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Public Health Service
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SUMMARY

A method for the study of foam in intimate contact with liquid is described in terms of its use in testing the pH-stability relationship of lung alveolar surfactant (LAS) foams. A minimum stability occurred near pH 4.5 which, considered in the context of present knowledge of the chemical composition of LAS, could indicate a disorientation of surface structure with a consequent weakening of the structure. The pH-stability relationship of LAS foam might contain elements significant to studies of the physiologic effects on lungs of such atmospheric pollutants as acid mists and vapors. Stability of a column of LAS foam floating on top of a liquid does not indicate how stable the foam will be when it is mixed intimately with the liquid.

INTRODUCTION

Foam stability has been studied by several techniques. In most of them, small bubbles of gas are generated at the bottom of a column of liquid. Surfactant, dissolved in the liquid, concentrates at the gas-liquid interface as the bubbles rise to the top of the column [1-16]. The unit of foam stability[3] incorporates time, volume of gas, and length of the column of accumulated foam as definitive measurements. Foams which are so stable that they pass out of the top of the vessel, even when the generation of gas bubbles is very slow, cannot be studied by such methods.

Lung alveolar surfactant (LAS), as it is collected from lungs[17], produces this sort of stable foam. However, a method for measuring the stability of LAS foam in contact with chemical agents is needed. Use of such a method could reveal facts concerning stabilizing forces that would be helpful in understanding the structure of units of LAS foam and perhaps of biological membrances[18,19]. Of more immediate importance, it could aid in development of the treatment of fulminating lung edema[20]. The latter problem has not as yet been considered in

studies of LAS. The method reported here measures relative foam stability. It was developed after several preliminary studies showed conventional techniques to be unsuited to the specific application desired. A set or sets of individual samples of foam are tested simultaneously. A permanent record is obtained and the results of each set are compared internally. Comparison of the individual samples of such sets was found to be reliable, but comparison of individual samples tested separately was not.

APPARATUS AND METHOD

TEST TUBE RACK

A modified test tube rack was constructed of aluminum stock and wood (Fig. 1). Its general dimensions, which are not critical, can be obtained by reference to the cm scale shown in the figure. The cm scales seen in Figs 2-4 are removed and replaced easily. They are held in position by light pressure produced by closeness of fit. Holes bored through the floor of the rack under each test tube permit light to pass upward through each tube from the corresponding 6-volt, 250-milliamp, 3,000-hour lamp, fixed beneath each hole and connected in parallel. A cardboard backing, which can be hung on the back of the rack was painted black on the area behind the test tubes to provide contrast. The test tubes were selected for uniformity of bore so that a given volume in any tube did not vary in height by more than 0.1 mm from the same volume in any other tube.

Photographs were taken using Polaroid* 3x5 P/N film with room light augmented by the lamps beneath the test tubes.

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* Mention of commercial products or companies does not constitute endorsement by the Public Health Service.

LUNG FOAM STABILITY

BUFFER SOLUTIONS

These were prepared with distilled water from approximately 0.15 M phthalate, phosphate, or Tris, giving a range in pH from 3.5 to 9 in steps of 0.5 pH units. The buffers were diluted with distilled water to 0.067M for some tests. In each set of samples, the difference in pH in adjacent test tubes (samples), was always 0.5 pH units.

ACQUISITION OF FOAM STOCK

About 2 liters of foam was secured from 6 hog lungs by filling them with and emptying them of distilled water to which had been added 8 gm NaCl and 0.275 gm $CaCl_2$ per liter[21]. This solution was used both to rinse foam from the lungs and to wash the foam. The foam was washed once by shaking it vigorously with an equal volume of liquid. Some detritus was visible in the liquid beneath the foam after their separation. Further washing was avoided, however, to increase assurance in the preservation of the original chemical composition and structure of the foam.

PREPARATION OF TESTS

Four 1/8-inch stainless steel ball bearings were placed in each test tube. The vessel containing the stock of foam and an equal volume of liquid was inverted vigorously 20 times and allowed to stand 2 minutes. The large end of a 50 ml volumetric pipet was inserted 2 inches into the foam and was filled with foam by suction applied through a piece of plastic tubing affixed to the delivery end of the pipet. An approximately equal preselected volume of foam was added as quickly as possible from the pipet to each of a set of the test tubes by allowing its height in each tube to rise about 1/2 cm above a fiducial mark made with crayon at the same height on each tube. The foam sample passing through the stem of the pipet into a test tube was observed for homogeneity of unit-of-foam (bubble) size. The set of samples was discarded whenever lack of homogeneity was noticed, although this became a rare occurrence after a little practice. A piece of blotter, 1 inch square and soaked with water, was placed in each tube near the top to assure a high relative humidity above the foam. The tubes were stoppered and drainage of liquid from the foam proceeded for 5 minutes. All liquid and enough foam were withdrawn through a long blunt hypodermic needle to bring the upper edge of foam even with the fiducial mark.

LUNG FOAM STABILITY

A volume of the liquid to be tested, equal to 1/2 the volume of foam, was injected beneath the foam in each tube with a calibrated syringe via the hypodermic needle. Notes concerning the set of samples were written on a 3x5 inch card, and this card was clipped to the cardboard backing of the test tube rack.

THE TESTING PROCEDURE

The test tubes were placed in the rack, the reference rulers and cardboard backing were put in position, the lamps were lighted, and a photograph was taken. The reference rulers, stoppers, pieces of blotter, and cardboard backing were removed. The stoppers were replaced and the rack inverted. Inversion proceeded through 180° each movement: to the right and back; to the left and back; away from the operator and back; toward the operator and back. Each inversion was done quickly; each reversion a little less quickly to eliminate any chance of shattering the test tubes with the steel balls. The process was repeated until unmistakeable evidence of net foam breakage was observed.

