calibrated at a single voltage. The 1-v Weston cell was used by Petering and Daniels (*2*), Manning (*1*), and Spoor (*3*). Although this is a convenient source of constant potential difference, it places the experiment at a region of the polarogram where the slope is steep (Fig. 1). Slight voltage changes in the system could thus cause considerable variations in the current at a constant level of oxygen. This hazard could be avoided by working at 1.6 v, where the current is relatively independent of the voltage over a wide range. Beckman (*4*) used the first oxygen reduction wave at 0.42 v, but the sensitivity to oxygen in this range is about half that at 1.6 v, and it is characterized by a very pronounced maximum (*5*).

The limiting currents (at 1.6 v applied) for each of the oxygen concentrations of Fig. 1 are shown in Table 1. There was a linear relationship between oxygen concentration and limiting current at 5.78–9.63 ppm with a slope of 0.44–0.46 μa/ppm. The ratio 0.32 at 1.43 ppm suggests a departure from linearity between low and high levels of oxygen. Variations in the residual current, however, could account for this discrepancy (*5*). A polarogram by Kolthoff and Lingane (*5*) indicated a sensitivity for oxygen of 0.67 μa/ppm, or about 45% greater than that obtained in the present experiments.

TABLE 1

LIMITING CURRENTS OF VARIOUS CONCENTRATIONS OF OXYGEN IN LAKE WATER

Oxygen (ppm)	Limiting current	
	μa	μa/ppm
1.43	0.46	0.32
5.78	2.63	0.46
8.62	3.89	0.45
9.63	4.20	0.44

The great sensitivity of the method is evident in the above data. However, troublesome variations made the present application unsuitable for critical determinations of respiration. A typical defection is shown in the dotted line of Fig. 1. At constant oxygen level (8.6 ppm) the galvanometer readings over the oxygen range were depressed as much as 3 cm from the usual values coincident with an observed increase in the mercury drop rate. This variation was associated with the diffusion of oxygen at the mercury surface, for it had its effect only below 2.2 v.

A supporting electrolyte was not used in the experiments; hence the wave height may have varied with the electrolyte content of the water at constant oxygen concentration. The seepage of potassium chloride from the calomel half-cell may be one source of trouble, although Spoor (*3*) recommended such a system to abolish variations in the limiting current at constant voltage.

Petering and Daniels (*2*) hoped to cancel out fluctuations in the readings that were due to temperature and accidental variations in the amounts of iron and other easily reducible substances by determining the current at 1.0 and 0.1 v. The oxygen concentration was then related to the difference between the currents flowing at these two voltages. However, in the present experiment (Fig. 1) there was a change of 12 cm in the galvanometer deflection at 0.1 v when the oxygen concentration was varied from 1.4 to 9.6 ppm. Also, the half-wave potential of the first oxygen wave may be expected at about – 0.05 v vs. the saturated calomel electrode (*5*). The 0.1 v reading is thus well above the decomposition potential for oxygen and should not be used to represent the residual current—that current which would flow in an oxygen-free solution at the working voltage.

The temperature coefficient of the process at 1.0 v was 1.3%/C degree at 4.3 ppm, and 1.6% at 8.0 ppm of oxygen. The latter value is equal to that applied by Manning (*1*), and both are within the range 1.3–1.6/degree quoted by Kolthoff and Lingane (*5*). Temperature and other variations that are a function of the total current obviously cannot be accounted for by the mere difference between the limiting and residual currents. A proportionality factor would have to be introduced. Frequent standardization against an air-saturated test solution (*5*) may yield more favorable results.

The dropping mercury electrode can be used to measure oxygen in flowing, unmodified lake water. However, the many variables which must be controlled or accounted for to obtain precise results render the method exceedingly difficult to apply in this manner.

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Relationship of Colloids to the Surface Tension of Urine

Robert A. Ravich

Institute of Applied Biology, Brooklyn, New York

The idea that colloids in the urine are of major importance in preventing precipitation, agglomeration, and conglomeration of crystalloids from a supersaturated solution has recently aroused renewed interest through the work of Butt and his associates. Ebstein in 1884 first suggested that the urinary crystalloids remain in the dissolved state through the protective action of the urine colloids (*1*).

Butt appears to have been the first to suggest that this protective action might be applied to the treatment of renal lithiasis (*2*). He found that the number of colloid particles visible in the urine when examined

under the ultramicroscope is increased by the administration of hyaluronidase (*3*), and presented clinical evidence which indicated that hyaluronidase may effectively delay or stop the formation of new stones in patients who recurrently form stones (*4*).

