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DISINFECTION BY THE FORMALIN-PERMANGANATE METHOD.

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In the thirteenth report of the Maine State Board of Health there appeared a paper by Dr. J. P. Russell and myself on a method of liberating formaldehyde from a solution of "formalin" by the use of potassium permanganate. That paper carried the work down to a point where, by using a fixed amount of the formalin solution, the time necessary for efficient disinfection was shown to be not over three hours, and possibly less. The State board of health adopted four hours as the minimum time for efficient disinfection, and then I began work on determining what amount of formaldehyde was to be considered the minimum with which to secure thorough disinfection in four hours time. Upon the results of that work, and the many new observations that have been made in the process of that work the present paper deals.

It has seemed best to make this paper include all the work that has been done at this laboratory upon the above method of disinfection. As a result the paper will include much that was in the first article, but in a form and substance considerable altered by the results and observations that have resulted from the continuation of the work along other lines. The work of determining the minimum of time for efficient disinfection was done in conjunction with Dr. J. P. Russell, then director of the laboratory; and in the latter part of the work I have to thank my assistant, Mr. H. F. Quinn, for his thorough and painstaking aid.

Early in the nineties interest was first widely aroused in formaldehyde as a disinfecting agent. Up to that time, and in many cases up to the present time, sulphur had headed the list of disinfecting agents. No doubt is to be cast on the efficiency of sulphur as a disinfecting agent, but its use is open to a

variety of objections such as the necessity of fire in preparing the gas, the long period of exposure necessary, and the destructive action it exercises on a large variety of fabrics, especially colored ones, and upon metallic objects. As a result of the above inconveniences attendant on the use of sulphur, the claims made for the new disinfecting agent caused not only considerable interest but wide investigation. These investigations not only revealed the fact that formaldehyde was an efficient germicide when it is intelligently used, but that cumbersome apparatus was apparently an essential part of the process.

While formaldehyde is now the most widely used of gaseous disinfectants those who have had to do with the work of actual disinfection have found the present methods, both of generating the gas or of liberating it from its water solution, far from satisfactory. At present there are three methods of obtaining the gas which are in general use, i. e. the lamps which form formaldehyde by the oxidation of methyl alcohol, generally through the catalytic agency of platinum black; the lamps or autoclaves which evaporate the water solution of formaldehyde known commercially as "formalin;" and the so called "sheet method" where the solution of formaldehyde is sprayed upon suspended sheets, from which it evaporates and diffuses throughout the room. At the present time the method described here, and in its first form published in 1904, has received considerable recognition.

One of the chief objections to the three earlier methods mentioned above was the long period of time necessary for the introduction of the requisite quantity of formaldehyde into the space to be disinfected. This naturally permits of a very considerable leakage of the gas and, more important still, prevents the bacteria from being exposed to the full strength of the gas at once. The first two methods also present the rather serious objection of having to use fire to generate or liberate the gas, thus introducing not only the need of constant attention but the element of danger from fire as well. Not only does the "sheet method" still more delay the process of diffusion of the gas throughout the room, but the long and disagreeable process of spraying the sheets fails to recommend itself to the majority of practical workers.

It has been and still is a vexed question as to whether formaldehyde exerts its germicidal power when in the gaseous condition or whether the gas must be condensed on the object to be disinfected before it can exert this germicidal action. The latter idea finds considerable substantiation in the fact that water vapor is necessary for efficient disinfection. Also von Brunn states that the greater part of the formaldehyde introduced into a room almost at once condenses on the walls and articles in the room. If it were to the condensed formaldehyde that the germicidal action is entirely due it would seem that more efficient disinfection should be secured in cold rooms, as the cold walls would precipitate a greater amount of the gas than would warm walls. This fact of a cold room precipitating more of the gas than a warm room is shown by results obtained by introducing into the same room the same amount of the gas once when the temperature of the room was high, and once when it was low and then determining the amount of gas present in the air in each case. It is always much smaller in the case of the cold room, and at the same time disinfection is not as efficient under these conditions as under warmer ones. These results would make it appear that the main part in disinfection is played by the gaseous formaldehyde, although there can be no doubt but that the condensed gases have considerable germicidal power also.

It had long been the opinion of the writer that, if it were possible to get an almost instant liberation of the entire amount of formaldehyde that was to be used, not only the time of exposure to the gas but the amount of the gas necessary for efficient disinfection could be very greatly decreased. In other words, it seemed probable that bacteria would be killed quicker and by a less amount of the gas if they were exposed at once to its full strength. How well this assumption has been borne out the results herein tabulated will show.

In December, 1903, Dr. Young called my attention to the fact that a gas having the odor of formaldehyde was liberated when permanganate of potassium was allowed to act on formaldehyde solution, and he requested that I see if the matter could be turned to any practical account.