A test tube was taken from the rack and its stopper removed; foam adhering to the inside wall of the tube was rinsed down with liquid from the bottom of the tube using the hypodermic needle and syringe; the remoistened blotting paper and stopper were restored to the tube, which was replaced in the rack in its former position. All tubes were so treated serially in ascending numerical order. Suitable notes were written on the 3x5 inch card, which together with the reference rulers and cardboard backing were fixed to the rack; and a second photograph was taken. Inclusion of the date and time in each photograph recorded the order of experimentation.

MEASUREMENT OF FOAM COLUMN HEIGHT

When measurement of foam column height was desired, the required photograph was enlarged to 8x10 inches. A straight-edge was used to span the enlargement including both cm scales. The upper edge of the straight-edge was placed in juxtaposition with the top of a column of foam the height of which was indicated simultaneously by both cm scales. The procedure was repeated to obtain the height of the bottom of the column of foam. The height of the column was the difference between the 2 readings.

LUNG FOAM STABILITY

This procedure was followed to obtain Figs. 5 and 6 where results were plotted as ratios of final foam column height/initial foam column height.

RESULTS

PHTHALATE BUFFER, pH 4.0-5.5. EFFECT OF STORAGE

Figures 2 through 4 illustrate the effect on LAS foam of changing the pH through the range of 4.0-5.5 with approximately 0.15 M phthalate buffer, and of using tap water. These photographs show levels of foam: before inversion, Fig. 2; after inversion 320 times through 180° in 14 min., Fig. 3 (plotted as ■ in Fig. 5); and after storage for 3 days at room temperature, Fig. 4. A relationship exists between pH and stability of the foam with a minimum stability near pH 4.5. Of the liquids used in this set of samples, tap water (pH ~ 8.3) had the least destructive action on LAS foam. During storage, cavities appeared in the foam, but without altering greatly the length of the column of foam.

PHTHALATE BUFFER, pH 3.5-5.5

Two sets of 5 samples each were run concurrently using 0.15 M phthalate buffer, pH 3.5-5.5 for each set. A minimum foam stability occurred at pH 4.5 in each set (Fig. 5, △ , ○).

Effect of Serial Sets

Four sets of samples with 5 samples in each set were run serially using phthalate buffer, 0.15 M, pH 3.5-5.5 (Fig. 5, ✚ , ✜ , ◪ , ■). Each set exhibited a minimum foam stability at pH 4.5. The first set was inverted 200 times in 6 min. The second set was inverted 220 times in 7 min., the third set 270 times in 8 min., and the fourth set 280 times in 8 min. It thus proved necessary to increase the number of inversions of each succeeding set in order to produce comparable results in corresponding samples of each set.

LUNG FOAM STABILITY

Effect of Molarity

Two sets of samples with 5 samples in each set were run concurrently using 0.15 M phthalate buffer for one set and 0.067 M phthalate buffer for the other set. The pH at each molarity ranged from 3.5 to 5.5 (Fig. 5, ▲ , △). A decrease in molarity of phthalate buffer from 0.15 M to 0.067 M increased the relative foam stability in the range of pH 3.5-5.5 except at pH 4.5 where a minimum and equal foam stability occurred at both molarities.

PHOSPHATE BUFFER, pH 6-8. EFFECT OF MOLARITY

A set of 10 samples run concurrently compared the effect on foam stability of 0.067 M and 0.15 M phosphate buffers in the range of pH 6-8 (Fig. 6, ● , ○). No real effect of pH or molarity was evident in this set of samples.

PHOSPHATE AND TRIS BUFFERS, pH 7-8. EFFECT OF SERIAL SETS

Three sets of samples with 6 samples in each set were run serially comparing the effect on foam stability of 0.067 M phosphate and Tris buffers in the range of pH 7-8 (Fig. 6, ▽ , ▲ , ◑ , ◐ , ◪ , ◩). No systematic difference in the effect of pH on foam stability was detected in this test. A systematic increase in resistance to net breakage of foam was apparent in succeeding sets of samples. The first set was inverted 200 times in 7 min., the second set was inverted 240 times in 8.5 min. to achieve similar results, and the third set still had more foam remaining than the first and second sets after 400 inversions in 12 min.

TRIS BUFFER, pH 7-9

Two sets of samples with 5 samples in each set were run concurrently using 0.15 M Tris buffers, pH 7-9 for each set (Fig. 6, ◬ , △). There was no apparent effect of pH on foam stability in this range.

TRIS BUFFER, pH 7-9. EFFECT OF MOLARITY AND SERIAL SETS

Two sets of 10 samples each were run serially to record the effect of molarity and pH in the range of pH 7-9 at molarities 0.067 and 0.15 M using Tris buffer (Fig. 6, ■ , □ , ✤ , ✣). Neither variable had

an effect on foam stability. An increase in resistance to net breakage of foam was observed in the second set when compared with the first set: the second set required 220 inversions in 7 min. to achieve a degree of net foam breakage comparable to that of the first set which was inverted 140 times in 5 min.

DISCUSSION

Convincing arguments have been developed to show that stable foam obtained from lungs by a wide variety of methods, including fulminating lung edema, originates at the lung alveolar surfaces[17]. It is this stable foam which might properly be termed lung alveolar surfactant (LAS). Accompanying liquid does not foam readily and foam produced from it is not stable[17]. The method used to obtain LAS foam for the present study was identical to most of those used previously, except for the appropriate addition of $CaCl_2$ to the wash liquid[21,22].

