THE MEASUREMENT OF SURFACE TENSION WITH THE BALANCE

THE theory of the ring method for determining surface tension was discussed last September in SCIENCE[1] by F. H. MacDougall. This method, which in some respects is superior to others, may be modified in the following way, in order to attain better agreement with the results of other methods.

In my former researches[2] I used a cylindrical ring of thin brass plate (on other metal) which was previously heated redhot in order that, when used, it would be completely wetted by the liquid, thus reducing the angle of contact to zero. The ring was suspended on a very light balance with a sliding weight and no doubt may as well be combined with the tensiometer of duNoüy; without the sliding weight the balance must be in the zero position with the wetted ring.

Then the balance is lowered slowly, till the ring touches the surface of the liquid and is pulled down into it by the surface tension. Then the sliding weight is put on the longer arm and shifted along the same till the pointer stands exactly on the same mark (zero) as at the moment when the ring first touched the surface, the under edge of the ring thus being lifted just to the level of the liquid. The pull measured at that height—the level weight—is $p = 4r\pi\alpha$, or if the ring be not infinitely thin and r', r'' are the inner and outer radius,

$$p = 2\pi(r' + r'')\alpha$$

being always smaller than the pull at the instant of rupture—the separating weight.

Thus I got for water at 20° C $\alpha = 74.85$ dynes per centimeter, a value differing but little from many obtained by other methods, for instance, by Grunmach[3] and Kolowrat-Tscherwinski[4] using the method of ripples. With other liquids than water and with watery solutions the angle of contact S with a metal ring can not always be made zero and must then be determined in each case. The following formula should then be used.

$$p = 2\pi(r' + r'') \propto \cos S$$

If the properties of anomalous water surfaces[5] be under investigation, when the tension varies with size and time, the balance method is the sole one applicable. But in that case a cylindrical ring can not be used, first because the measurement must be made in very short time and the above course is not fast enough; secondly, because the tension of the surface enclosed by the ring would not be the same as on the outer surface.

Therefore I used in most of my investigations an auxiliary balance with a very small platinum ring, 6 mm in diameter, which acts like a disk taking the enclosed water surface with it in breaking off. The separating weight was read and afterwards transformed into the corresponding surface tension by the aid of an empirically established table.

AGNES POCKELS

BRAUNSCHWEIG, GERMANY

THE DETERMINATION OF VIABLE LACTOBACILLUS ACIDOPHILUS[1]

CONSIDERABLE difficulty has been experienced recently in this laboratory in the quantitative determination of viable L. acidophilus in cultures containing certain strains of this species.

It has been the custom in the laboratory for the past five years to use the casein-digest, skim milk digest, galactose agar described by Kulp and Rettger[2] (1924) for the routine plating of acidophilus milk. The milk sample and dilutions are shaken fifty times, in the manner recommended in the Standard Methods of Milk Analysis[3] (1923). The agar plates are incubated at 37° C. for forty-eight hours and the colonies counted with the aid of a 4× hand lens. Type of colony is determined by observation with the low power of the microscope.

It developed that acidophilus milk prepared with a certain strain (Scav.), which had never given a very high count by this method, without any apparent reason began to give L. acidophilus counts which were alarmingly low. New materials had been employed in the preparation of the casein-digest plating medium, and therefore it was thought that some of these new ingredients were exercising an inhibitory effect upon the bacteria. Five other strains were plated with this same medium. Three of these strains grew very well, while two did not. This was sufficient evidence to indicate that the ingredients were not at fault.

Comparative platings of the six strains were made in the routine medium and in whey agar containing one per cent. galactose and prepared according to the method of Rettger and Cheplin[4] (1921). All strains gave higher counts in the whey agar than in

[1] The author wishes to express gratitude to Prof. L. F. Rettger for his kindness in criticizing and correcting this manuscript.

[1] MacDougall, SCIENCE, 62, 1604, p. 290.

[2] A. Pockels, Wied. Ann., 67, p. 668 (1899).

[3] *Ann. d. Phys.*, 9, 1283 (1902).

[4] Journ. d. Russ. Phys. Chem. Ges., 36, 265 (1904).

[5] A. Pockels, *Nature*, 43, p. 437, 1891; 46, p. 418, 1892; 48, p. 152, 1893; *Ann. d. Phys.*, 8, p. 855, 1902.

[2] Kulp, W. L., and Rettger, L. F., 1924, "A Comparative Study of L. acidophilus and L. bulgaricus." *Jour. Bact. 9*, 357–394.