The reaction in question has long been known, and has probably been employed by many, as by myself, in classroom to

illustrate the formation of an acid by the direct oxidation of an aldehyde. Base states that the final results of the reaction are probably these:

$$4KMnO_4+3H.COH+H_2O=4MnO(OH)_2+2K_2CO_3+CO_2.$$

Theoretically formic acid (H.COOH) is formed by the oxidation of formic aldehyde (H.COH) just as the latter results from the oxidation of methyl alcohol (CH_3OH). This reaction can be brought about by adding to the formaldehyde a substance that rapidly gives up part or all of its oxygen, such as the dichromate or permanganate of potassium. In reality the presence of an excess of oxygen carries the reaction a step farther and results in the decomposition of some of the formic acid into water (H_2O) and carbon dioxide (CO_2).

The amount of heat generated by the formation of the formic acid is very great and, under proper conditions, this total amount of heat is liberated in a very short space of time. When proper proportions of the permanganate and formaldehyde solution are mixed oxidation at once begins and the sudden and great amount of heat generated by this oxidation is sufficient to cause the evaporation of a large amount of the aldehyde before it is oxidized, *and to do this very quickly.* The permanganate is reduced to a lower oxide of manganese with the liberation of much oxygen. The reaction is very vigorous and attended by much effervescence but, best of all, this amount of formaldehyde which is evaporated and thus removed from the oxidizing action of the permanganate, *is avilable within five minutes of beginning of the reaction and in its maximum amount.*

Here seemed to be the desired method with all three of the desiderata, i. e. instant availability of the maximum amount of the gas; absence of need of skilled attention, and absence of all danger from fire. As an additional advantage experience has shown that no special apparatus is needed with it. Work was at once started to determine the practicability of the method for actual disinfection.

At first hand this method may seem an anomalous one as the disinfecting agent is obtained by a process that involves the destruction of a part of it but, as I was working along the idea that instantaneous exposure of the orgaism to the full strength of the gas would decrease the amount of the latter needed, it

seemed as though in the end the cost of the operation would be considerable decreased. Also its very simplicity was greatly in its favor.

At the time that work was started along these lines I was unaware that this method had ever been advanced as a means of practical disinfection, although no claims of originality were presented with the first report that was made. When it had been shown that the method could be used successfully it developed that the method was first suggested in 1902-3, but it was given no publicity until 1904, when it was described in a paper by Dr. G. A. Johnson of Sioux City, Iowa; the paper being read before the Sioux Valley Medical Association. Even then it attracted little attention, and the work at this laboratory was the first subjection of the method to systematic tests.

All the work has been performed using the ordinary commercial solution of formaldehyde supplied to the State board of health; an article that rarely assays above 36% of formaldehyde by weight. The permanganate has been the fine needle-shaped crystals of commerce, not the large c. p. octohedral crystals. In the work of 1904, in determining the amount of formaldehyde available through the use of this reaction, the c. p. crystals were used, but were first powdered. In the latter work the commercial article has been used entirely. All determinations of the formaldehyde have been made by Romjin's potassium cyanide method, as described on page 393 of Sutton's "Volumetric Analysis," eighth edition, experience having shown that this method was especially reliable for use with the very dilute solutions of formaldehyde that were obtained by aspirating the charged air from rooms.

The first work was to determine the proportions of the two reagents that would furnish the greatest yield of the gas. Results of experiments in 1904, the experiments being conducted in glass beakers, led me to decide on the use of 3.75 grams of permanganate to 10 cubic centimeters of formaldehyde solution. Practical work in the disinfecting room showed that this amount of permanganate was too small when large quantities of the reagents were employed, a moist residue being left in the generator that contained considerable formaldehyde. As a result, since the experiments then reported, I have carried on others using as large quantities of the reagents as would be

used in actual disinfection. *The new proportions decided upon are 4.75 grams of permanganate to 10 cubic cetimeters of formaldehyde.*

It was of interest to observe, after considerable work had been done, using these proportions, that Base in an independent investigation decided on the proportions of 5 grams of permanganate to 10 cubic centimeters of formaldehyde solution. In the 1906 circular of the Illinois board of health on disinfection the proportions recommended were about 2 grams of permanganate to 10 cubic centimeters of formaldehyde. Work at this laboratory has shown that when these proportions are used a layer of formaldehyde solution remains over the manganese residue in the generator at the end of the reaction, a thing that shows great waste of formaldehyde. The air aspirated from a room charged under these conditions has never shown as much as 9% of the formaldehyde that was put into the generator, the usual amount being about 5%. As this report goes to press a second edition of this same circular comes to hand in which the proportions are practically as in my first report. In view of the recent work I think that the minimum proportions should be *4.75 grams of permanganate to 10 cubic centimeters of formaldehyde solution.*

