentrations reported were pointed out as being rather high from a biological point of view. Since then numerous other soils have been found which show hydrogen-ion concentrations close to or even higher than the point where many bacterial species suffer injury. Sharp and Hoagland[2] found such soils, using an electrometric method very similar to that used in this laboratory. Gillespie and Hurst[3] found that such soils are very common in northern Maine, and were led to suggest that the relatively high hydrogen-ion concentration of the Caribou loam might be responsible for its relative freedom from potato scabs, a suggestion which has been strongly supported in the case of the common potato scab by a study, soon to be published elsewhere, of the growth of the causal organism at different hydrogen-ion concentrations. The existence of such soils would seem to place beyond question the widespread occurrence of "truly acid" soils, the acidity of which is *biologically* significant.

Sharp and Hoagland state "the work of Gillespie, paralleled by that presented in Table II, conclusively proves that there may be an excess of H ions in the solution bathing the soil particles." They also state that it is not necessary to associate soil acidity with physical adsorption.[4]

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[1] Gillespie, *J. Wash. Acad. Sci.*, 6, 7 (1916).

[2] *J. Agr. Res.*, 7, 123 (1916).

[3] *Soil Science*, 4, 313 (1917).

[4] It may be well to note that both Gillespie, and Sharp and Hoagland, found

Nevertheless, soil acidity has long been associated with adsorption, and the action of neutral salts on soils and the behavior of litmus paper with soils and with soil extracts appear to offer special difficulties.[1]

We wish, therefore, to describe some experiments on the action of the so-called humus on neutral salts and on the behavior of litmus paper toward various acid solutions, and to discuss the bearing of such experiments on the nature of soil acidity.

The Action of Humus on Neutral Salts.

Sharp and Hoagland found by means of the hydrogen electrode that the hydrogen-ion concentration of extracts of acid soils prepared with neutral chlorides is greater as a rule than that of extracts prepared with water. Barium chloride was more active than sodium or potassium chloride. Previous to this work we had regarded with suspicion many investigations on the titratable acidity of salt extracts of soil, since in many cases no mention was made of the purity of the salt used, and the presence of any salts of weak acids would have robbed the results of much of their significance, because an increase of hydrogen-ion concentration was not shown, but only inferred. We have also seen the effect of sodium chloride and sodium acetate on soils by the use of indicators, and have found that a soil may yield on extraction with sodium acetate much more titratable acid than on extraction with sodium chloride (this is the usual finding), and the hydrogen-ion concentration be the higher in the sodium chloride extract. When different concentrations of sodium acetate are used, it is found that the smaller the concentration, the smaller is the quantity of titratable acid liberated, but the greater is the hydrogen-ion concentration. This suggested that this process, though not necessarily purely chemical, as maintained by Truog,[2] has its chemical aspect, since it seems to be limited by high hydrogen-ion concentrations. In extending this work we chose humus rather than soil, and treated it with varying quantities of potassium, sodium or barium chloride, to find out whether there was any limiting hydrogen-ion concentration. There was no indication that the process was not practically instantaneous.[3]

certain so-called alkali soils to yield extracts which were alkaline without boiling off carbon dioxide. The term alkali soil signifies a soil containing salts of the alkali metals, and has no reference to alkalinity, except in the case of the so-called black alkali. Hoagland has recently reported experiments which suggest strongly that the relatively feeble degrees of alkalinity observed by the above workers may have however a distinct biological significance. (*Soil Science*, 3, 547 (1917).)

[1] J. E. Harris, *J. Phys. Chem.*, **21**, 454 (1917).

[2] *J. Phys. Chem.*, **20**, 457 (1916).

[3] We chose humus rather than soil for this purpose because humus extracts offer no such difficulties to electrometric measurement as are often offered by soil extracts. Such difficulties are stated by Sharp and Hoagland to be due to the nitrate content of soils; we confirmed this practically quantitatively for one soil by comparing the behavior of the extract with that of known solutions of the same buffer action and nitrate

Electrometric Measurements.—The measurements were made in a Clark electrode vessel provided with the modified three-way stopcock suggested by Cullen.[1]

The electrode vessel was filled with hydrogen, then partly filled with the fluid to be tested, and shaken mechanically for 5 minutes to bring about equilibrium. The shaking was then stopped, the liquid contact with saturated potassium chloride solution made with mixing, in a tube about 7 mm. in internal diameter, and the total potential measured at once with an accurate potentiometer of the low resistance type, against a well seasoned and tested saturated potassium chloride calomel electrode. In all the tables the potential recorded is this total potential difference, in millivolts. The platinum electrode was always freshly coated with palladium black after removal of the previous coating by nitric acid, and in the usual way washed with distilled water, subjected to cathodic polarization in dil. sulfuric acid, and washed again abundantly. According to Cumming and Gilchrist[2] and Clark and Lubs,[3] absolutely constant potentials are not in general observed, or to be expected, but the most reproducible and reliable value is obtained soon after liquid contact is made. As a rule the potential varies a little after liquid contact is made, but the change in 5 minutes is ordinarily small. In most cases we observed the changes after 5 minutes, the vessel not being shaken after the first measurement, and the electrode being totally immersed in the liquid. When the potential was inconstant will be noted below. The measurements were made in a constant temperature compartment provided with electrical temperature control set at 30°; for most of these experiments the electrical control was not used, but the temperature only slowly changed with the room temperature, generally changing not more than 0.5° in half a day, and the inconstancies of potential could not have been due to the temperature variations.