Butt, Hauser, and Seifter (*4*) attribute the protective effect of the colloids to the influence they exert upon the surface tension of the urine. They found that hyaluronidase both increased the number of colloids and decreased the surface tension of the urines of patients treated with this enzyme.

During the past six years the surface tension values of serial urine specimens of a large number of patients have been routinely followed in this laboratory. A capillary tube calibrated to permit direct reading of the surface tension in dynes/cm has been employed. The design, calibration, and use of this urotensiometer have been described by Revici (*5*). Determination of the surface tension with this instrument is as simple as the reading of a clinical thermometer, and can be made a routine part of any urinalysis.

The urinary excretion of tensioactive agents has been found to be altered in various disease states (*6*), but the exact nature of these tensioactive substances is unknown. It therefore seemed worth while to determine whether the urinary colloids could be directly related to the surface tension, as suggested by Butt, Hauser, and Seifter (*4*), using the urotensiometer for this purpose.

Consecutive urine specimens from 50 persons were used. A drop of urine was placed on a clean glass slide, covered by a clean cover glass, and examined under the ultramicroscope. The colloid particles are readily identified as tiny pinpoints of light possessing erratic Brownian motion. The number of these particles in an average high dry field (×440) was estimated, and the specimens were divided into groups according to the number of colloid particles present.

The surface tension of the same urine specimens was determined, using the urotensiometer. The tip of the calibrated capillary tube was introduced into the specimen, and the urine was drawn up above the top mark of the scale by gentle mouth suction and then expelled twice in order to clear the lumen of the previous specimen. Urine was then drawn up a third time to the same level, and the tube removed from the specimen and read. The level at which the descent of the column first came to a stop or where the rate of descent was distinctly slowed was recorded as the surface tension of the specimen in dynes/cm (*5*). The urine specimens were divided into groups according to the surface tension values found.

When the urine specimens were grouped according to the number of colloids rendered visible under the ultramicroscope, 4 specimens showed no colloid activity, 11 showed 1–5 colloid particles/high power field, 5 had 6–20 particles, 15 had 21–40 colloid particles, and 15 had more than 40/high power field.

When the urines were divided on the basis of the surface tension values of the specimens, 21 had surface tension values of 70–72 dynes/cm, 13 had values of 67–69 dynes/cm, and 16 had values below 66, ranging as low as 60 dynes/cm.

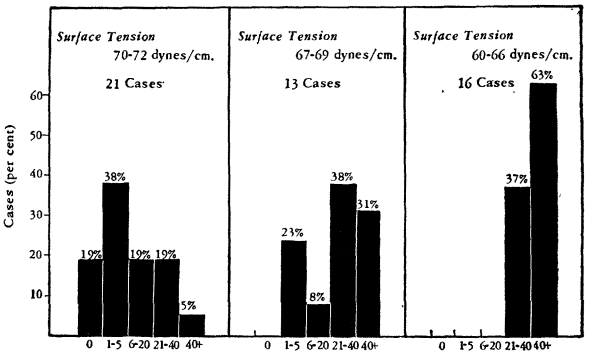

FIG. 1. Number of colloids per high power field.

In Fig. 1, the correlation between the number of colloid particles/high power field and the surface tension of the urine is shown graphically. It can be seen that the urines having surface tension values of 66 dynes/cm or less contained 21 or more colloid particles in each high power field. Conversely, those urines which showed no colloid activity had surface tension values of 70–72 dynes/cm. The urine specimens with 1–20 colloid particles/high power field all had surface tension values above 67 dynes/cm. However, some urine specimens with a high level of colloid activity (i.e., more than 20/high power field) had surface tension values of 70–72 dynes/cm.

Our findings indicate that there is a correlation between the number of colloids present and the surface tension of individual urine specimens. Generally, urine specimens exhibiting a high degree of colloid activity have low surface tension values, whereas specimens in which little or no colloid activity is apparent have high surface tension values. Similar findings apparently led Butt, Hauser, and Seifter (*4*) to attribute the protective action of the urinary colloids to an influence exerted by the colloids upon the surface tension of the urine.

The finding that a significant number of urine specimens in the group studied had high surface tension values, even in the presence of a large number of colloid particles, indicates that the colloids do not actually determine the surface tension of the urine. Thus, although a general inverse relationship exists between the number of colloid particles and the surface tension of the urine, it appears that the surface tension is not determined by the number of colloids present, or vice versa.