Experience has shown that no change should be made in the original method of bringing the reagents together. The permanganate is to be put into the generator first and the formaldehyde solution poured upon that. The reagents are never to be mixed in the reverse order. If the operator's courage is good it is of advantage to quickly shake the contents of the generator after putting in the reagents, as the pouring of the formaldehyde solution upon the permanganate is apt to disturb the even distribution of the latter, so heaping it up in places that it may be some time before the interior of the mounds thus raised are acted on by the solution. Experience has failed to show any advantage to be gained by the addition of sulphuric acid to the formaldehyde solution in order to increase the yield of oxygen from the permanganate. In fact it seems to be slightly detrimental to the reaction.

In order to get as rapid and vigorous action as possible the permanganate must be in powdered form, or in the long needle-shaped crystals of the commercial article. If the large chem-

ically pure crystals are used they must first be powdered. The reason for this is of course plain. Chemical action takes place between two substances only when they are in actual contact, and varies in intensity as the amount of surface which the substances present to each other for contact. As a result, the greatest amount of chemical action in a given time will take place when the substances present the greatest amount of surface for mutual action. In the large crystals the action does not reach the material in the interior of the crystals until the surfaces have been eaten away. This necessarily increases the time required for the reaction and involves loss of available permanganate, as the greater part of the material in such a crystal lies not on the surfaces but in the interior. The greatest possible exposure of surface of the permanganate gives the highest and quickest yield of the gas. As a result the permanganate should always be in a fine crystal or in powdered form.

There is little to be said in regard to the kind of generator to be used in this work as any dish, with sides high enough to prevent the solutions from boiling over, will answer the purpose. So far as bacteriological results go there is no preference to be given to any kind of generator. In our report of 1904 a special form of generator was described but was not recommended, as it was desired to make the method of operation and the apparatus as simple as possible. It is now to be stated as the belief of the writer that the apparatus then described can be discarded with more profit than it can be used.

The reaction is so violent, and so much frothing and effervescence attends it that either very high or very wide dishes must be used as generators. In our work a variety of materials have been used as generators, such as tall glass beakers, tall earthern jars, pails both of tin and galvanized iron, wide bottomed and shallow tins such as the ordinary dishpan, and agate lined ware with moderately high sides and wide bottoms. As a result of my experiments I am led to favor the wide bottomed and comparatively shallow dishes. Glass does not make an acceptable generator for, apart from the score of fragility and cost of large enough beakers, glass is a very poor conductor of heat. This, coupled with its fragility, constitutes a serious drawback to the use of glass, for the production of sudden and great heat at the bottom of the dish results in too sudden a strain within

the glass itself, and the bottom often breaks out. In addition, and on account of the poor conductivity of glass, a considerable amount of formaldehyde gas coming in contact with the cold upper walls of the dish is converted into the solid white modification known as paraformaldehyde ($C_3H_6O_3$), and this latter is deposited on the sides of the generator, resulting in the loss of this amount of formaldehyde for disinfecting purposes.

In the work of 1904 earthern jars and pails of tin and galvanized iron were used entirely. The earthern crocks are not recommended as there results too great an absorption of heat by them, this resulting in less heat being available for evaporating the formaldehyde solution and either consequent loss of the latter or increase in the amount of permanganate that must be used. There was here but slight tendency of the gas to polymerize. The tin and galvanized iron pails were superior to the earthern jars in not absorbing as much heat, but offered no indications of superiority as far as polymerization of the gas was concerned.

One great drawback to the use of all these tall dishes was the spattering out of the reagents. The amount of actual loss of formaldehyde for disinfecting purposes was of course slight from this source, but it necessitated the use of some wide dish in which to set the generator or else covering the floors in the immediate vicinity of the latter. Attempts were made to obviate this difficulty by changing the shape of the dish in which the gas was liberated with the result that it was found that the use of a wide shallow dish, such as a dishpan, will prevent all spattering of the reagents and also all apparent polimerization. The dishes to be so used need not have sides more than 8 inches in height *but must have wide bottoms.* A good rule to follow in deciding on the size of the dish to be used is to choose one whose bottom is such that it will just be hidden from sight when the requisite amount of the permanganate is poured in and evenly distributed. Proceeding thus practically the entire surface of the permanganate that is used is in contact with the formaldehyde solution and the reaction proceeds evenly, where, if the permanganate lies to some depth on the bottom of the dish, after the surface action between the reagents is evenly under way the hot formaldehyde solution works down into the layers of the permanganate below, and sets up a more

violent reaction than is proceeding above it. The products of this more violent reaction are forcibly thrown up through the effervescing liquid above, causing a spattering of the reagents. An even and uniform union of the reagent, while attended by effervescence, is not accompanied by spattering, and this reaction is obtained when the permanganate and formaldehyde solution are spread out in such a thin layer as to be practically all in contact at once.