[3] Standard Methods for Milk Analysis (1923).

[4] Rettger, L. F., and Cheplin, H. A., 1921, "The Transformation of the Intestinal Flora, with Special Reference to the Implantation of Bacillus Acidophilus," Yale University Press.

the routine medium. However, three strains continued to give lower counts than it was thought they should.

Valley and Rettger[5] (1926) have shown that carbon dioxide is necessary for bacterial development, and that while many bacterial species will grow and multiply normally in the presence of the ordinary atmospheric CO_2 (about 0.03 per cent.), others are accelerated in their growth when they are provided with an atmosphere containing from 1 to 10 per cent. or even more CO_2 gas. L. acidophilus was found to belong to the latter group.

They showed more recently[6] that added CO_2 in the gaseous environment is responsible for much larger plate counts of L. acidophilus and for the development of larger, and more typical colonies. The degree to which added CO_2 favored development varied with the different strains of the organism.

An application of this principle in the plating out of the more backward strains of L. acidophilus was regarded as a possible way out of the present dilemma. Accordingly, whey agar platings were made from one of the "low count" strains. The plates were placed in a can with two dextrose agar plates freshly inoculated with Bact. coli. The Bact. coli growth was expected to increase the CO_2 content of the can, which was closed with a fairly tight-fitting cap. These plates, together with a set of open-air whey agar control plates of L. acidophilus, were incubated at 37° C. for forty-eight hours. The results of this preliminary experiment are given in Table I.

TABLE I[7]

COLONIES PER CUBIC CENTIMETER OF MILK PLATED IN WHEY GALACTOSE AGAR

In closed can with Bact. coli.	In open can; no Bact. coli.
280 M	190 M
600 M	250 M
680 M	Few
100 M	50 M
260 M	51 M
900 M	450 M
100 M	45 M
160 M	90 M

M – Millions.

The superiority of the method involving the use of the closed tin and Bact. coli plates is very apparent, the counts ranging from two to five times the number obtained by the open-air method.

The amount of CO^2 generated from the Bact. coli plates is probably not appreciable until after the first five or six hours of incubation. In order to avoid this delay in CO_2 increase, added CO_2 was in part supplied in subsequent experiments directly from a CO_2 tank just before incubation. Air-tight containers of measured volume were employed. Definite amounts of CO_2 gas were introduced into these containers, and after incubation CO_2 determinations were made.

Milk cultures of five different strains of L. acidophilus were employed in these experiments. Five sets of dilution plates were made from each milk culture, with whey-galactose agar as the plating medium. The agar plates were arranged for incubation as follows:

Set No. 1—Plates in open can.
Set No. 2—Closed can (not air-tight) with Bact. coli plates.
Set No. 3—Plates in air-tight jar with 5 per cent CO_2.
Set No. 4— " " " " " 10 per cent CO_2.
Set No. 5— " " " " " Bact. coli plates.

The results of a typical comparative experiment are given in Table II.

TABLE II.

L. ACIDOPHILUS PER CUBIC CENTIMETER OF MILK

Strain	Set No. 1	Set No. 2	Set No. 3	Set No. 4	Set No. 5
R–1–1	430 M	620 M	760 M	620 M	680 M
Ala.	N. C.	N. C.	25 M	45 M	30 M
Cas.	N. C.	N. C.	160 M	235 M	50 M
Scav.	N. C.	300 T	190 M	160 M	64 M
4 B	14 M	38 M	50 M	46 M	30 M
Residual CO_2	——	——	5 %	9%	2%

M–Millions; T–Thousands; N. C.–No colonies on plates.

These results require very little comment. Carbon dioxide in amounts varying between one and ten per cent. of the total gas in the container causes an increase in the growth of L. acidophilus. Some strains are more susceptible to the CO_2 than others. Strains R–1–1 and 4 B are rat strains; the source of "Ala" is unknown, while "Cas" and "Scav" are of human origin. According to these experiments, the human strains are apparently more dependent upon CO_2 for growth in agar plates than the rat strains. However, insufficient data are at hand to warrant a final statement to this effect. The plates exposed to CO_2 in amount varying from 5 to 10 per cent. of the container volume yielded the highest counts of L. acidophilus.

Based upon the results of the foregoing experiments, the following procedure for the quantitative

[5] Valley, George, and Rettger, L. F., 1926, "Carbon Dioxide Requirements of Bacteria," *Jour. Bact., 11,* 78–79.

[6] Report in preparation and as yet unpublished.

