AN AERATION TRAIN FOR THE STUDY OF PRODUCTS OF BACTERIAL METABOLISM[1]

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OBJECTIVES

The chemical end-products of bacterial metabolism have been used since the early days of the science as a means of classifying bacteria; and systematic bacteriology rests in large part upon such determinations. In recent years the unique advantages offered by bacteria for the elucidation of the basic problems of cell physiology have been increasingly recognized.

The study of bacterial metabolism in the past has generally been concerned either with the products of protein metabolism (summarized by Rettger (1928)) or with the products of carbohydrate metabolism (reviewed by Kendall (1928)). In the former case, analyses of the medium give at least a fair picture of the situation. In carbohydrate metabolism, on the contrary, the acidity of the medium may, as shown by Merrill (1930) for mycobacteria, bear no relation whatever to actual decomposition of sugars. On the other hand, the study of carbon dioxide production in the Smith fermentation tube or in similar pieces of apparatus can only permit the most general conclusions. As shown by Keyes (1909) and Rogers, Clark and Davis (1914) and others, diffusion from the open surface of such a medium is so considerable and so variable a factor that the method throws little light on basic metabolic changes.

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Rogers, Clark and Davis (1914) themselves obtained highly significant results by collecting and analyzing all the gases produced by colon-group organisms,—results which established a clear differentiation between the *Esch. coli* and *Aero. aerogenes* types on the basis of the ratio of carbon dioxide to hydrogen in the gases which they liberate. A further advance was made by Novy, Roehm and Soule (1925) who collected the gas formed by bacteria in a compensation manometer so that the rate of gas production could be watched as well as the composition of the gas determined. Soule (1928) on the basis of this method was able to compute for several bacterial and protozoal types respiratory quotients which varied from 0.8 to 1.3.

A simple respirometer in which CO_2 produced by yeast was measured has been used by Rahn (1929, a and b) to establish time-number-product relationships, and the micro-respirometer developed by Warburg (1926) has been recently used for bacteriological investigations by Eaton (1931) and by Burk and Lineweaver (1930).

These studies have contributed very materially to our knowledge of quantitative bacterial metabolism. For the prosecution of certain investigations which are in process in the laboratories of the Department of Public Health of the Yale Medical School, we desired to attain certain ends which no previous technic made possible. Our desiderata were as follows:

a. The cultivation of bacteria in a medium which should be kept free of as large a proportion as possible of the waste products of metabolism so as to permit the study of a reasonably normal cycle. That ordinary test tube cultivation is unfavorable for optimum growth conditions has been increasingly remarked of late. Rogers and Whittier (1930) by continual ingress of fresh medium have kept *Str. lactis* and *Esch. coli* at maximum population for one month without onset of a death phase, and Magoon and Brunstetter (1930) by aerating cultures have obtained greatly increased growth.

b. The determination of the rate of bacterial growth so that metabolic activity could be correlated with definite phases of the life cycle and metabolic products correlated with the number of cells present at a given time.

c. The simultaneous determination of all the ammonia formed (as a net end-product of protein metabolism) and of all the carbon dioxide formed (as an end-product of carbohydrate metabolism),— whether retained in the medium or given off from it. It is scarcely possible to draw sound conclusions as to the so-called "respiration" of microörganisms without knowing something of ammonia production also on account of the interaction of acidic and basic substances in a complex organic medium.

The apparatus briefly presented two years ago (Walker (1930)) has proved so successful for this purpose and seems to have so many possible applications as to warrant full description. Several of its features are included in aeration methods developed at about the same time by Krishna (1928) and by Merrill (1930).

GENERAL PLAN OF APPARATUS

The general plan of the apparatus used is shown in figure 1. It involves three major sections as follows:

A culture unit in which the bacteria are grown in any appropriate medium which is constantly aerated by a current of air which has been freed from ammonia and carbon dioxide. This unit is shown at *E* in figure 1.

A purification train for removing ammonia and carbon dioxide from the incoming air. This is shown at *A–D* in figure 1.

An absorption train in which the ammonia and carbon dioxide carried off by the aerating current can be collected. This is shown at *F–H* in figure 1.

Purification train

The purification train includes four units, as follows:

Unit *A* (fig. 1). A Milligan spiral gas-washing bottle containing a strong solution[2] of KOH for the absorption of CO_2. Repeated tests showed that removal of CO_2 was consistently 100 per cent complete.