Orienting Experiments.—Humus was prepared from Scottsburg silt loam from Indiana as follows: The soil was extracted with 2% sodium hydroxide solution, the silt allowed to settle, the alkaline liquor siphoned off and treated with enough sulfuric acid to cause the formation of a brown flocculent precipitate. This was filtered off, redissolved in 1% sodium hydroxide, the solution filtered from residual silt and again acidified with sulfuric acid. The precipitate thus obtained was once more "purified" by the above procedure. Washed repeatedly with distilled water with the aid of a centrifuge, the preparation, which at first yielded clear supernatant fluids, began to go into colloidal solution and yielded increasingly denser

content. Very dilute sulfuric acid solutions were used for this. Solutions of greater buffer action do not show much disturbance due to such quantities of nitrates.

[1] Clark, *J. Biol. Chem.*, **23**, 475 (1915).

[2] *Trans. Faraday Soc.*, **9**, 174 (1913).

[3] *J. Biol. Chem.*, **25**, 479 (1916).

and more colored washings. After two washings the hydrogen-ion exponent (p_H of Sörensen) was determined neglecting diffusion potentials[1] and found to be 3.6; after 4 washings, 4.1; and after five, 4.15. After 5 washings a portion of it was washed with normal potassium chloride solution; the humus was precipitated, leaving the supernatant fluid water-clear, and the supernatant fluid had the exponent 3.3. On washing further with normal potassium chloride the exponent increased very slowly, reaching after the eighth washing the value 4.65. Some of the humus treated with potassium chloride was treated with dil. sulfuric acid to extract potassium, and washed 6 times with water, until the exponent of the last wash-water was 4.55, and when this material was then treated again with normal potassium chloride it yielded a supernatant fluid, the exponent of which was 3.26.

The original humus preparation was reprecipitated once more with sulfuric acid from alkaline solution, washed 4 times with water, until the last washing showed the exponent 4.1 and contained considerable quantities of humus, and then the humus was treated with normal potassium chloride. The exponent of the clear fluid resulting was then 3.46. The concentration of humus was not controlled well in these experiments, but the process seemed to a considerable degree both reproducible and capable of reversal.

Further Experiments.—In further experiments the procedure was the following: Ten cc. of humus suspension were treated with 10 cc. of saline solution of the desired concentration, the mixture permitted to settle a few minutes or centrifuged, and a portion decanted and examined electrometrically. In all cases the addition of salt precipitated the humus from its suspension or colloidal solution more or less completely. In the last experiment (No. 5) 10 cc. of a given mixture containing no humus were treated with the same volume of saline solution and the mixture examined. In all cases the concentrations reported for humus, salt, or other substance are calculated for the 20 cc. of mixture, assuming no change of volume on mixing. Since the Bjerrum extrapolation does not in general determine the diffusion potentials with certainty we have preferred to report all the results in terms of the total potential, rather than to apply the corrections for diffusion potentials and to calculate the hydrogen-ion exponents. Neglecting diffusion potentials, a decrease of total potential of 10 millivolts would correspond to a decrease of about 0.17 in the value of the exponent.

[1] The diffusion potentials of the supernatant fluids from this humus were estimated according to Bjerrum's principle (see Walpole, *J. Chem. Soc.*, 105, 2501 (1914)) and found to be 2 or 3 millivolts or less for the water suspensions, and less than 0.1 millivolt for the potassium chloride extracts. They were neglected in calculating the exponents.

Experiment 1.—The humus was prepared from Superior clay, a soil from Wisconsin, as follows: The soil was treated with 2% sodium hydroxide solution and the crude humus precipitated by acetic acid. The precipitate was redissolved in 1% sodium hydroxide solution, the silt filtered off, and the humus precipitated with acetic acid, washed with distilled water, and dried at 50°. This humus yielded 25.3% of ash, which contained carbonates, was strongly alkaline, and contained a large quantity of aluminium, a small quantity of iron, and traces of calcium. The humus contained 3.7% nitrogen, on the ash-free basis. Another reprecipitation of the humus did not affect appreciably the ash or nitrogen content. For use, the dried humus was once more dissolved in 1% sodium hydroxide solution, precipitated with acetic acid, washed sufficiently free from acid with the aid of a centrifuge, and suspended in distilled water. The mixtures represented 0.85% of humus. The results, obtained at 21°, are given in Table I.

TABLE I.—COMPARATIVE EFFECTS OF SODIUM AND BARIUM CHLORIDES ON HUMUS FROM SUPERIOR CLAY SOIL.

Salt.	Concentration in gram equivalents per liter.	Potential in millivolts.	Change of potential due to salt.
NaCl.............	0	546.5	..
	0.17	512.20	—34.3
	0.43	507.80	—38.7
	0.86	504.45	—42.0
	1.71	499.80	—46.7
	3.10	499.45	—47.0
$BaCl_2$.............	0	546.5	..
	0.007	508.0	—38.5
	0.04	499.2	—47.3
	0.14	495.5	—51.0
	0.21	495.7	—50.8
	0.36	496.5	—50.0
	0.72	496.8	—49.7

Table I shows large decreases in the value of the potential due to the addition of sodium or barium chloride to the humus suspension; these decreases of potential point to considerable increases of acidity. Neglecting diffusion potentials the hydrogen-ion exponent of the humus plus water was 5.1, of the distilled water, 6.2; and of 4 *M* sodium chloride solution, 7.7. The electrometric measurements of half-saturated barium chloride gave erroneous results as shown by indicator tests and as indicated by the electrometric behavior. The potentials drifted badly, as much as 9 millivolts in 5 minutes. The barium chloride solution was however certainly less acid than the humus itself. The mixtures of barium chloride and humus behaved much better, the drifts being about 1 millivolt in 5 minutes. The mixtures with sodium chloride showed drifts of about 0.3 millivolt in 5 minutes.