From the very even and excellent results obtained with these dishes I would recommend the use of these wide bottomed and comparatively shallow pans as generators, the fact being borne in mind that there will be no spattering of the contents if the bottom of the dish be so wide that the requisite amount of permanganate just conceal it, and the sides be 8 inches high. It was with the purpose of obtaining a thin and even layer of the permanganate that I recommended, in a previous paragraph, that the contents of the generator be shaken somewhat after adding both the reagents. The rather low walls of these dishes also offer little chance for sudden cooling of the gas and consequent polymerization. The width of the dishes also makes any additional flare to the tops unnecessary, the column of gas not being thrown straight up to the ceiling but beginning to spread out as soon as it rises above the top of the generator.

This reaction between the formaldehyde and permanganate does not result in the formation of any products that are harmful either to the texture or color of any materials that may be in the room when it is disinfected. The only products that get into the room are those that are gases, and these are water vapor, formaldehyde, carbon dioxide, a very little formic acid and a little oxygen. None of these would be expected to have any effect on the material left in the rooms, and a number of experiments have shown that they do not. The considerable amount of carbon dioxide which occurs is the product of a secondary reaction by which the formic acid, which is first formed, is broken up in the presence of an excess of oxygen and of heat into carbon dioxide and water vapor. This decomposition of the formic acid cannot be regarded as detrimental to the principal reaction as it removes a compound which might be undesirable in large amount, and at the same time adds to the heat of the first reaction.

In the work reported in 1904 the determination of the yield of formaldehyde was made not by measuring the gas in the room into which it had been introduced but by generating the gas in a specially designed flask, the products of the reaction being led into absorption bulbs, and a check being arranged in the generating flask by means of which the large amount of formaldehyde that condenses on the cold walls of the flask and ordinarily runs back upon the permanganate to be revaporized was itself collected. The result of taking all possible precautions to collect all the formaldehyde given off was the surprisingly large yield there recorded as compared with those I have obtained by aspirating the air from the room where the disinfection was actually going on.

In the work just finished determinations were made of the amount of the gas in the air drawn from the room where disinfection was in actual progress, and it was intended to repeat the experiments of the two years previous. One determination was made in the latter way, yielding 74.6% of formaldehyde, but the apparatus was broken in the process of the second experiment. At the same time the amount of formaldehyde in the air drawn from the charged rooms had been determined. The great difference in the results showed that we were dealing with widely divergent conditions and, as I wished the results set forth in this paper to represent the figures to be obtained in actual disinfection, the older method was abandoned and determinations only made on the air that was aspirated from the charged room.

The room in which the latter experiments were carried on was built into a room in the laboratory, a jog in one end of the laboratory room making it possible to get a good-sized room by building a wall from the jog to the opposite wall of the laboratory. This wall was built of matched pine boards and the side within the test room was papered. In this wall was put a heavy door, 3 x 7 feet, with a window set into the upper portion through which observations of the instruments in the test room could be had. This door was perforated with inch holes which were tightly closed with cork stoppers. Through these holes test objects could be introduced and withdrawn without opening the door or allowing escape of the gas. The room in question measured 20 x 4½ x 10 feet high. The wall to the south

was the one of matched boards just described. The north wall was of the same size and papered save for a strip running around the wall about three feet from the floor, and being three feet wide. This strip was of painted plaster, having once been used as a blackboard. Midway in this wall and 8 feet from the floor was a long narrow window 1 x 9½ feet, and opening into another room. The west wall was 4½ x 10, and a duplicate of the one just described except for the window. The east wall was of the same size as the west one but had a window let into it two feet above the floor, the window measuring 3¼ x 6¼ feet. Around the north and east walls ran four coils of pipes from the hot water heater, so as to permit of work in it during cold weather. The door and the windows were no more tight than in an ordinary room nor was any effort made to make them tighter, as it was desired to work under ordinary disinfecting conditions. No cracks were covered or patched while the experiments were under way. This is true both of the cracks by the door and the windows. The capacity of this room, including the jog into which the east window was set, was 862 cubic feet.

For drawing air from this room the following apparatus was devised. A glass tube passed into the test room through two rubber stoppers set tightly into one of the holes in the door. This tube projected two feet into the room and terminated in a funnel. The end of this tube which was outside the test room was connected with the first of a series of three Dreschsel gas wash-bottles. The last Dreschsel bottle was connected with a bottle holding a little more than ten liters of water, from which a siphon tube discharged the water. In each of the three Dreschsel bottles was placed 75 cubic centimeters of distilled water; the siphon bottle filled to the ten liter mark, and all joints and connections rendered air tight by sealing them with paraffin wax. When in operation the water was siphoned off at such a rate that at least 30 minutes were required to draw off the ten liters. The amount of water that had to run from the siphon bottle before the air began to bubble through the Dreschsel bottles was measured and, after ten liters had been run out from the siphon bottle, this measured amount was run off in addition to make correction for the amount that had to run from the siphon before the partial vacuum was established

that was necessary to start the bubbling through the Dreschsel bottles.