The application described here tests what we believe to be the ultimate resolving power of the method and makes evident the value of a technique which provides direct internal comparison of individual samples tested simultaneously. It is applicable to the study of specific problems of a theoretical nature such as the structural stability of LAS foam surfaces[22]. A more important, immediately practical, but directly related problem is the establishment of criteria for non-toxic antifoams effective against the foam of fulminating lung edema.

The procedure, as reported, was developed to achieve internally consistent results. The stock of foam agglomerated into an increasingly coherent "clot" of uncertain uniformity. Vigorous shaking of the stock was accordingly performed under standardized conditions which resulted in a uniform and readily flowing mass of foam for sampling. Vigorous shaking of the stock resulted in breakage of more labile units of foam. Consequently, an increased number of inversions was required in succeeding serial tests to achieve a similar degree of net breakage of foam at a given pH.

LUNG FOAM STABILITY

After obtaining suitable samples from the stock of foam, the individual samples, in their respective test tubes, were allowed to drain as completely as practicable in order to achieve a similar mass of foam for each sample of the set. Under these standardized conditions LAS foam formed a mass with a consistency similar to that of whipped egg white.

The problem of mild dispersal of this mass into the test liquid was then confronted. Intimate dispersal was necessary as is illustrated in Fig. 4: the liquid ceased to have an effect upon the foam after separation of foam and liquid. Intimate mild mixing of foam and liquid could not be obtained without an aid to penetration of the mass of foam by the liquid. Borosilicate glass balls, of 3/16 inch diameter, penetrated the foam too slowly. The metal balls used in these experiments penetrated the foam readily, but not violently. The time interval between each abrupt inversion was about 2 seconds resulting in mild conditions but thorough mixing when the test tubes were also inverted through the different specified directions. Failure to do this produced channeling by the liquid and balls through undisturbed banks of foam.

The fact that intimate contact between foam and liquid was required for their interaction lent some economy to the experimental procedure. When several sets of samples were run serially, each completed set could be put aside until the succeeding sets were finished. The final photographic record of the several sets could then be taken with each set placed in the rack in its appropriate position. We suggest that the reason for the relative stability of the foam contiguous with, but not stirred into, the liquid is related to the solubility in the liquid of foam stabilizers[19,21,22]. In the absence, or after cessation of stirring, any liquid remaining in contact with foam becomes saturated with the stabilizing agents of the foam surfaces. Crowding together of the units of foam permits no free access of fresh liquid to individual units under static conditions.

However, if one wished to study individual units submerged in chemical agents, complex equipment would be required in order to secure control over the experimental variables. Also, many individual experiments would be necessary to evaluate the results by application of statistical methods. The use of large populations of units, mixed intimately with the test substance, circumvents these obstacles and is desirable in a test designed to ascertain relative effects on net foam stability of a large number of materials.

LUNG FOAM STABILITY

The method was developed specifically for the study of LAS foam. It must be distinguished carefully from methods designed to measure physical parameters of LAS as it might exist on open surfaces. Nevertheless it is reasonable to assume that factors which have an effect upon LAS in the form of a closed surface (unit of foam) might have definite, but as yet unspecified, effects upon LAS in the form of an open surface. LAS is thought to exist as an open surface at the normal air-alveolar interface.

Stability of the LAS foam was influenced by the pH of the bathing fluid in the zone of pH 4-5 with a minimum stability near the middle of this zone. Prior studies indicate that LAS forms a structure similar to that of cell membranes with phospholipids and proteins as the probable dominant organic components[18,19,21,22]. A minimum foam stability at a definite pH (near pH 4.5) could then indicate a disorientation of structure through a preferential precipitation of a structural component with a consequent diminution of foam stability.

LAS probably occupies the largest surface area of the body which is exposed to the external environment. The demonstrated pH-foam stability relationship might therefore be of interest when related to tendencies toward or factors favoring production of physiologic effects on lungs by acidic atmospheric pollutants. Examples of these are mists of sulfuric acid and gases containing oxides of sulfur or nitrogen.

ACKNOWLEDGMENT

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Fig. 1. Schematic representation of the test tube rack to show details of construction. The rack has been cut in two in this figure at its vertical plane of symmetry.