Measurements were also made with 0.17 and 1.7% humus, which need not be reported in detail. The results showed that the potential was lower, the more concentrated the humus. The lowest potential observed was 489 millivolts, found for a mixture of equal volumes of 3.4% humus and saturated barium chloride solution.

Experiment 2.—In this and the rest of the experiments potassium chloride, recrystallized from distilled water, was used instead of sodium chloride, and the barium chloride was recrystallized. The humus mixtures with the recrystallized barium chloride behaved very well, showing changes of potential of only 0.2 millivolt or less in 5 minutes; the behavior of the humus mixtures with potassium chloride was equally satisfactory. Solutions of either potassium or barium chloride without humus showed inconstant potentials which were only very roughly reproducible; the potentials however agreed with indicators in showing the solutions to be nearly neutral, compared with the humus suspensions. Such unsatisfactory electrometric behavior of neutral salts seems characteristic; the buffer action of many impurities would secure better behavior. Mixtures rich in neutral salts and poor in buffer action are very easily polarized. The temperature was electrically controlled at 30.0°. The results are given in Table II.

Table II.—Comparative Effects of Potassium and Barium Chlorides on Humus from Superior Clay Soil.

Salt.	Concentration in gram equivalents per liter.	Potential in millivolts.	Change of potential due to salt.
KCl	0	537.7	..
	0.02	524.4	—13.3
	0.10	514.15	—23.5
	0.40	508.4	—29.3
	1.00	504.95	—32.7
	2.00	501.7	—36.0
$BaCl_2$	0	537.7	..
	0.01	506.5	—31.2
	0.06	499.3	—38.4
	0.25	495.6	—42.1
	0.62	492.1	—45.6
	1.24	486.4	—51.3

Experiment 3.—A dried preparation of humus from Volusia silt loam was dissolved in dil. sodium hydroxide solution, filtered, then treated with conc. hydrochloric acid and the resulting precipitate washed on the filter. When the humus was washed by the use of a centrifuge the supernatant fluid obtained was itself a heavily colored colloidal solution. A sufficient volume of this solution was filtered through paper and used for the experiment. On filtering, the material passed through the paper, leaving only a slight stain, which also passed through when washed with a little distilled water.

Ten cc. of colloidal solution were treated with a mixture of 0.5 cc. of 30% potassium chloride solution plus 9.5 cc. of distilled water. The humus appeared to be completely precipitated, leaving the supernatant fluid clear but not quite colorless. This fluid showed a potential 21 millivolts lower than that shown by 10 cc. of colloidal solution plus 10 cc. of water. When 10 cc. of colloidal solution was treated with a mixture of 9.5 cc. of the potassium chloride solution and 0.5 cc. of water, the supernatant fluid obtained was quite colorless and showed a potential 16 millivolts lower than that found when the smaller quantity of potassium chloride was used. To determine whether this additional change of 16 millivolts was due to a continued reaction of potassium chloride with humus already precipitated, we treated the colloidal solution with potassium chloride solution in the proportions: 10 cc. colloidal solution plus the smaller quantity of potassium chloride solution (0.5 cc.), and removing the precipitated humus before making up the volume to 20 cc., then, taking 10.5 cc. of the supernatant fluid from this precipitation, we made up the volume in one case with 9.5 cc. of distilled water and in another case with 9.5 cc. of the potassium chloride solution. We found then that of these two preparations the one with more potassium chloride showed a potential 12.5 millivolts lower than the other. Considering that the humus was not precipitated in quite the same concentration, and that a little more humus was precipitated on the addition of the larger quantity of potassium chloride, it appears that nearly the same change of potential is produced by the increase of potassium chloride whether the mass of humus is present or not; in other words the continued effect on the potential of the larger quantities of potassium chloride, after the precipitation is practically complete, is not due to a continued reaction with precipitated humus, but mainly to something else, (possibly to effects such as those which are the subject of Expt. 5). The experiment, performed at 23°, is given in Table III. The supernatant fluid mentioned in the table is the one obtained by treatment of 20 cc. of colloidal solution with 1.0 cc. of the 30% potassium chloride solution and centrifuging. In the table, "KCl" means the 30% solution. All the mixtures were centrifuged before testing.

TABLE III.—PROTOCOL OF EXPERIMENT 3.

No.	Mixture.	Potential in millivolts.	Potential after 5 minutes.
1	10 cc. colloidal solution + 10 cc. H_2O	449.2	448.9
2	10 cc. colloidal soln. + (0.5 cc. KCl + 9.5 cc. H_2O)	428.15	428.10
3	10 cc. colloidal soln. + (9.5 cc. KCl + 0.5 cc. H_2O)	412.25	412.15
4	10.5 cc. supernatant fluid + 9.5 cc. H_2O	434.50	434.40
5	10.5 cc. supernatant fluid + 9.5 cc. KCl	422.05	422.00
6	10 cc. H_2O + 10 cc. KCl	587.0	613.0

Experiment 4.—A colloidal suspension was prepared from the same material as that used in the previous experiment, and the comparative effects

of potassium chloride and barium chloride on it were studied as in Expts. 1 and 2, and all the diffusion potentials were estimated by the Bjerrum extrapolation. The measurements were made at 24°. The results are shown in Table IV.