When the requisite amount of air had passed through the apparatus, and pressure conditions had been equalized the wash-bottles were disconnected and their contents were emptied into a 500 c. c. flask; each bottle washed with three changes of water, and the wash water added to the contents of the 500 c. c. flask. Into this flask was then run 10 c. c. of a standard solution of potassium cyanide (KCN), the whole thoroughly agitated and allowed to stand for five minutes. Then 10 c. c. of N-10 silver nitrate solution, to which had been added ten drops of 75% nitric acid, were run into the flask; the whole mixed; distilled water added to the 500 c. c. mark; 100 c. c. filtered and the excess of silver determined by Volhard's method. (See Sutton, "Volumetric Analysis." Eighth edition, page 155.) From this result the amount of formaldehyde in the ten liters of air drawn from the room was obtained, and the percentage of formaldehyde in the room determined. As an example of the calculation the following will suffice:

The reagents used were N-10 silver nitrate ($AgNO_3$) and N-10 ammonium sulphocyanide (NH_4SCN), the two solutions being accurately adjusted to each other, also a standard solution of potassium cyanide (KCN). The potassium cyanide solution was of such strength that when 10 c. c. of it were treated with 10 c. c. of the silver nitrate solution 1.5 c. c. of the silver solution was left uncombined.

In this case the time required to draw the ten liters of air was 34 minutes, the temperature of the room was 54° F, and the contents of the Dreschsel bottles, when treated as above described, showed the presence of 5.0 c. c. of uncombined silver solution. By calculation this showed that an amount of formaldehyde corresponding to 3.5 c. c. of N-10 silver nitrate had been absorbed from the ten liters of air, this corresponding to 0.0105 grams of formaldehyde. ($3.5 \times 0.003 = 0.0105$).

One cubic foot is the equivalent of 28.315 liters; therefore a cubic foot of air in the test room would contain 0.02973 grams of formaldehyde ($0.0105 \times 2.8315 = 0.02973$). The formaldehyde used was of 35.27% strength as determined by the cyanide method above used, and 431 c. c. were used in the room, this corresponding to the proportion of 500 c. c. of formaldehyde

to 1,000 cubic feet, the room having a capacity of 862 cubic feet. 431 c. c. of 35.27% formaldehyde having been used the actual weight of formaldehyde was 153.01 grams in all, or 0.176 grams per cubic foot. $(431 \times 35.27 \div 862 = 0.176)$

$$\frac{0.02973 \times 100 = 16.8\%}{0.176}$$

By this method the following determinations were made:

Number.	Temperature.	Formalin used c. c.	Permanganate used, grms.	Formaldehyde found per cubic foot.	Formaldehyde introduced per cubic foot.	Percentage formaldehyde present.	Time of drawing the air.
1	83	431	204.7	0.06370	0.176	36.2	34 min.
2	74	431	204.7	0.05946	0.176	33.7	38 min.
3	74	431	204.7	0.05521	0.176	31.3	35 min.
4	80	431	204.7	0.05967	0.176	33.9	40 min.
5	64	431	204.7	0.04247	0.176	24.1	35 min.
6	55	431	204.7	0.02973	0.176	16.8	34 min.

A feature especially noticeable in these results is the very marked decrease in the amount of formaldehyde obtained when the temperature fell below 65° F. There are two explanations for this. As the temperature of the room drops the amount of condensation of the moisture is increased and this condensing moisture undoubtedly carries down with it very considerable amounts of formaldehyde. Also below 65° F. polymerization of the formaldehyde seems to begin and the amount of this polymerization increases rapidly as the temperature drops, this being evinced by the appearance of a hazy cloud which appears in the room as soon as the gas is liberated, but which soon disappears by settling out. In the results tabulated above I began drawing air from the room at the end of twenty minutes after the beginning of the reaction except in the case of No. 6, when I wated until the hazy appearance had disappeared. I did this since the paraformaldehyde has no value as a disinfectant, but would react with the cyanide solution just as the formaldehyde itself. The fact that paraformaldehyde has no disinfecting power in itself may aid in explaining why formaldehyde disin-

fection is not as efficient in cold as in hot weather, and why but little liberation of the gas is to be obtained under these same conditions with the "sheet method."