Unit *B*. A similar bottle containing a strong solution[3] of H_2SO_4 for absorption of NH_3.

[2] Specific gravity of KOH solution approximately 1.3.

[3] Specific gravity of H_2SO_4 approximately 1.7.

Unit *C*. A bottle of distilled water (several hundred cubic centimeters) to serve as a trap preventing mechanical entrainment of acid from unit *B*.

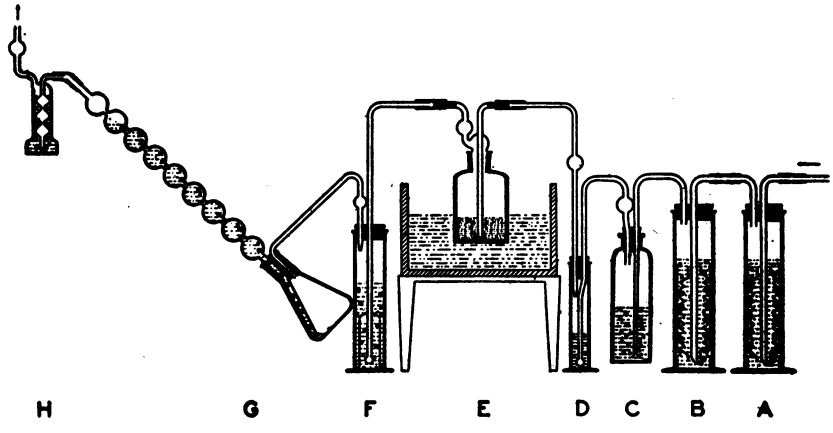

FIG. 1. TRAIN FOR REMOVAL AND DETERMINATION OF FREE CO_2 AND NH_3 FROM BACTERIAL CULTURES GROWING UNDER THE INFLUENCE OF CONTINUOUS AERATION

KEY TO FIGURE 1—AERATION TRAIN

Division I. Purification train

A. Milligan spiral gas-washing bottle.
Contents—strong solution KOH, for removal of CO_2 from incoming air.

B. Ditto. Contents—strong solution H_2SO_4, for removal of NH_3.

C. Glass bottle.
Contents—distilled water for trap against mechanical entrainment from *B*.

D. Small cylinder.
Contents—distilled water and bromthymol blue or other colorimetric pH indicator, for control on atmospheric purification.

Division II. Culture unit

E. Sterile Dreschel "low form" glass-stoppered gas washing bottle, capacity 250 cc.
Contents—100 cc. or more culture medium and organisms, for aeration during incubation in water bath.

Division III. Absorption train

F. Cylinder, 8 by 1½ inches, with Folin ammonia absorption bell.
Contents—100 cc. 0.05 normal H_2SO_4, for absorption of NH_3 aerated from culture; results determined by neutralization and direct Nesslerization of aliquot portion.

G. Erlenmeyer flask, capacity 250 cc. and Brady-Meyer absorption tube.
Contents—75 cc. standard solution $Ba(OH)_2$ for absorption of CO_2 from culture; results determined by titration of residual $Ba(OH)_2$, with standard HCl and thymolphthalein, CO_2 remaining precipitated as $BaCO_3$.

H. Bowen potash bulb.
Contents—saturated $Ba(OH)_2$ for control on efficiency of absorption of Brady-Meyer tube (*G*).

Unit *D*. A test cylinder containing distilled water tinted with bromthymol blue as a check on the efficiency of units *A* and *B* in removing CO_2 and NH_3 and on the efficiency of unit *C* in trapping entrained acid. This proved to be a highly important element in the apparatus. After about a day's aeration, the principal trap bottle (unit *C*) would absorb enough acid by entrainment to begin to yield up acid to unit *D*. As soon as this occurred and the color in unit *D* began to change, the apparatus was stopped and units *C* and *D* were rinsed and replaced.

It is of interest to note that when the apparatus was working normally, the reaction in unit *D* was slightly on the alkaline side of the neutral point. This alkalinity was only maintained during the process of aeration with CO_2-free air and was presumably due to traces of alkali in the distilled water (or dissolved from the glass) made manifest by removal of CO_2,—an explanation apparently consistent with observations of Fawcett and Acree (1929).