TABLE IV.—COMPARATIVE EFFECTS OF POTASSIUM AND BARIUM CHLORIDES ON VOLUSIA HUMUS.

Salt.	Concentration in gram equiv. per liter.	Appearance of supernatant fluid.	Potential in millivolts.	Potential after 5 minutes.	Change of potential due to salt.	Diffusion potential correction in millivolts.
0	0	thick	463.5	463.1	..	—0.4
KCl	0.02	thick	449.45	449.40	—14.0	—0.15
KCl	0.2	clear but yellow	441.35	441.25	—22.1	—0.05
KCl	1.0	very light color	434.30	434.25	—29.2	—0.03
$BaCl_2$	0.02	still less color	436.85	436.85	—26.7	—0.05
$BaCl_2$	0.2	almost no color	430.30	430.35	—33.2	+0.83
$BaCl_2$	1.0	same	420.70	420.70	—42.8	+2.0

As in Expt. 2, barium chloride exerts a considerably greater effect than potassium chloride at equivalent concentrations on the potential and also on the precipitation of the humus. Some of the difference in the effects on the potential is due however to diffusion potential, but how much may be due to this can not be stated at present, since the Bjerrum extrapolation may not be exact for these mixtures, though the sign of the correction is probably reliable. Fairly large differences were seen by Harned in the action of potassium and barium chlorides on the hydrogen electrode potential of decinormal hydrochloric acid; these differences were attributed by him to differences of diffusion potential (he did not apply the method of Bjerrum, but calculated the diffusion potentials).[1]

The fact that such differences can be observed in the absence of colloidal or undissolved substance makes it unsafe to conclude that the barium reacts more extensively with the humus than does potassium. The same applies to sodium.

Experiment 5.—A number of solutions were tested in an effort to find a mixture which would give with potassium chloride effects on the hydrogen electrode potential comparable with those seen in the case of humus. The procedure was similar to that used for the humus preparations; 10 cc. of the given mixture were treated with 10 cc. of potassium chloride solution. The results are shown in Table V. The concentrations of potassium chloride and other substances entered in the table are those calculated for the mixture examined, assuming no change of volume to occur on mixing. The mixtures were not prepared with precision, but the results are comparable with those obtained with humus. As in the previous cases, the total potential observed between the hydrogen electrode and the saturated potassium chloride calomel electrode is given in millivolts.

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[1] THIS JOURNAL, 38, 1986 (1916). The calculation in our case can not be made.

TABLE V.—EFFECT OF POTASSIUM CHLORIDE ON THE HYDROGEN ELECTRODE POTENTIAL (INCLUDING DIFFUSION POTENTIAL) OF VARIOUS SOLUTIONS.

	Mixture.	KCl. mols per liter.	Potential in millivolts.	Potential after 5 minutes.	Change of potential due to salt.	Diffusion potential correction, in millivolts.
1	0.102 *N* HCl	0	312.55	312.45	..	—3.4
		0.1	312.20	312.05	— 0.3	—3.25
		1.0	309.40	309.25	— 3.1	—2.0
		2.0	301.40	301.15	—11.1	—1.55
2	0.001 *N* HCl	0	446.3	446.6	..	—0.3
		0.1	445.65	445.70	— 0.6	0.0
		1.0	442.25	442.25	— 4.0	..
		2.0	438.70	437.95	— 7.6	0.00
3	0.01 *N* H_2SO_4	0	373.4	373.55	..	+0.3
		0.2	372.2	..	— 1.2	..
		1.0	367.75	367.70	— 5.7	—0.25
4	0.001 *N* H_2SO_4	0	428.5	428.5	..	..
		0.2	427.55	..	— 1.0	..
		1.0	426.8	..	— 1.7	..
		2.0	419.3	418.8	— 9.2	..
5	0.013 *N* $Al_2(SO_4)_3$, 0.0005 *N* H_2SO_4	0	473.2	47315	...	0.0
		0.2	477.9	..	+ 4.7	0.0
		0.8	482.5	482.8	+ 9.3	..
		2.0	476.5	478.5	+ 3.3	0.0
6	0.05 *M* H_3BO_3, 0.0085 *N* NaOH	0	745.8	746.0	..	—0.2
		0.2	737.40	737.60	— 8.4	—0.2
		0.8	731.80	732.10	—14.0	—0.05
		1.4	729.45	729.80	—16.3	..
		2.0	727.65	727.95	—18.1	0.0
7	0.05 *M* H_3BO_3	0	613.0	614.0	..	—1.4
		0.2	577.0	582.0	—36.0	—0.05
		0.8	614.0	629.0	+ 1.0	0.0
		1.4	616.0	628.0	3.0	0.0
		2.0	603.0	622.0	—10.0	0.0
8	0.05 *M* H_3BO_3, 0.00002 *M* H_2SO_4	0	530.1	530.7	..	..
		0.2	519.8	521.8	—10.3	..
		1.0	581.5	604.0	+51.4	..
		2.0	561.0	593.0	30.9	..
9	0.05 *M* H_3BO_3, 0.005 *M* H_2SO_4	0	372.0	372.15	..	—0.1
		0.2	371.9	..	— 0.1	..
		1.0	367.05	367.50	— 5.0	—0.25
10	0.067 *M* phosphate	0	518.65	518.45	..	—0.4
		0.2	507.80	508.25	—10.8	—0.3
		1.0	505.0	505.0	—13.6	—0.2
		2.0	500.0	502.0	—18.6	..
11	0.0067 *M* phosphate	0	541.3	544.0	..	..
		0.2	523.2	527.1	—18.1	..
		1.0	536.0	546.0	— 5.3	..
		2.0	525.0	541.0	—16.3	..