In considering the amount of formaldehyde gas found in a room it is to be remembered that the amount found by aspirating the air from the room does not represent the whole amount that was liberated in the room, as there is a very considerable amount of condensation of the gas on the walls and objects in the room. The amount of this condensation is said to depend on the surface of the walls and objects in the room, and that it takes place almost as soon as the gas is introduced, being greater upon fabrics and vegetable material than upon the metals. Proof of this very considerable condensation of the formaldehyde gas is had in practical disinfection for, even after a room has been disinfected and so aired that there is no odor of the gas, warming the room will cause the development of such an odor of the formeldehyde that it is often impossible to stay in the room. This odor is undoubtedly caused by the vaporization of the condensed gas from the walls and objects in the room. It is to be remembered that this condensed solution of formaldehyde has a disinfecting value of its own apart from the formaldehyde gas in the room, but that, while this condensed formaldehyde is available for surface disinfection, it is upon the gas, *as a gas,* that we have to depend for all disinfection of the interior of fabrics. As a result it is well to see that the amount of formaldehyde solution used is well on the side of safety if disinfection is to be attempted at temperatures below 60° F. Whenever possible the temperatures should be above this point.

The bacteriological work was carried on in the test room described above. The work of 1904 had been carried on in the rooms of a tenement house under the usual disinfecting conditions, and using 1,000 cubic centimeters of the formaldehyde solution to 1,000 cubic feet. Just before the work of reducing the amount of formaldehyde was started the New Hampshire state board of health announced that as good results could be obtained with 500 c. c. as with 1,000, and work was started with this amount, all decrease being from that as a starting point after it was proved that that amount was efficient.

In the bacterial work we used cultures of the following organisms:—Subtilis, pyocyaneus, typhoid, colon, albus, aureus, diph-

theria, anthrax, pneumococci, throat, prodigiosus, tetragenus, and mixed cultures from throat swabbings from persons suspected of having diphtheria. In the tabulations these latter cultures are marked "mixed," and include cultures some of which did, and some of which did not contain diphtheria bacilli. The cultures used were some on argar, some on blood serum, and some in bouillon, the majority being in the latter. Part of the cultures were exposed open to the gas and part were buried between layers of cloth to test the penetrating power of the gas. The cloths used to bury the cultures in were silk, cottonflannel, and ticking.

The infected slips that were exposed to the gas were made of glazed paper, filter-paper, silk, cottonflannel, and ticking. It was found necessary to sterilize the strips of cottonflannel and ticking before inoculating them, as they were usually found to contain subtilis. This was done by exposing the strips, in a closed bottle, to a temperature of 180° C. for an hour.

The technique of handling the cultures was as follows. On a piece of glazed paper were placed five of the strips to be inoculated with the organisms, then a full loop of a 48 hour, 37.5° C. culture of the organism was taken up on a sterile platinum loop and spread on each of the five strips. If this were done from a bouillon culture of course the entire loopfull was absorbed while of the argar or serum cultures streaks resulted. The bottom of the glazed supporting paper was then bent up, forming a trough, and into this trough the infected strips were shaken. The paper with its five identical cultures was then placed in the test-room in the desired position.

When the cultures were to be buried the following method of procedure was used. A piece of tin six inches square had a circular hole cut in the middle of it, the hole being three inches in diameter. Over one of these tins were spread the requisite number of thicknesses of the cloth and upon the last piece were placed the five infected strips, inoculated as described above, care being taken that they were placed over the circular hole in the tin. The same number of thicknesses of cloth as was put below were now placed above the infected strips, and above that a tin cover the exact duplicate of the tin bottom described. The four sides of the square were then clamped down so that no air

could enter between the folds of the cloth, and thus making it possible for the gas to get at the organisms only by penetrating through the cloth where it was exposed by the holes in the tins. It is unnecessary to add that controls were put up from all the cultures used in the work. In the test-room the cultures were distributed on the floor, on the walls, and suspended from the ceiling. Some were laid on the cracks of the window frames, but just as good results were obtained in one position as in another, showing an equal diffusion of the gas.

After the organisms had been exposed to the gas for four (4) hours the test-room was opened and, while airing it, a leaf of the glazed paper with its five inoculated strips was removed from the room, the strips lifted by sterile forceps and dropped into separate tubes of sterile bouillon of a +1 reaction. These tubes were then incubated at 37.5° C. for 196 hours. All tubes in which growths of bacteria appeared were examined microscopically to see if the growth was of the organism with which the strip was inoculated or was the result of contamination in transferring to the bouillon tubes. Also strips of the same materials as the cultures were exposed on were put into the room and exposed for four hours. They were then put into tubes of sterile bouillon and a loop of the various cultures added. This was to see if enough formaldehyde condensed on the papers to inhibit the growth of the different organisms. In no case was the growth inhibited even when the strips added to the organism were five times the area of the strips on which the cultures were exposed. It may be noted that few cultures showed a growth at a period later than 48 hours after incubation, and none later than 96 hours.