No part of the purification train required sterilization as was shown by long-continued aeration of sterile media in control experiments. The slight tendency of bacteria to leave a liquid phase and the filtering effect of the cotton plug in the inlet of the culture unit proved amply sufficient to guard against contamination.

Culture unit

The culture unit (*E*, fig. 1) consisted of a sterile Dreschel "low form" glass-stoppered gas-washing bottle with a capacity of 250 cc. and containing a known amount (100 to 175 cc.) of inoculated culture medium. It was immersed in a water bath kept at constant temperature (37°C.) by thermo-electric regulated heat. Variations were usually within a few tenths of a degree in either direction.

Two culture units containing the same medium, each with an absorption train, were aerated in parallel from a single purification train in the water bath at the same time,—one for the culture under observation and the other for an uninoculated control. The aeration rate commonly employed in our experiments was 2.5 to 3.5 liters of purified air per hour for each *E* vessel.

The Dreschel bottles after sulphuric-dichromate cleaning, were prepared by plugging inlet and outlet arms with non-absorbent cotton and covering the arms with glass caps pushed over cotton packing. A strip of paper was inserted between the ground glass stopper and its seat and the assembly then sterilized by dry heat in excess of 160°C. for over one hour.

At the start of an experiment the sterile ingredients of the media, in solutions of suitable stock concentration, were measured aseptically according to a dilution schedule into the Dreschel bottles and control plates made from each stock solution. Two filled Dreschel bottles (one for culture and one for uninoculated control) were placed in the water bath side by side and aerated, without absorption units (*G* to *H*), for one to three hours or more, during which time they rose to the temperature of the water bath and any excess free gases were expelled.

When ready for inoculation, the Dreschel bottles were lifted from the water bath to a table and similar volumes of bacterial suspension and sterile water were added respectively to the culture and the control. After being shaken, samples for plating and for chemical medium analyses were rapidly and aseptically removed from both, the bottles returned to the water bath, aeration resumed and the absorption units connected. Similar manipulations occurred at each subsequent sampling period, except, of course, that no bacteria or water were added. Caution was exercised to keep the bottles out of the water bath for the briefest possible time.

The volume of culture in the 250 cc. Dreschel bottles was planned to approach approximately 100 cc. at the midpoint of experiments. Accordingly a starting volume of 100 to 175 cc. was employed, dependent on the amounts to be withdrawn at sampling periods. Record was kept of the number of cubic centimeters thereafter removed,—hence the volume present during any stated period was known,—and the observed gas yields for each period were multiplied by an appropriate factor to convert them to yields per 100 cc. of culture volume. The record of sample portions removed was confirmed at the close of experiments by measure of the terminal volume, which showed substantial agreement with the calculated remainder.

At the start and close of each gas collection period, the menstruum was analyzed for NH_3 and CO_2. The content of each in the control medium was then subtracted from that in the culture and the net increase in medium content for the period calculated. The methods used involved modifications of the well-known procedures of Van Slyke and Cullen (1914) and Van Slyke and Stadie (1921), respectively. The modifications were conditioned by the very minute values with which we were dealing and will be described in a subsequent communication (Walker and Winslow, 1932). To the NH_3 and CO_2 yields thus revealed by net increase in menstruum content over an observed period were added the amounts of these gases which had been continuously removed by aeration during the period, their determination being as follows.

ABSORPTION OF AMMONIA

Ammonia was absorbed in unit *F*, which was an 8 by $1\frac{1}{2}$-inch cylinder equipped with a Folin ammonia absorption bell and containing 100 cc. of approximately 0.05 normal H_2SO_4. Early tests showed that anticipated yields of free NH_3 would in many instances be too small for accurate determination by titration. After neutralization, direct Nesslerization of an aliquot according the Standard Methods of Water Analysis (1925) of the American Public Health Association and American Water Works Association was therefore tried, modified by the fact that preliminary clarification was unnecessary.