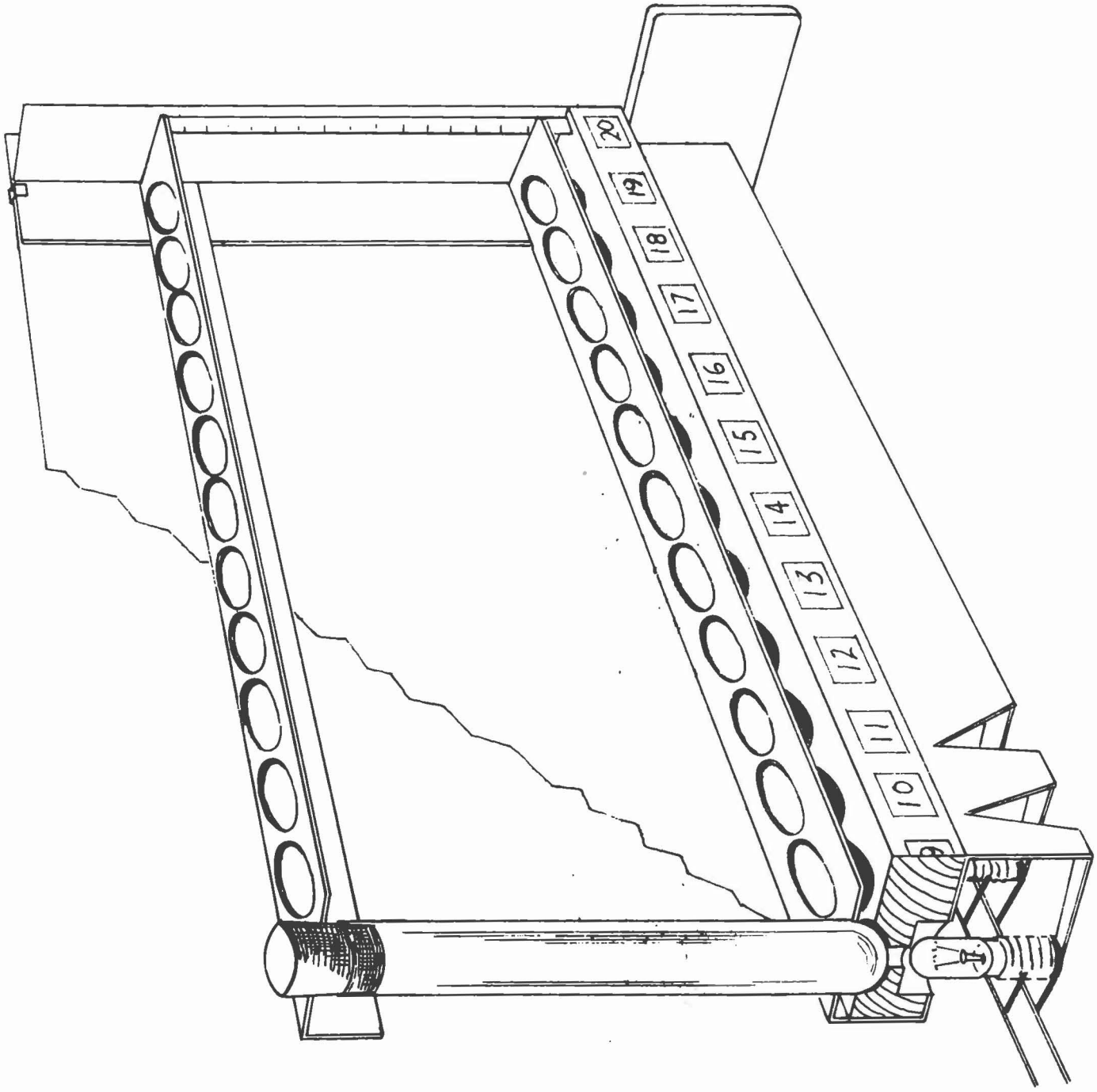
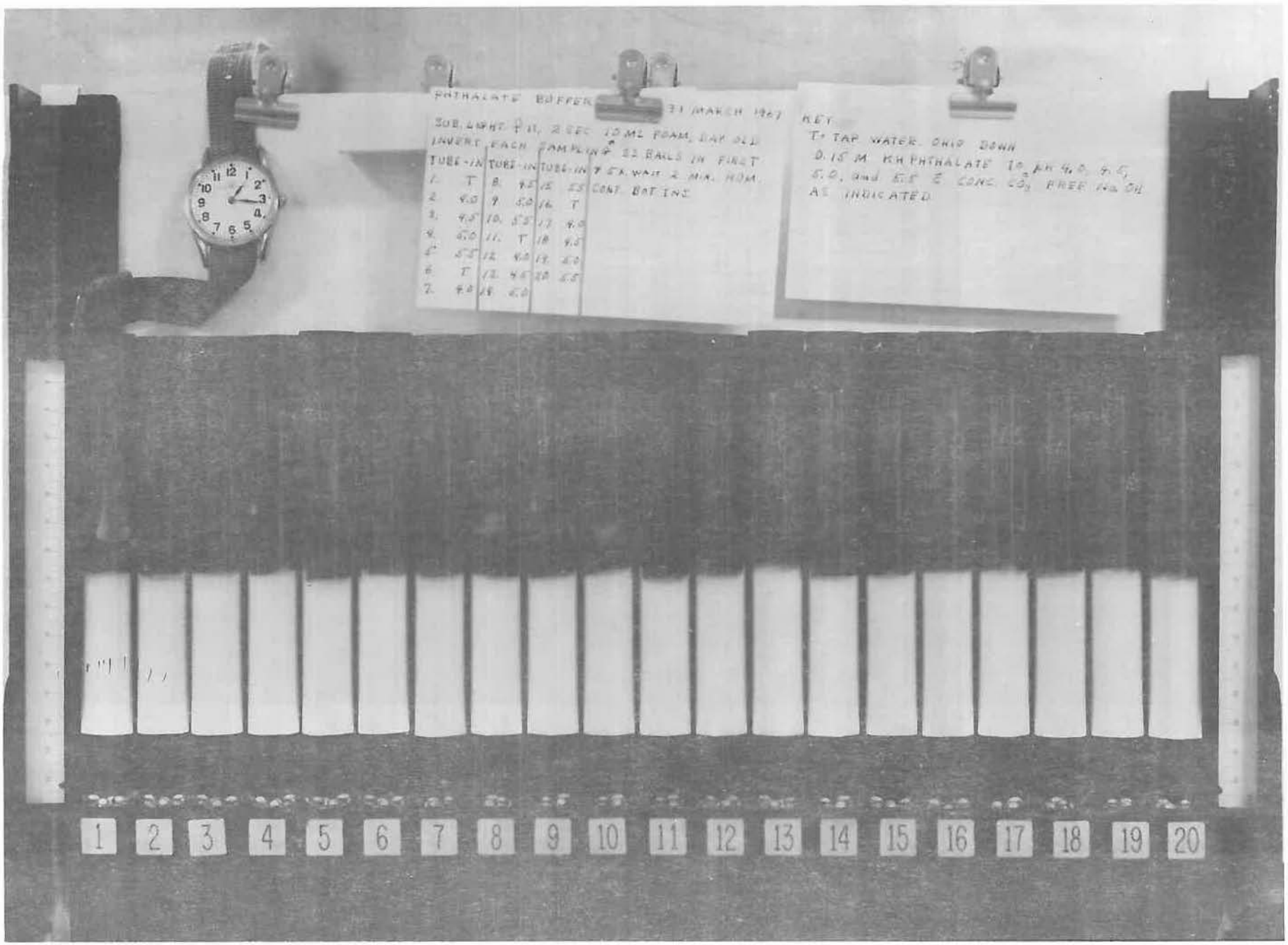