The first set of mixtures, which were 0.102 N with hydrochloric acid and contained varying concentrations of potassium chloride, gave results close to Harned's precise results for 0.1 N hydrochloric acid, except that the change of potential found here for 2 mols of potassium chloride is somewhat higher. In the second set the concentration of the acid is smaller, 0.001 N, but the effects are of the same order of magnitude, compared with the larger humus effects, as may be seen in Fig. 1. Mixtures 3 and 4 contained two concentrations of sulfuric acid, and the effects are about as large as those shown by hydrochloric acid. Mixture No. 5 contained aluminum sulfate, filtered clear, and a trace of sulfuric acid added; the changes of potential were of opposite sign to those of the rest of the mixtures, and pointed to a decrease of hydrogen-ion concentration. The next four mixtures contained 0.05 mol of boric acid per liter but had different initial hydrogen-ion concentrations, and because of this fact they should also possess different buffer capacities—in fact No. 6 and No. 9 should have a considerable buffer action, whereas Nos. 7 and 8 should have very little. Corresponding to these differences were great differences in electrometric behavior: Nos. 6 and 9 were capable of satisfactory measurement; and Nos. 7 and 8 showed very serious drifts of potential in 5 minutes after the first measurement, and the changes of potential due to addition of potassium chloride were apparently very high, but very irregular and of course uncertain because of the inconstancy of the potential. Mixture No. 6, of partially neutralized boric acid, showed fairly high effects: these are not shown in Fig. 1 in part because such a mixture could not be present at the hydrogen-ion concentrations of the humus preparations and also because as large effects were seen in the case of Mixture No. 10. Mixtures 10 and 11 were examined because Palitzsch reported, incidentally in his study of methyl red,[1] large effects on the hydrogen-ion concentration of "0.25 sec." phosphate mixtures when sodium chloride was added; the effects were of

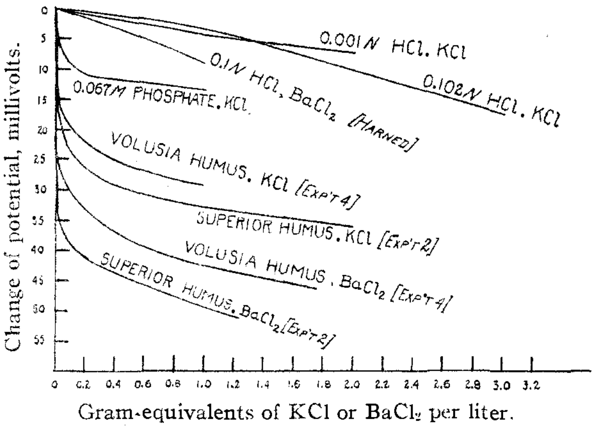

Fig. 1.—Changes of potential resulting from the action of salts on humus or on solutions. A drop in the curve means a fall in the potential.

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[1] *Biochem. Z.*, 37, 131 (1911).

the same order of magnitude as our humus effects. The same mixture of phosphates was used by us in these experiments, *i. e.*, Na_2HPO_4 and KH_2PO_4 in the molecular ratio 0.25 to 9.75, and the final phosphate concentrations, after addition of the potassium chloride solution, were the same as in his experiments. In the case of mixture No. 10, the electrometric behavior was satisfactory; such a mixture could exist at the hydrogen-ion concentrations of the humus preparations, and the effects are fairly large, as shown in the figure. In the case of the more dilute mixture, No. 11, the effects are about as large, but irregular, and the potential inconstant.

Fig. 1 shows the comparative effects of potassium and barium chlorides on humus preparations and on some solutions. Harned's values for the effect of barium chloride on 0.1 *N* hydrochloric acid are also given. Diffusion potential corrections were not applied in any case. Not all the results can be clearly shown in one figure, but the most significant results are shown. It may be well to note that the curve for Superior clay humus and sodium chloride would very nearly coincide with the curve shown for Volusia humus and barium chloride, so that with the Superior clay humus the spread of the curves for the three salts resembles the spread of the curves found by Harned for 0.1 *N* hydrochloric acid and the same three salts.

There seems to be no reason to doubt that the large decrease of potential seen when potassium chloride is added to humus is due to a large increase of hydrogen-ion concentration, which in turn is due mainly to a reaction between the potassium chloride and the humus. The action of salts on the hydrogen-ion activity of true solutions and the uncertainties due to diffusion potentials make it difficult to draw any conclusions as to the mechanism of the reaction between salts and humus.