The absorbent to be used was crucial since it must retain NH_3 and pass CO_2 but must not be too acid for Nesslerization. The problem was worked out with the last named criterion as a starting point. The Nessler's reagent contains a strong base necessary to proper color development. Obviously, if any acid solution were to be directly Nesslerized, arrangement must be made to leave a sufficient excess of free alkali to avoid depression of color. The critical zone for HCl or H_2SO_4 was found to lie in the vicinity of N 0.025 to 0.050. A concentration of N 0.025 could be neutralized (in relation to methyl orange) by slightly less than half the alkali contained in the amount of reagent regularly used in Nesslerization. A concentration of N 0.050 was not neutralized

by one-half the reagent but was rendered alkaline on addition of the second half. Tests were then made by adding standard NH_4Cl to acids, Nesslerizing, and comparing the developed color with that in acid-free standards prepared from the same NH_4Cl solution. The results showed that N 0.050 HCl partially but definitely suppressed color development, while N 0.005 had no deleterious effect. However, it was further learned that by neutralizing N 0.050 acid with NaOH prior to adding the Nessler's reagent, inhibition of color could be avoided.

It was deemed inadvisable to attempt using stronger acids, for alone they would seriously exceed the tolerance of the Nessler's reagent and, if neutralized, they might introduce additional error. Therefore it was tentatively resolved to absorb the aerated

TABLE 1

Series-absorption of aerated NH_3 from peptone cultures of Esch. coli

ABSORBENTS, 2 IN SERIES	LENGTH OF AERATION	NH_3-N FOUND	
		First absorbent	Second absorbent
	hours	*mgm.*	*mgm.*
100 cc. N 0.05 H_2SO_4	16	0.696	<0.003
100 cc. N 0.05 HCl	23	0.560	<0.010
100 cc. N 0.50 HCl	72	11.000	<0.004

ammonia in N 0.05 HCl or H_2SO_4 and to determine the amount by direct Nesslerization, the tubes during dilution, and before addition of the reagent, to receive an amount of 5 N NaOH equal to one-hundredth the volume of the acid aliquot sampled.

Obviously, the next procedure was to prove that N 0.05 acid would absorb NH_3 completely, would not appreciably retain CO_2 and would not exhibit volatility or mechanical entrainment deleterious to the $Ba(OH)_2$ in the succeeding unit (*G*). To test these points a series of experiments were performed.

Adequacy for NH_3 absorption was proven by continuously aerating the gases from an incubating peptone culture of organisms (*Esch. coli*) into two (*F*) units in series, each containing 100 cc. of N 0.05 acid. The results of two such tests and of one with stronger acid are presented in table 1.

It is evident that a high degree of absorptive efficiency was obtained. In the light of a large body of later experience it seems probable that the amounts of ammonia found in the second serial absorbents as shown above do not represent a carry-over from their predecessors, since they are of a magnitude appearing in almost any use made of this method and may be related to absorption from rubber stoppers or connecting tubes.

Non-retention of CO_2 by the acids was established in two ways. First, gases produced by a culture of organisms in standard lactose broth were passed for nineteen hours through an (*F*) unit containing 100 cc. N 0.05 HCl. It is probable that in this time about 100 mgm. of CO_2 were blown through the cylinder. The culture was then cut out of the line and air from the purifying train passed at a very brisk rate directly into the cylinder, which was now followed by an absorbent vessel containing saturated $Ba(OH)_2$. In one-half hour no turbidity appeared. After removing half the contents of the cylinder for Nesslerization, the remaining 50 cc. were briefly aerated to remove any room atmosphere absorbed during withdrawal of the first half. The saturated $Ba(OH)_2$ was again connected, sufficient strong H_2SO_4 added to the N 0.05 HCl to make a concentration of nearly 20 per cent acid and air was then blown rapidly through the entire assembly for eight hours. The barium hydroxide remained free from turbidity.

The second method of testing non-retention of CO_2 was as follows: The gases from an actively growing culture of *Esch. coli* in 1 per cent peptone + 0.1 M NaCl were aerated for two hours through an (*F*) unit containing 100 cc. N 0.05 H_2SO_4. Analysis of the succeeding unit (*G*) showed that 16.4 mgm. CO_2 had passed through *F* in this time. Simultaneously, air from the same purifying train had been passed into a parallel (*F*) unit through an equal volume of peptone medium containing no organisms. Aliquots from the two (*F*) units, which had therefore respectively experienced aeration with CO_2-containing and CO_2-free air, gave identical readings for dissolved gases when analyzed for CO_2 in a Van Slyke blood gas apparatus.