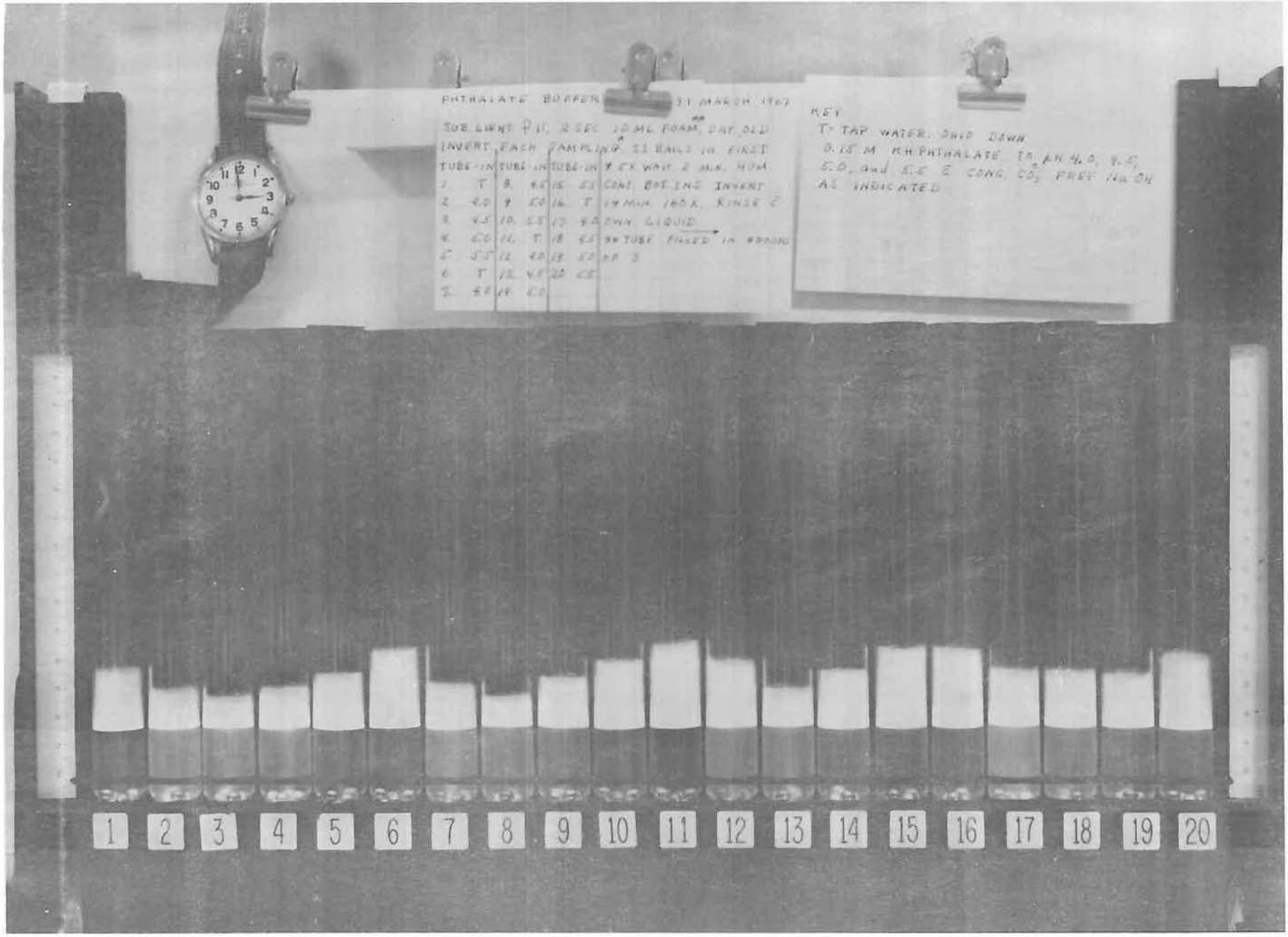