[7] The writer is grateful to his wife for the determination of the bacterial counts employed in this table.

determination of viable L. acidophilus in acidophilus milk and other cultures seems warranted at this time.

The medium recommended here is whey-peptone-galactose agar.

Whey is made by the method advocated by Rettger and Cheplin[8] (1921), which is essentially as follows: Skimmed milk is heated to 85–90° C. The amount of 10 per cent. HCl necessary to precipitate the casein in 10 cc amounts of the hot milk is determined. From this the volume of acid required to precipitate the casein in the entire volume of milk is calculated. The whey is removed from the precipitated casein by filtration through three or four thicknesses of cheesecloth. The filtrate is adjusted to a reaction of pH 6.8 with 10 per cent. NaOH and placed in flasks. The cotton-plugged flasks are autoclaved at fifteen pounds extra steam pressure for twenty minutes. The lact-albumen is completely coagulated and after this precipitate has settled to the bottom of the flask, the supernatant whey is decanted off and filtered. It is the custom in this laboratory to employ one and one half liter flasks and to fill them only two thirds full to avoid boiling over, on sudden cooling after autoclaving. Whey prepared in this manner can be used when needed.

The method for the preparation of agar is as follows. To 1000 cc of whey add 5 grams peptone. Adjust reaction to pH 6.5. Add 10 grams Difco granular agar and autoclave to dissolve the agar. Add 10 grams galactose and filter through absorbent cotton.

The milk sample and all dilutions are treated as stated in the first part of this paper.

Carbon dioxide gas amounting to from 5 to 10 per cent. of the container volume is added immediately before incubation.

A satisfactory and readily available container is the Kodak Developing Tank made by the Eastman Kodak Company and sold by their agents in all parts of the country. The tank should be about five inches in diameter and eight inches high, the one used in developing five by seven films. The accompanying cut[8] with appended note illustrates the set-up for L. acidophilus work.

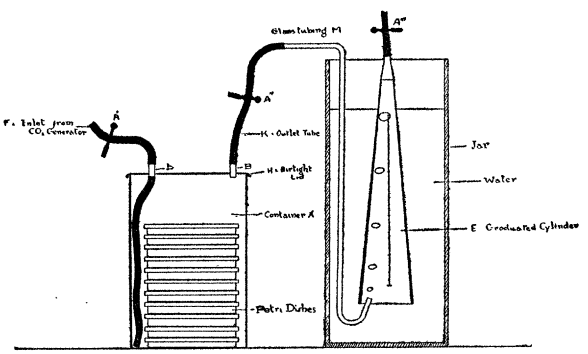

FIG. 1

EXPLANATION OF FIGURE 1

The agar plates are placed in the container X and the lid H screwed on tightly. The inlet tube F is connected with a CO_2 generator and the outlet tube K with the glass tubing M, the open end of which is placed in the mouth of the inverted graduated conical cylinder E. A Sedgwick-Rafter funnel is employed, but any large bruette or graduated cylinder will serve the purpose. The jar holding the inverted cylinder is filled with water.

The volume of the container X must be known. By subtracting from the total volume of the can 40 to 50 cc for each agar plate the approximate volume of the container is obtained. The clamps A′ and A″ are opened and CO_2 allowed to enter the container through F, forcing a corresponding amount of the contained air through K. The volume of displaced water in E is a direct measure of the expelled air. A volume of CO_2 equal to from 5 to 10 per cent. of the total atmospheric volume of X should be displaced. A′ and A″ are then tightly closed and the rubber tubing disconnected from the CO_2 generator and the gasometer jar. The apparatus is now ready for incubation.

Methods of incubation and counting have already been described briefly.

WALTER L. KULP
LABORATORY OF GENERAL BACTERIOLOGY,
YALE UNIVERSITY

SPECIAL ARTICLES

THEORIES OF A NEW SOLID JUNCTION RECTIFIER

AT the Physical Society meeting held at Washington on April 23 and 24, this year, the writer described a new type of electronic rectifier. The rectifier unit consists of a disc of copper having an oxide formed on its surface. It is found that, under suitable conditions, current flows more readily from the oxide to the copper than in the reverse direction, the phenomenon being very much like that observed in a high-vacuum electronic-discharge tube, in which electrons flow more readily from the hot cathode to the anode than from the anode to the cathode. The new rectifier acts as if a minute electronic cell existed at the junction between the copper and the oxide.

The rectifier operates with quite large current den-

[8] The writer is indebted to Mr. George Hunt for this drawing.