In addition to non-interference with Nesslerization, efficiency

for NH_3-retention, and non-retention of CO_2, three other properties of unit (*F*) were controlled. Possibility of interference with the following alkaline unit (*G*) was first tested. To this end, purified air was passed successively through a culture bottle containing sterile distilled water, then through an (*F*) unit containing N 0.05 acid, then through a tube of distilled water and in some cases an (*H*) unit with saturated $Ba(OH)_2$. At the end of the aeration period, brom thymol blue was added to the distilled water which followed the (*F*) unit. In some experiments, the aeration was maintained for twenty hours and in others for ninety hours, but no blown-over acidity was detected by the brom thymol blue from either N 0.05 H_2SO_4 or N 0.05 HCl. As points of additional interest, the (*F*) units and the sterile distilled water flasks were Nesslerized and the saturated $Ba(OH)_2$ terminal units watched for turbidity due to $BaCO_3$ or $BaSO_4$. The negative results of all these blank runs proved the efficiency of the purifying train while confirming the non-volatility or non-entrainment of the NH_3 absorbents at the aeration rate employed.

The possibility of false positive NH_3 results due to entrainment of culture medium into unit (*F*) was next studied by aeration of sterile media. Results showed that only very small amounts of ammonia could be traced to this source from peptone and lactose-peptone media in a much longer period of aeration than was actually used in our metabolism studies.[4] From Dolloff's medium, which contained 76.5 mgm. of NH_3-N per 100 cc., more definite amounts were aeratable.[5] Since no method to prevent this could be discovered and since all yields from metabolism studies were based on subtraction from observed culture products of amounts obtained from parallel and simultaneous aeration of sterile control media, this matter had to be allowed to rest with the latter type of control.

The final factor investigated was the NH_3 content of the sul-

[4] In five tests aeration of sterile 1 per cent peptone followed by dilute acid absorbents revealed that in time periods as long as twelve to twenty hours the average amount of NH_3 carried over from the medium would not be expected to exceed 0.001 to 0.007 mgm.

[5] In 4 experiments aeration of sterile Dolloff's medium for twenty-four hours into N 0.05 H_2SO_4 showed an average of 0.129 mgm. of NH_3-N carried over.

phuric acid itself. The amount of NH_3–N in a routine determination attributable to this source was found to be between 0.002 and 0.004 mgm. per 100 cc. of N 0.05 H_2SO_4 absorbent. Thereafter the amount equivalent to this content, in cubic centimeters of standard NH_4Cl, and proportionate to the aliquot acid Nesslerized, was deducted from all readings as observed against the color standards.

In Nesslerization, fresh color standards were prepared when used in preference to keeping permanent standards. The distilled water was collected from a Barnstead still and stored in 6-liter Pyrex flasks equipped for siphon delivery, the incoming air being drawn through a tube of sulphuric acid to exclude atmospheric NH_3. This water gave a very faint color reaction with Nessler's solution which may have been largely dilution of the color of the reagent, since an addition of standard solution to the extent of only 0.001 mgm. of NH_3–N produced a visible color response. The 50 ml. color comparison tubes conformed to the American Public Health Association Committee's specifications.

ABSORPTION OF CARBON DIOXIDE

Carbon dioxide absorption was accomplished in units *G* and *H*. Unit *G* was a 250 cc. Erlenmeyer flask equipped with a Brady-Meyer absorption tube. This device is used in the steel industry for the determination of carbon (Brady, 1914). The CO_2 absorbent was standardized $Ba(OH)_2$ of about N/10 concentration. Twenty-five or 50 cc. were measured by a burette into the Erlenmeyer flasks and CO_2-free distilled water added to make the final volume 75 cc., the amount needed for proper occupancy of the Brady-Meyer bulb tube. The culture and control flasks were kept tightly stoppered between filling and use, at which latter time the bulb-tubes and connecting tubes, assembled in their 2-hole stoppers, were introduced. When aeration was completed, the fluid was allowed to return to the flasks from the bulb tubes, the latter being then carefully rinsed down with 40 cc. CO_2-free water from a protected siphoning reservoir. The flasks were tightly stoppered and set aside for subsequent titration. Traces of atmospheric CO_2 probably gained entrance in

the filling or rinsing down processes or by stopper perfusion but since results were reported as differences between cultures and controls and the parallel flasks were in all manipulations given the same handling, it is unlikely that serious error occurred from such sources.