20
19
18
17
16
15
14
13
12
11
10
9

Fig. 2. Four sets of 5 samples each, run concurrently, each set starting, respectively, with tubes 1, 6, 11, and 16. Tubes contain 10 ml foam over 5 ml liquid, consisting from left to right in each set of experiments, of tap water, then phthalate buffer, 0.15 M, pH 4.0-5.5 in 0.5 pH increments. The phrase "Ohio Down" on the reference card refers to the state of flooding of the Ohio River. The influence of this variable on the quality of LAS foam is under study, but no information has been obtained which is applicable to the work presented here.


PHTHALATE BUFFER 31 MARCH 1967
SUB. LIGHT 9:11, 2 SEC 10 ML FOAM, DAY OLD
INVERT EACH SAMPLING 22 BALLS IN FIRST
TUBE-IN TUBE-IN TUBE-IN 7 SEC. WAIT 2 MIN. HOM.
1. T 8. 9.5 15. 5.5 CONT. BATING
2. 9.0 9. 5.0 16. T
3. 9.5 10. 5.5 17. 9.0
4. 5.0 11. T 18. 9.5
5. 5.5 12. 9.0 19. 5.0
6. T 13. 9.5 20. 5.5
7. 9.0 14. 5.0
KEY
T= TAP WATER. CHLO DOWN
D. 15 M KH PHTHALATE TO PH 9.0, 9.5, 5.0, and 5.5 & CONC. CO_2 FREE H_2O OH AS INDICATED
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Figs. 3 and 4. Continuation of Fig. 2. In order: after inversion 320 times through 180° in 14 minutes; after storage at room temperature for 64.5 hours.


PHTHALATE BUFFER 31 MARCH 1967
SUB-LIGHT P.U. 2 SEC 10 ML FOAM, DAY OLD
INVERT EACH SAMPLING 22 BALLS IN FIRST
TUBE-IN TUBE-IN TUBE-IN
1 T 8 4.5 15 5.5
2 4.0 9 5.0 16 T
3 4.5 10 5.5 17 4.0
4 4.0 11 T 18 5.5
5 5.5 12 4.0 19 5.0
6 T 13 4.5 20 4.5
7 4.5 14 4.0
KEY
T= TAP WATER, DWID DOWN
0.15 M KH PHTHALATE TA, AN 4.0, 4.5, 5.0, and 5.5 E CONC. CO_2 FREE 1×10^{-4} OH AS INDICATED
CONT. BOTTING INVERT
IN MIN 180 X. RINSE E
DWN. LIQUID
XX TUBE FILLED IN ERROR
OF 3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