Butt and his associates observed that administration of hyaluronidase produced a higher degree of colloid activity and a reduction in the surface tension of the urine of treated patients (*4*). Our findings indicate that the apparent retardation of stone formation observed in the patients treated with this enzyme might have been due to the reduction in surface tension, the increase in colloid activity, or perhaps to some other unknown factor fundamental to both.

In pursuing this aspect of the stone problem further, it would be of interest to study the surface tension values along with the degree of colloid activity and to determine whether some common factor influences both of these. In the past, surface tension determinations have been difficult to perform, requiring considerable time, intricate instruments, and the solution of complicated formulas. The simple urotensiometer designed by Revici (*5*) obviates these difficulties and makes it possible to perform accurate determinations on all urine specimens as a routine procedure. Preliminary studies have indicated that urine surface tension determinations may increase our knowledge of the mechanisms involved in stone formation and may be helpful in the management of renal lithiasis (*7*).

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Production of Folinic Acid from Folic Acid by *Lactobacillus casei*[1]

T. J. Bond

Department of Chemistry, Baylor University, Waco, Texas

It has been reported (*1*) that naturally occurring folinic acid (citrovorum factor) appeared, on the basis of microbiological assay, to be some fifteen times as effective as folic acid in competitively reducing the toxicity of x-methyl folic (*2*) acid for *Lactobacillus casei* in a defined synthetic medium. Accumulated evidence (i.e., *3–6*) appears to place folinic acid as at least one metabolically active form of folic acid and makes it highly probable that dietary folic acid is converted to folinic acid, and that the latter active principle is the naturally occurring factor. Further support for this belief is found in noting that the methods employed in the isolaton of folic acid from natural sources are sufficient to convert folinic acid to folic acid (*7*).

In this laboratory enhanced folinic activity has been observed in the livers of normal, healthy rats growing on an adequate diet supplemented with added folic acid. In a comparative fashion, it was thought that investigation of possible biosynthesis in microorganisms would be profitable.

During experiments designed to ascertain the possible metabolic function(s) of folinic acid, the biosynthesis of this factor by each of several microorganisms was observed. *L. casei* was selected as the organism for the purpose of reporting the production of folinic acid by a microorganism growing in a defined synthetic medium.

TABLE 1

FOLINIC ACID CONTENT OF *Lactobacillus casei* MEDIA

Folic acid (μg/50 ml)	Folinic acid* (μg/50 ml)	
	Uninoculated medium	Inoculated medium
10	00	.1
30	00	1.8
100	00	23.0
300	00	28.0
1000	00	50.0
3000	00	90.0

* Amounts based upon folinic acid SF (synthetic folinic acid) as the standard.

An enriched medium, essentially that of Rogers and Shive (*8*), but modified to contain purines and varying amounts of folic acid, was prepared in double strength and diluted before sterilization with an equal volume of phosphate buffer at pH 7. A small portion of each test (10 ml) was removed from uninoculated blanks as medium controls. The remainder (40 ml) in each case was heavily seeded with 1 ml of a highly turbid 10-ml saline suspension of saline washed cells of an actively growing 24-hr culture of *L. casei*, ATCC No. 7469, carried routinely on glucose-yeast-agar in this laboratory.

After 24 hr incubation (static culture) at 37° C, the cells were removed by centrifugation, the medium was neutralized with sodium carbonate, and the relative folinic acid content in each medium, inoculated and uninoculated, was estimated microbiologically, using *Leuconostoc citrovorum*, ATCC 8081, as the test organism and synthetic folinic acid-SF[2] as the standard (Table 1). The assay medium was essentially that described by Snell *et al.* (*9*) but was modified to contain asparagine, folic acid, pyridoxine, and inositol.

All assays were incubated 16–18 hr at 37° C. Graded growth responses were measured turbidimetrically with a Klett-Summerson photoelectric colorimeter, using light filter No. 54.

At the conclusion of the incubation period it was observed that the pH of the medium containing *L. casei* was moderately acid in spite of the presence of the added phosphate buffer. Since folinic acid activity is destroyed by mild acid hydrolysis (*10*), the amount of this factor remaining in the medium (Table 1) at the conclusion of the incubation period will not necessarily present a true picture of the actual conversion of folic acid to folinic acid by this microorganism. However, these data will serve to illustrate that such a transformation is accomplished.

Samples of medium containing folinic acid activity were compared bioautographically with synthetic folinic acid, employing paper chromatograms developed

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[2] Supplied through the courtesy of Eli Lilly and Company, Research Laboratories, Indianapolis, Indiana.