The amount of CO_2 absorbed was determined by back titration of the free $Ba(OH)_2$ remaining unprecipitated, the volume of which was deducted from the original $Ba(OH)_2$. The advantages of thymolphthalein as an indicator for this type of titration were pointed out by Schollenberger (1928). Its use has been found very satisfactory.

The permanent standard for this part of the work was N/10 HCl, prepared by volumetric dilution from a purified stock solution, the strength of which had been fixed by distillation at constant boiling point. The final figure for its value was based on an ultimate standard of carefully weighed portions of dried potassium acid phthalate titrated through an intermediary of CO_2-free NaOH. The permanent N/10 HCl gave in terms of the phthalate, on two standardizations, made a year apart by different laboratory workers, values of N 0.1029 and N 0.1028.

The $Ba(OH)_2$ solution was prepared by dissolving weighed crystals in boiling CO_2-free distilled water and was clarified by filtration into a volumetric flask freed of atmospheric CO_2 by aeration. When cooled and diluted with CO_2-free water the solution was stored in a large reservoir equipped with a Squibb's automatic-O type burette. Soda-lime tubes were placed on the air vent arm of the burette and between the reservoir and the rubber bulb supplying air pressure. The solution was then standardized in terms of its volumetric ratio to a known working solution of N/10 HCl, using the mean of values obtained in titrating the acid with the alkali in the presence of two indicators (alizarine sodium monosulfonate and phenolphthalein) lying close on either side of true neutrality. A minor error which would have been incurred in making the subsequent experimental titrations with thymolphthalein, the end point of which is near pH 10, was avoided by the subtraction of the blank from the sample. The $Ba(OH)_2$ solution as stored in the protected

Squibb's burette was found to retain its strength very satisfactorily.

The distilled water used in all CO_2 work was prepared as follows: Atmospheric CO_2 was expelled from a clean 6-liter Pyrex flask by aeration with CO_2-free air. Meanwhile, the Barnstead still with dissolved gases escaping in the steam waste exhaust was set in operation and the early yield discarded. A 2-hole stopper with short straight inlet tube and a capillary outlet tube was connected directly to the discharge from the still. The condensed water was thus collected with avoidance of serious exposure to room atmosphere, since it was presumed that little back diffusion would occur through the capillary outlet from which air and hot water vapor were continually displaced by the collecting distillate. After being cooled the flask was stored for siphon delivery by a soda-lime protected burette. The air intake for the flask was protected by a tube of strong NaOH solution. Water from this reservoir would for many days exhibit a pH of 6.9 to 7.1 with brom thymol blue if tested as soon as drawn and without shaking. In the light of the recent work of Fawcett and Acree (1929) it seems possible that the above procedure may not have yielded a strictly CO_2-free water, but it always appeared satisfactory for our purposes.

One other feature was given attention. This was the question of whether the Erlenmeyer flasks (unit *G*) containing the experimental yields could be safely titrated in the open or would require passage of a stream of CO_2-free air while being held under the burette. The possibilities were that the unprotected $Ba(OH)_2$ might absorb atmospheric CO_2, or that the aerated $Ba(OH)_2$ + $BaCO_3$ might lose CO_2 during titration. The results of trial titrations under several conditions indicated that flasks of $Ba(OH)_2$ diluted proportionately to those employed in the metabolism studies, whether containing $Ba(OH)_2$ or $Ba(OH)_2$ + $BaCO_3$, could be titrated unstoppered, with gentle shaking, without recourse to the aeration technic. This more common procedure was thereafter followed.

The final aeration unit (*H*), a Bowen potash bulb containing saturated $Ba(OH)_2$, served to control completeness of absorption

in the preceding unit (*G*). So long as the standard solution in (*G*) was not overloaded by CO_2 its absorption performance was shown by this control to be 100 per cent complete. In metabolism experiments in which over 100 mgm. of CO_2 were harvested in unit *G* no evidence appeared of any carry-over to unit *H*. The similar bulb at the end of the aeration train containing the sterile control medium served incidentally as a constant control on the freedom from CO_2 of the air which was being fed by a *Y* junction from the one purification train (*A–D*) into the parallel culture and control trains (*E–H*).