While the test objects were being exposed to the gas observations were being made on the temperature and humidity changes that were in progress in the test-room. The rise in temperature was very slight, averaging 3-4° F., and the temperature soon dropping down to that of the outside air. The observations on the humidity of the room were made with a hair hygrometer, and these observations showed that the gas was equally diffused throughout the room in all cases in twenty (20) minutes, and usually this condition was established in fifteen (15) minutes. Below are given the results of the work in cutting down the

amount of formaldehyde necessary for efficient disinfection, and the average increase in humidity is appended to each of them.

The first series of tests was made using formaldehyde solution in the proportions of 500 c. c. to 1,000 cubic feet. The capacity of the room being 862 cubic feet this necessitated the use of 431 c. c. of the formaldehyde solution and 205.7 grams of permanganate. (47.5 grams of permanganate to 100 c. c. of formaldehyde solution.)

Tests were made with these proportions on four separate days, the temperatures on the successive days being 74° F., 72° F., 79° F., and 76° F. The outside humidity on these same days was 88, 50, 63, and 65, the measurements all being made on a hair hygrometer. The humidity within the test-room after the reaction had been over for twenty minutes was 99, 62, 79, and 80 on the same days, and at the end of the four hours of exposure the humidity was never less than 8% higher than that of the outside air.

The cultures used were serum, argar and bouillon cultures of subtilis, pyocyaneus, typhoid, colon, albus, aureus, diphtheria and mixed cultures from throat swabs, varying from 24 to 72 hours in age.

The total number of cultures exposed was 578. Of these 235 were exposed open to the gas, and 343 were buried, as described, in silk, cottonflannel and ticking, from one to four thicknesses being employed. These cultures were exposed to the action of the gas for four (4) hours, and then put into tubes of bouillon and incubated at 37.5° C, for 196 hours, as described under the section on technique. The results are as follows:

500 C. C. TO 1,000 CUBIC FEET.

Culture.	Number.	Growth.	No growth.
Subtilis	30	5*	25
Pyocyaneus	30	0	30
Albus	30	0	30
Aureus	20	0	20
Colon	35	0	95
Diphtheria	108	0	108
Typhoid	130	0	130
"Mixed"	135	0	135
Total	578	5	573

* The 5 subtilis growths were from open cultures made from heavy argar smears.

After the above work, the quantity of formaldehyde solution was cut down to 400 c. c. to the 1,000 cubic feet, this necessitating the use of 344.8 c. c. of formaldehyde solution and 163.7 grams of permanganate in the test-room. Tests were made with these proportions on four separate days, the temperatures being 72°, 74°, 75° and 80° F. The out-of-doors humidity was 85, 76.5, 68 and 72, and the humidity of the test-room twenty minutes after the reaction was over was 90, 94.5, 85, and 90. This represents an average rise of 17% in the humidity of the room due to the reaction.

The cultures used were serum, argar and bouillon cultures of diphtheria, tetragenus, albus, subtilis, colon, pneumococci, typhoid, throat, aureus, anthrax, prodigiosus, pyocyaneus, and mixed cultures from throat swabs, 403 were open and 40 were buried cultures. The cultures ranged from 24 to 72 hours in age. The time of exposure to the gas was again four (4) hours, and the time of incubation was 196 hours at 37.5° C.

400 C. C. TO 1,000 CUBIC FEET.

Culture.	Number.	Growth.	No growth.
Diphtheria	22	0	22
Typhoid	86	0	86
"Mixed"	43	0	43
Subtilis	31	7*	24
Anthrax	18	0	18
Colon	42	0	42
Albus	41	0	41
Throat	35	0	35
Pneumococci	29	0	29
Tetragenus	25	0	25
Aureus	14	0	14
Prodigiosus	33	0	33
Pyocyaneus	24	1†	23
Total	443	8	435

* Four of the positive subtilis growths were in open cultures and three in buried cultures.

† The pyocyaneus growth was from an open culture.

After this work the formaldehyde solution was cut to 300 c. c. to the 1,000 cubic feet. This necessitated the use of 258.6 c. c. of the formaldehyde solution and 122.8 grams of permanganate in the test-room. Tests were made with these proportions on five separate days, the temperature being 80°, 83°, 81°, 79°, and 80° F. The out-of-doors humidity was 63, 65, 73, 67, and 79, and the humidity of the test-room was 80.5, 81.5, 84.5, 84, and 92 when the reaction had been over for twenty minutes. This represents an average rise of 15.1% in the humidity of the test-room due to the reaction.