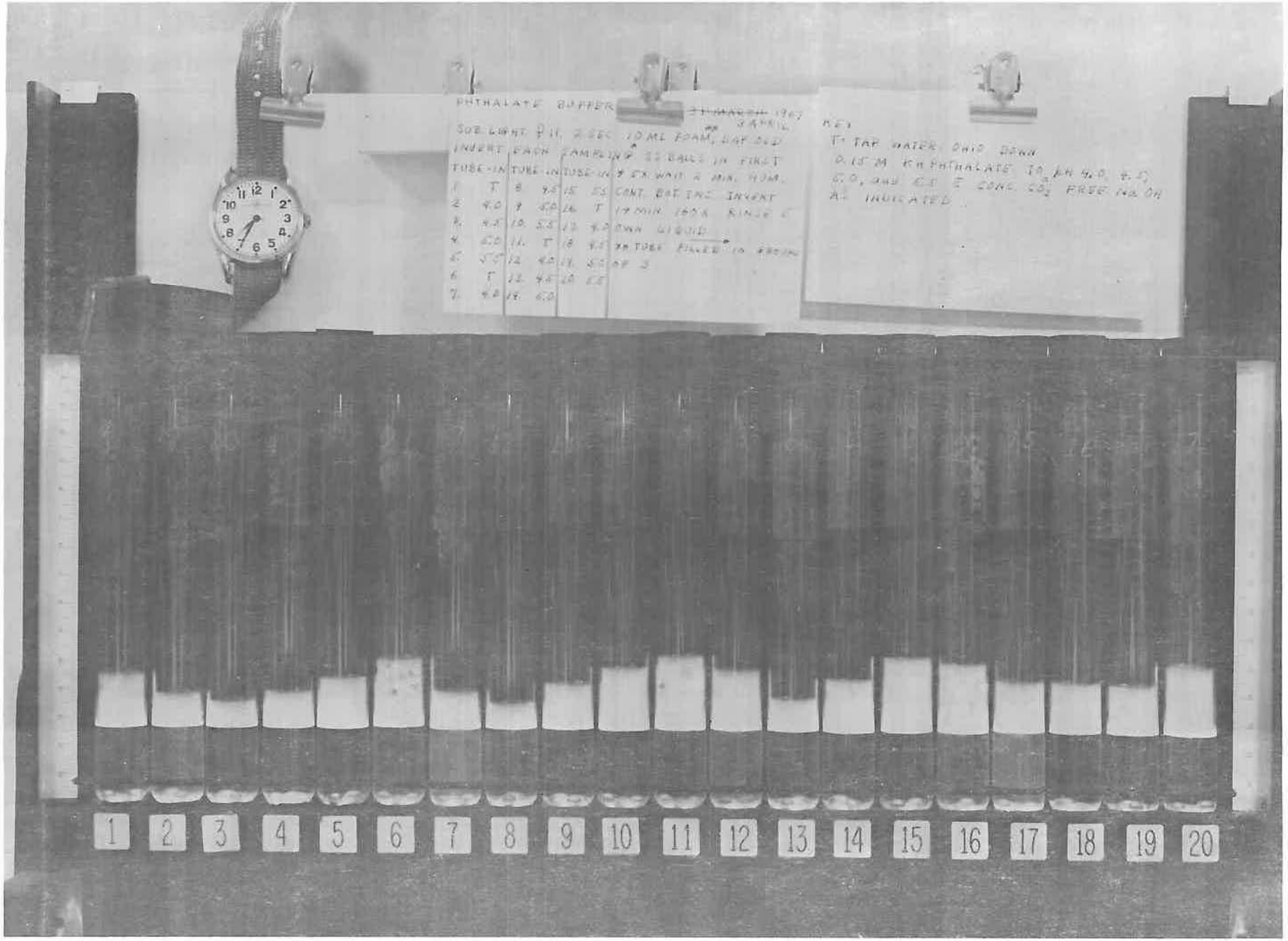

PHTHALATE BUFFER
SUB LIGHT 7 11, 2 SEC 10 ML FOAM, LAP OLD
INVERT EACH SAMPLING
TUBE-IN TUBE-IN TUBE-IN
1 T 8 4.5 15 5.5
2 4.0 9 5.0 16 T
3 4.5 10 5.5 17 4.0
4 5.0 11 T 18 4.5
5 5.5 12 4.0 19 5.5
6 T 13 4.5 20 5.5
7 4.0 14 6.0
KEY
T= TAP WATER, OHIO DRAW
0.15 M KH PHTHALATE TO pH 4.0, 4.5, 5.0, AND 5.5 + CONC CO_2 FREE NaOH
A= INDICATED
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Fig. 5. Plots of foam stability vs pH using 0.15 M phthalate buffer, except △ which is 0.067 M. Each value recorded on the ordinate is the ratio of final:initial length of column of foam for each sample. Use of this technique compensates for small differences in initial volume of foam. Information following each key symbol below is, in order: (1) initial ml of foam, (2) number of inversions through 180°/min required, (3) remarks, if any. □, 10, 320/14, see Fig. 2. ◬, ○, 10, 280/9. ✚, 8, 200/6. ✜, 8, 220/7, serial with ✚. ◪, 8, 270/8, serial with ✚ and ✜. ■, 8, 280/8, serial with ✚, ✜, and ◪. ▲, △, 4, 100/3.