SAFETY VALVES

For use with our aeration, which was supplied by compression from a laboratory tap, considerable preliminary time was spent in perfecting a safety valve which would satisfactorily permit escape of excess pressure without permitting kick-back of fluids in the long train. The valves finally developed are shown in figure 2. In all our later work only the pressure valve (right) was employed. The principle underlying the valves is evident from the figure and its key. This part of the apparatus has been highly satisfactory in that it not only prevented kick-back but has even permitted aeration through the train to continue while excess pressure was escaping. Many an experiment was thereby saved from accidental ruin.

The complete aeration train was operable under a head of about 3 feet of water (equal to 3 inches of mercury) in the safety valve. The usual rate of aeration was 2.5 to 3.5 liters of air per hour per culture flask.

The rubber stoppers and tube connections employed in our apparatus were cleaned after the method recommended by Novy, Roehm and Soule (1925), which involved boiling in dilute alkali and in acid, thorough rinsing, and autoclaving in glycerol, after which they were preserved in glycerol to insure a moist, tight seal. Whenever possible, glass junctions were kept end against end in order to minimize rubber surface exposure.

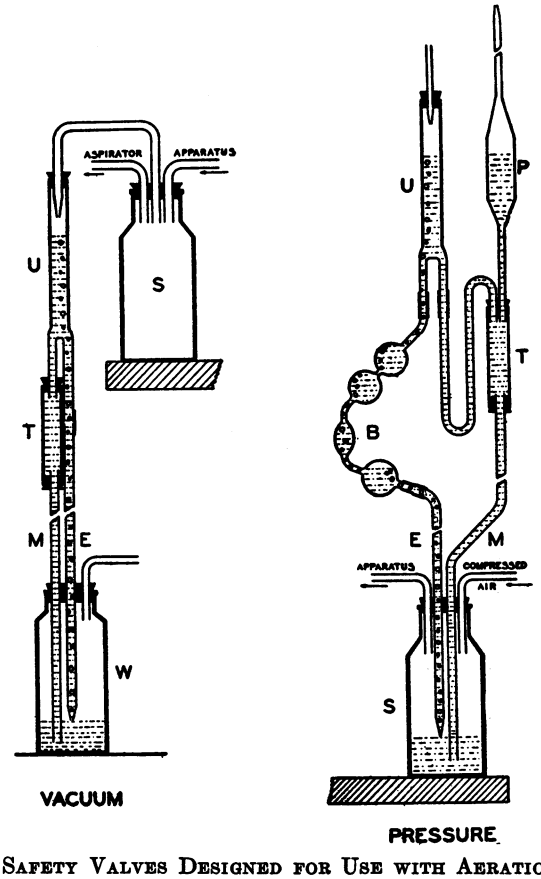

FIG. 2. SAFETY VALVES DESIGNED FOR USE WITH AERATION TRAIN

KEY TO FIGURE 2—SAFETY VALVES FOR AERATION TRAIN

	Function	*Composition*
S	Safety bottle and pressure seal	Large-mouth, heavy glass bottle
S	Vacuum safety bottle	Large-mouth, heavy glass bottle
W	Vacuum water seal	Large-mouth, heavy glass bottle
E	Pressure escape column	Glass tubing, constricted tip
M	Pressure maintenance column	Glass tubing
U	Common reservoir uniting (*E*) and (*M*)	Test tube 8 inches by 1 inch with *U*-base inlet tubes
T	Trap against air rebound	Glass tube, 5 inches by 1 inch
P	Pressure maintenance reservoir	100 cc. pipette, inverted
B	Escape air-arresting bulbs	Potash bulb

S may rest on table with apparatus.
W may rest on floor.
Supporting stands and clamps not shown.

SUMMARY

A method has been described which makes it possible to cultivate bacteria in a medium continuously aerated by a stream of air which has been freed from ammonia and carbon dioxide; and to determine with accuracy the yielded amount of both ammonia and carbon dioxide in the air which has passed through the culture. The pitfalls in such a procedure are so many that it has seemed justifiable to describe in considerable detail the methods as finally worked out. The determination of the ammonia and carbon dioxide remaining in the culture medium can be made by slight modifications of standard technics used in the physiological laboratory which will be described in a subsequent communication (Walker and Winslow (1932)).

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