The mechanism of the reaction between soils and neutral salts is in dispute. Harris maintains that when a portion of soil is repeatedly extracted with saline solution, the total acid substance yielded by sodium chloride or potassium nitrate is very much less than that yielded by sodium acetate.[1]

He mentions a difference in the total acid yielded by potassium nitrate and sodium chloride (this difference is small compared with the difference seen by him in the action of ether and sodium acetate), and points out that it is difficult to account for the liberation of so much acid on the assumption that the reaction is purely chemical, and insists that it is a case of adsorption, following the typical adsorption curve. It is fair to point

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[1] *J. Phys. Chem.*, **18**, 355 (1914); **21**, 454 (1917). The total acid substance can not of course be actually measured, because only a certain fraction is given off at each treatment, but the totals may be comparatively estimated for different salts by comparing the rates. Such an experiment would seem to demand careful attention to technique, especially with regard to endpoint because of the buffer action of strong solutions of sodium acetate, in order that no accumulative error be introduced at each titration. No difficulties were reported by Harris.

out that the fact that sodium acetate reacts so much more extensively with soil than does potassium nitrate or sodium chloride (in a single treatment) may well be due to the buffer action of sodium acetate, which permits the liberation of a considerable quantity of acetic acid without too high a hydrogen-ion concentration resulting. We also appreciate the difficulty of accounting for the large apparent increase of hydrogen-ion concentration occurring when humus is treated with potassium chloride, on the assumption that the process is purely chemical, but we do not seek to draw a sharp line between chemical and adsorptive processes.

Even if such a sharp line could be drawn, and the liberation of acid from neutral salts by soils or humus be placed in a category of purely adsorptive processes, we do not see how this would indicate the absence of truly acid bodies in soils that exhibit the adsorptive property. Truly acid and adsorptively unsaturated bodies may be present together, and indeed probably are. The fact given by Harris that repeated extraction of soils with saline solutions gives different total results according to the nature of the salt would go to show that we can not sharply titrate in this (indirect) way the quantity of acid substance. It is common knowledge that different methods of determining lime requirements (these are titrimetric methods in principle) give different results, and this of itself has led to the suspicion that the acidity of soils is of a peculiar kind, and not a "true acidity." Truog[1] maintains that soils may be titrated with various bases with the same result, or treated with various chlorides with the liberation of equal quantities of acid, if suitable experimental conditions be chosen. Harris replies in effect that the personal equation enters into the choice of conditions.[2] It is time to recognize that titratability is not a criterion by which the presence of true acids in all mixtures can be tested. We know from the modern theory of titration[3] that acids can not always be titrated in mixtures. Thus acetic acid can not be accurately titrated in mixture with any substance having a large buffer effect at the turning point of phenolphthalein, nor can the total quantity of acid substance always be titrated in such mixtures. It is apparent that the behavior of soils toward attempts to determine the quantity of acid substance offers no evidence whatever against the presence of true acids in the so-called acid soils.

These considerations do not discredit the usefulness of such titrimetric methods as that of Veitch. Such a method is capable of determining the quantity of lime which must be added to bring a soil to an approximately neutral condition, if neutrality is considered not in the stoichiometrical sense, but in the physico-chemical sense. Whether such methods can serve to solve fundamental problems of soil fertility is doubtful, since

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[1] *Loc. cit.* and *J. Ind. Eng. Chem.*, **8**, 341 (1916).

[2] *J. Phys. Chem.*, **21**, 454 (1917).

[3] See for instance Hildebrand, THIS JOURNAL, **35**, 847 (1913).

titrimetric methods have been found insufficient in other fields of applied biology, notably bacteriology.[1]

It is of course true, as suggested by Harris, that the small quantity of acid which is extracted from soils by water and detected by means of the hydrogen electrode (or by suitable indicators) can not be the *cause* of the liberation of the large quantities of acid when the soil is treated with a salt. Neither can it be the cause of the binding of large quantities of lime or other bases. Actually, the hydrogen-ion concentration of soil extracts is *dependent* in high degree on the undissolved acidic or adsorptively unsaturated substances, and if these are permitted to bind large quantities of lime or other bases, the soil extract which may then be obtained will have a very different hydrogen-ion concentration. In any case, with mixtures such as soil, the hydrogen-ion concentration can not in general be said to be *due* to any one acid substance, and, since soil acidity has a manifold connotation, we can not safely make at this time any broad statements as to the "cause of soil acidity."

The Behavior of Litmus Paper.

The fact that water extracts of many soils apparently fail to turn litmus paper, though the litmus paper may promptly be turned if pressed into close contact with the moist soil, has led to the statement that the action is a purely adsorptive process and therefore incapable of demonstrating acidity in a chemical sense. The further conclusion has also been drawn that truly acid soils do not in fact exist, save in the rare cases where the litmus paper is turned by the soil extract. Ramann[2] for instance substitutes the term "adsorptively unsaturated" for the term "acid" soil.

The view that when soil acts on litmus paper the soil selectively adsorbs the base of the blue litmus salt, leaving the red dye on the paper, has recently been stated by Harris[3] to constitute a plausible explanation of the action. It seems to us that some such explanation is conceivable but hardly plausible, as the actual mechanism of the process would offer difficulties. Furthermore there is no logical necessity of giving one and the same explanation for the action of litmus paper and the action of neutral salts on soils. The behavior of litmus paper can be explained in a simple way by means of the following experiments, which illustrate strikingly some of the principles involved in the application of litmus paper, principles which have been discussed in a very thorough manner by Walpole.[4]

We prepared a solution containing 0.5 cc. of 0.2 N hydrochloric acid per liter, *i. e.*, a 0.0001 N solution, using ordinary distilled water. When 5 cc. of this solution were treated with 2 drops of an aqueous solution of methyl

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[1] See Clark and Lubs, *J. Bact.*, **2**, 1, 109, 191 (1917).