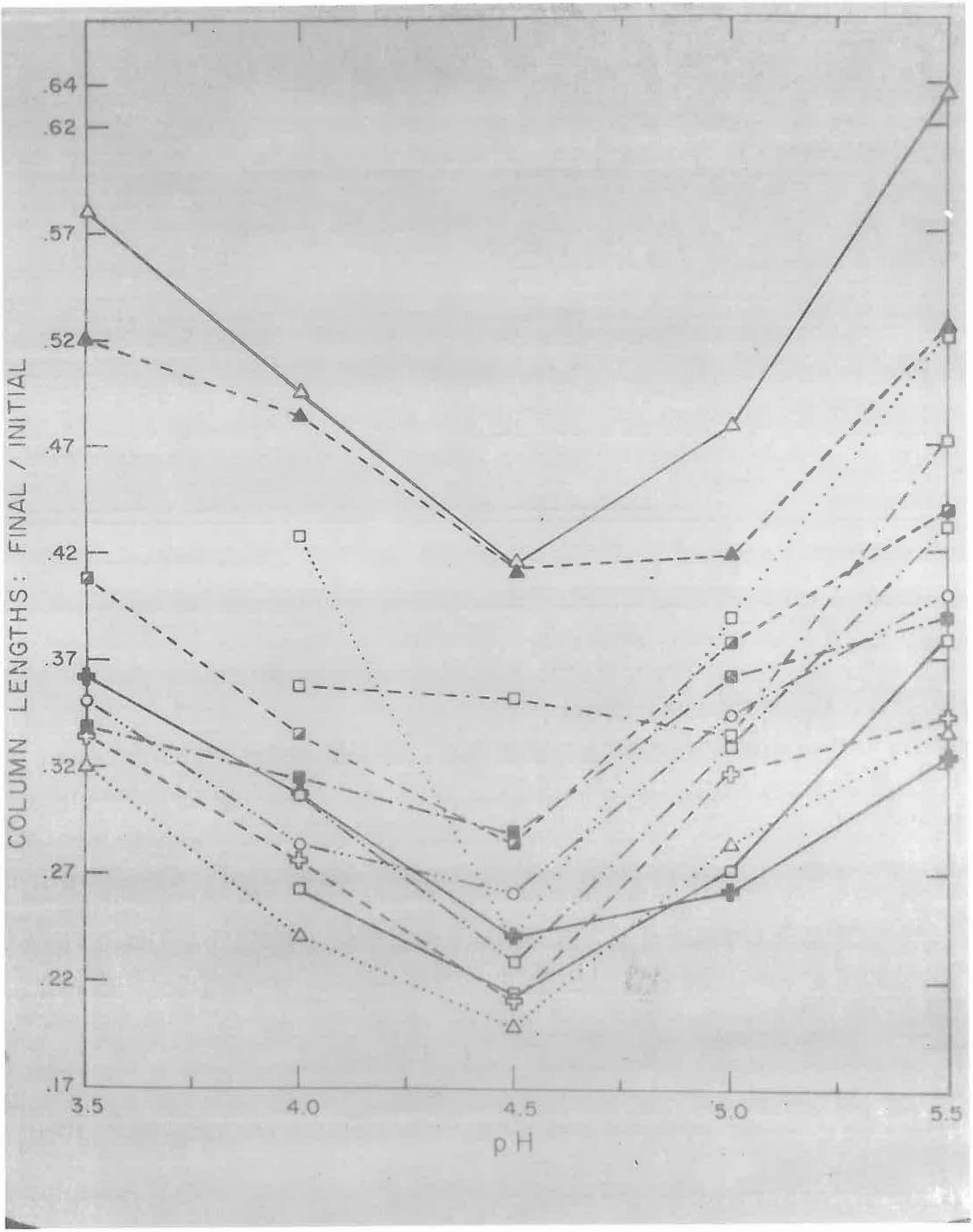
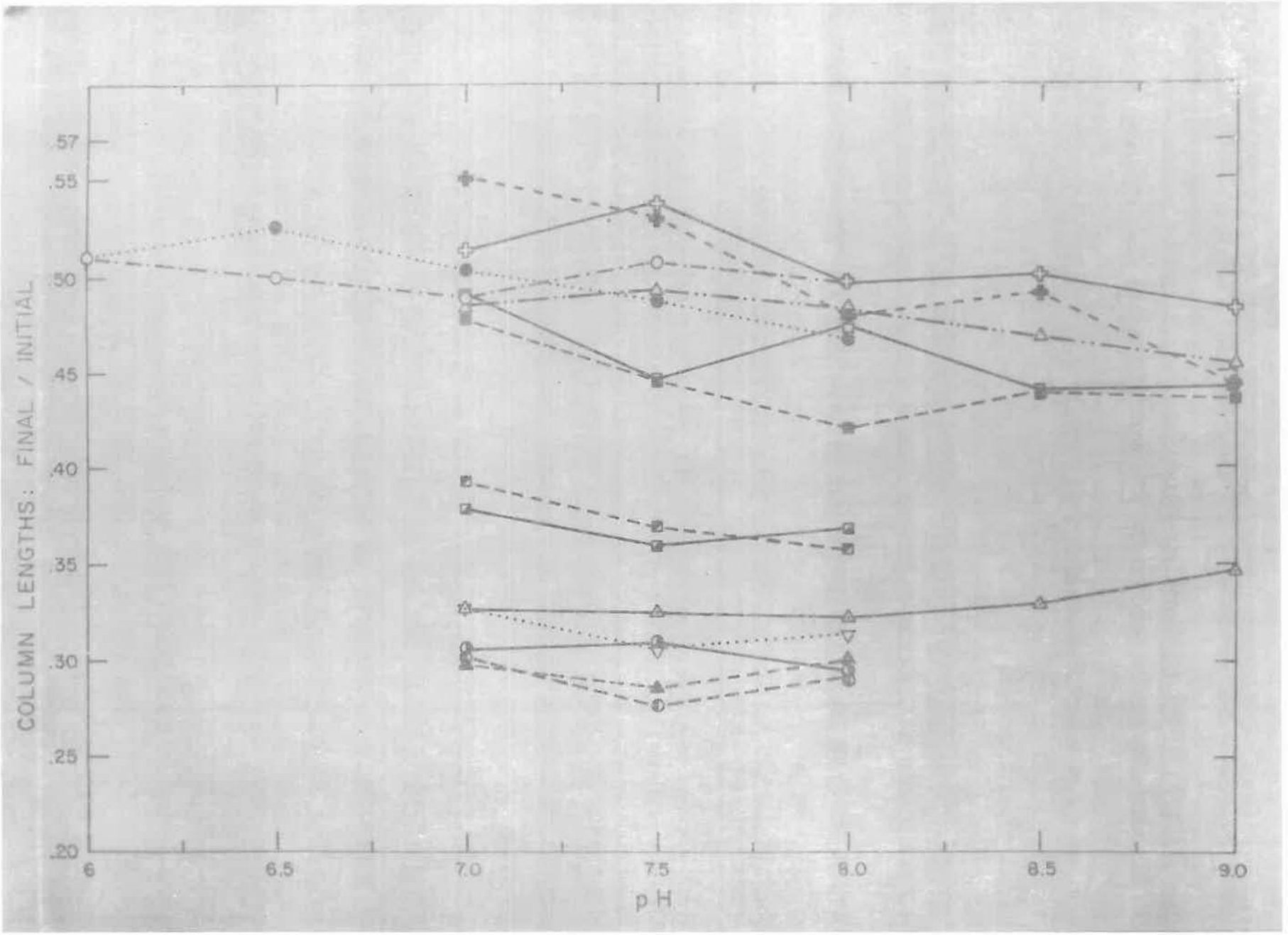

COLUMN LENGTHS: FINAL / INITIAL
.64
.62
.57
.52
.47
.42
.37
.32
.27
.22
.17
3.5
4.0
4.5
5.0
5.5
pH

Fig. 6. Plots of foam stability vs pH. See legend to Fig. 5 for method of determining values recorded on the ordinate. Information following each key symbol is, in order: (1) type of buffer, where P = phosphate and T = Tris, (2) its molarity, (3) initial ml of foam, (4) number of inversions through 180°/min required, (5) remarks which, at times, obviate some other information. ● , P, 0.067 M, 4, 140/5. ○, P, 0.15 M, 4, concurrent with ●. ▽, P, 0.067 M, 7, 200/7. ▲, T, 0.067 M, 7 concurrent with ▽. ◑, P, 0.067 M, 7, 240/8.5, serial with ▽ and ▲. ◐, T, 0.067 M, 7, concurrent with ◑. ◪ , P, 0.067 M, 7, 400/12, serial with ◑ and ◐. ◪ , T, 0.067 M, 7, concurrent with ◪. ◬, △, T, 0.15 M, 10, 280/8.5. □, T, 0.067 M, 4, 140/5. ■, T, 0.15 M, 4, concurrent with □. ✣, T, 0.067 M, 4, 220/7 serial with □, and ■. ✚, T, 4, concurrent with ✣.


COLUMN LENGTHS: FINAL / INITIAL
.57
.55
.50
.45
.40
.35
.30
.25
.20
6
6.5
7.0
7.5
8.0
8.5
9.0
pH