[2] E. Ramann, "Bodenkunde," 3rd edition, Berlin, 1911.

[3] *J. Phys. Chem.*, **21**, 454 (1917).

[4] *Biochem. J.*, **7**, 260 (1913).

red[1] a red color instantly developed, which enabled us to measure the hydrogen-ion concentration in the usual way, by comparison with standards. The hydrogen-ion exponent was found to be 4.8.[2] When strips of Squibb's sensitive neutral or the blue litmus paper were placed in the hydrochloric acid solution, *no changes*, relative or absolute, could be observed in the papers at the end of 5 minutes, a control experiment also being performed with distilled water. The acid solution showed a faint pink tinge due to the leaching of the litmus coloring matter. At the end of 13 minutes, both papers in the acid solution had acquired only a slight pink tint around the edges. When however neutral and blue litmus paper strips were placed in *M/20 phthalate buffer mixtures* having a hydrogen-ion exponent of 4.8, they were instantly reddened.

The contrast was even more striking in the case of another solution which was prepared by diluting one drop of 0.2 *N* hydrochloric acid to one liter. Colorimetric test of this solution with methyl red showed an exponent of 6.1, with bromocresol purple an even more acid exponent, 5.8. Colorimetric test of the distilled water which was used as a control, showed an exponent of 6.3 with bromocresol purple; with methyl red the color was a yellow which could not be used for measurement, as it lay outside the useful range. The experiment with litmus strips was similar to the one just described. At the end of one hour no changes could be seen in any of the papers when removed from solution and examined at once. After 2 hours a slight reddening was beginning to show in spots on both the neutral and the blue papers, in both the acid solution and the distilled water, the effect of the acid solution being but very slightly greater than the effect of the water. Now when blue and neutral litmus papers were dipped into the buffer mixtures it was found that not only the buffer mixture having a hydrogen-ion exponent of 5.8, *but even the buffer solution having the very much less intense acidity of 6.8 reddened the papers instantly*. The 6.8 buffer mixture did not appreciably affect the color of red litmus paper of the same make. The 7.0 (neutral) buffer mixture turned both red and blue papers instantly to an intermediate tint.

These experiments show that the speed with which litmus paper turns depends in very large measure on the buffer action of the solution under test, and one may easily be led to absolutely wrong conclusions as to the relative intensity of acidity in various solutions, soil extracts, or even moist soils, if this factor is left out of consideration. Unless special precautions are taken, litmus paper can not be used to arrange a group of soils according to their relative acid intensities.

The importance of the buffer factor is due to the fact that the litmus

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[1] Prepared by dissolving methyl red in a slight excess of alkali and diluting.

[2] This means that the hydrogen-ion *concentration* was $1/10^{4.8}$. It is more convenient to use only the exponent, however, the exponent 7 indicating neutrality.

paper has a certain acidity or alkalinity of its own which has to be changed by the action of appreciable quantities of alkali or acid before the paper can show the reaction of the fluid in which it is immersed. All indicators have this failing to some extent, but litmus, even in solution, is a poor indicator, and on paper it is even worse from this point of view.

The reason why moist soil turns litmus paper far more readily than does the soil extract is simple, and to harmonize its behavior with the presence of "true acidity" in soils does not require, as Harris suggests, that the hydrogen ions must be bound in a mysterious manner to the soil particles. The solubility of the acidic substances of most soils is very small. When litmus paper is dipped into a soil extract the reaction of the extract in the neighborhood of the paper is changed in overcoming the reaction of the litmus paper itself. If a small volume of extract is used, much of its acid substance may be thus used up; if a sufficiently large volume is used, time will be required for diffusion, a greater quantity of dye may leach from the paper, and a negative result still be obtained. When the litmus paper is brought into contact with the moist soil, the acid used up by the litmus paper is supplied again by the soil particles to the solution *near the litmus*, and the speed of the process may be infinitely greater.

There is therefore no ground for classifying soils into two types, "truly acid," and otherwise, by means of the behavior of litmus paper toward the soil extract and the moist soil, as was done by Harris in his more recent article.

The work of Walpole would lead us to suppose that many soil extracts thought to be neutral to litmus might in fact turn litmus paper if a good paper be used and exposed to a considerable volume of the extract for a sufficient length of time. He allowed in some cases 24 hours to elapse. However, in addition to the difficulties mentioned by him we have in many soil extracts, even after the use of a centrifuge, the further difficulty due to the obscuring effect of floating and suspended material. To filter the extracts through porcelain candles or even through paper before testing is of course inadmissable, unless it can be shown that the treatment does not change the reaction.

Soil chemists do not as yet generally realize the progress which has been made in the development of brilliant and reliable indicators. A careful treatment has recently been given by Clark and Lubs,[1] who themselves prepared new and useful indicators,[2] which we found useful in soil work. Paranitrophenol was discarded in this laboratory in the first preliminary experiments, though recently stated to be a splendid indicator by Christensen,[1] who wrote without knowledge of the newer indicators or

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[1] *J. Bact.*, **2**, 1, 109, 191 (1917).

[2] *J. Wash. Acad. Sci.*, **5**, 609 (1915); **6**, 481 (1916).

[3] *Soil Science*, **4**, 115 (1917).

of the application of them together with the hydrogen electrode to soil study.

The litmus paper test, as applied to moist soil, is simple, and with suitable precautions it can be made to yield information of some practical value. It is likely that some other indicator will eventually be found more useful. The choice of a simple test should await more definite information on the relation of hydrogen-ion concentration to the various biological activities of soil.

Summary and Conclusions.

The action of humus on solutions of sodium, potassium, and barium chlorides of different strengths was studied by means of the hydrogen electrode. The measurements showed that on the addition of chlorides to humus solutions or suspensions the potential of the gas chain was very considerably lowered, showing that there was a large increase of hydrogen-ion concentration.

Similar effects of smaller magnitude were seen in the action of potassium chloride on true solutions in the absence of humus or undissolved substance; these effects make it unsafe to draw conclusions as to the detailed mechanism of the process in the case of humus.

At equivalent concentrations barium chloride exerted the greatest effect on the potential of humus preparations. The direction of the diffusion potentials and the uncertainty of their magnitude would seem to make it unsafe to conclude from these experiments that barium reacts more extensively with humus than potassium.

Some experiments with litmus paper served to call attention to the difficulties and limitations of the litmus paper test as applied to soils and soil extracts. These experiments make it plain that there is no ground for a recent classification of soils which respond in the moist state to blue litmus paper into two types: "truly acid" and otherwise, according to the behavior of the litmus paper toward the aqueous soil extract.

Litmus paper can not be used, without suitable precautions, to arrange soils in the order of their intensities of acidity.

No proof has yet been brought forth that soils can act on litmus paper solely by virtue of their adsorptive capacity. The behavior of litmus paper toward the moist soil and the soil extract can be explained in a simple way without reference to adsorption.

It has been pointed out that it is dangerous to make broad general statements as to the cause of soil acidity.

Against the positive evidence, obtained by means of the hydrogen electrode and suitable indicators, in favor of the common occurrence of truly acid soils, there has been produced no valid negative evidence, based on adsorption or on the behavior of soils toward neutral salts. Truly acid soils are not rare, but very common and widely distributed in the United States.

[Contribution from the Carbohydrate Laboratory, Bureau of Chemistry United States Department of Agriculture.]

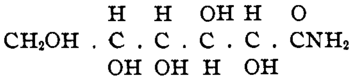
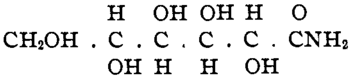
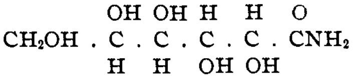
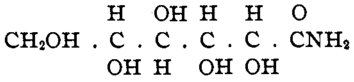
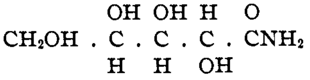
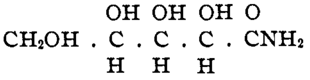
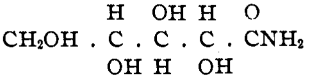
THE ROTATORY POWERS OF THE AMIDES OF ACTIVE α-HYDROXY ACIDS.

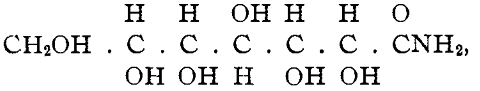
By C. S. Hudson.

Received February 13, 1918.

Weerman[1] has recently published measurements of the rotatory powers in water of the amides of seven monobasic acids of the sugar group. A comparison of the configurations that have been established for these acids or their amides by Fischer with their rotations is shown in the following table:

Table I.—Configurations and Specific Rotations.

Amide.	Fischer's configuration.	α-Carbon OH position.	Specific rotation.
d-Gluconic amide.......	 $\mathrm{CH_2OH\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{O}{C}NH_2}$	Below	+33.8°
d-Galactonic amide.....	 $\mathrm{CH_2OH\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{O}{C}NH_2}$	Below	+36.7°
l-Mannonic amide......	 $\mathrm{CH_2OH\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{O}{C}NH_2}$	Below	+29.9°
d-Gulonic amide........	 $\mathrm{CH_2OH\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{O}{C}NH_2}$	Below	+16.1°
l-Arabonic amide.......	 $\mathrm{CH_2OH\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{O}{C}NH_2}$	Below	+38.4°
l-Ribonic amide........	 $\mathrm{CH_2OH\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{O}{C}NH_2}$	Above	—15.7°
d-Xylonic amide.......	 $\mathrm{CH_2OH\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{O}{C}NH_2}$	Below	+44.5°

It will be seen that *the dextrorotary amides have the hydroxyl group below the asymmetric α-carbon atom, while the levorotary ones have it above.* Recently Dr. E. Yanovsky has measured for me the rotation of *d*-α-glucoheptonic amide,  $\mathrm{CH_2OH\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{OH}{\underset{H}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{H}{\underset{OH}{C}}\,.\,\overset{O}{C}NH_2}$, and Dr. L. H. Chernoff

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[1] Dissertation "Over de Inwerking van Natriumhypochloriet op Amiden van Onverzadigde Zuren en Oxyzuren," published by A. H. Kruyt, Amsterdam, 1916.