The cultures used were serum and bouillon cultures of pneumococci, colon, anthrax, subtilis, typhoid, throat, albus, diphtheria, tetragenus, aureus, prodigiosus, and mixed cultures from throat swabs. 503 were open and 105 were buried cultures. The cultures varied from 48 to 72 hours in age. They were exposed to the action of the gas for four hours, and then incubated at 37.5° C. for 196 hours.

300 C. C. TO 1,000 CUBIC FEET.

Culture.	Number.	Growth.	No growth.
Diphtheria	30	0	30
Typhoid	97	0	97
"Mixed"	37	0	37
Subtilis	30	2*	28
Anthrax	54	0	54
Colon	70	0	70
Albus	48	0	48
Throat	57	0	57
Pneumococci	57	0	57
Tetragenus	21	0	21
Aureus	5	0	5
Prodigiosus	76	0	76
Pyocyaneus	36	0	36
Total	618	2	616

* Both subtilis growths were from open cultures.

The amount of formaldehyde solution was again cut down, this time to 250 c. c. to the thousand cubic feet. In this work 215.5 c. c. of the formaldehyde solution and 102.3 grams of permanganate were required. Tests were made with these proportions on four separate days, the temperatures being 81°, 79°, 79°, and 79° F. The out-of-doors humidity was 84.5, 78, 70, and 66, and the humidity of the test-room at the end of twenty minutes after the reaction had started was 91, 88, 83, and 81, representing an average rise of 11.1% due to the reaction.

The cultures used were serum and bouillon cultures of typhoid, pyocyaneus, colon, prodigiosus, throat, anthrax, subtilis, pneumococci, albus, diphtheria, and mixed cultures from throat swabs. The cultures ranged from 24 to 96 hours in age, the prodigiosus culture being the only 96 hour one. The time of exposure to the gas was four hours and the time of incubation was 196 hours at 37.5° C.

The total number of cultures exposed was 409. Of this number 282 were open and 127 were buried cultures.

250 C. C. TO 1,000 CUBIC FEET.

Culture.	Number.	Growth.	No growth.
Diphtheria	20	0	20
Typhoid	54	1*	53
"Mixed"	31	3†	28
Subtilis	15	5‡	10
Anthrax	30	0	30
Colon	51	0	51
Albus	41	0	41
Throat	41	0	41
Pneumococci	34	0	34
Prodigiosus	51	0	51
Pyocyaneus	41	0	41
Total	409	9	400

* The typhoid growth was from an open culture.

† The subtilis growths were all from open cultures.

‡ The three "Mixed" growths came from swabs that contained the diphtheria bacillus and a tetrad. Examination of the growth obtained here showed *no diphtheria bacilli*, but that the growth was composed entirely of the above mentioned cocci.

The amount of formaldehyde solution was once more reduced, this time to 200 c. c. to a thousand cubic feet. This required 172.4 cubic centimeters of formaldehyde solution and 81.9 grams of permanganate. Tests were made on three separate days, the temperatures being 80°, 85°, 82°. The out-of-doors humidity was 60, 86, 66, and the humidity in the test-room twenty minutes after the reaction was over was 73, 95, and 76, representing an average increase of the humidity of 10.6% due to the reaction.

The cultures used were serum and bouillon cultures of colon, albus, diphtheria, pneumococci, pyocyaneus, typhoid, prodigiosus, throat, tetragenus, subtilis, and mixed cultures from throat swabs. The total number of cultures exposed was 292. Of this number 233 were open and 59 were buried cultures. The cultures ranged from 24 to 72 hours in age. The time of exposure to the gas was four hours, and the time of incubation was 196 hours at 37.5° C.

200 C. C. TO 1,000 CUBIC FEET.

Culture.	Number.	Growth.	No growth.
Diphtheria	21	0	21
Typhoid	44	0	44
"Mixed"	17	3*	14
Subtilis	6	6†	0
Colon	39	0	39
Albus	31	0	31
Throat	25	0	25
Pneumococci	22	0	22
Prodigiosus	36	0	36
Pyocyaneus	31	2‡	29
Tetragenus	20	0	20
Total	292	11	281

* The growths from the "Mixed" cultures were composed entirely of tetrads, as in the case under the proportions of 250 c. c. to a thousand cubic feet. The diphtheria bacilli were all killed. These cultures were made from the same blood-serum growth as were those that gave growths in the former experiment.

† The subtilis growths were from open cultures.

‡ The two growths from the pyocyaneus cultures were from buried cultures in two thicknesses of cotton-flannel. The parent culture was an old serum culture.

SUMMARY.

Quantity.	Total.	Negatives.	Positives.	Subtilis.
500 c. c.	578	573	5	5
400 c. c.	443	435	8	7
300 c. c.	618	616	2	2
250 c. c.	409	400	9	5
200 c. c.	292	281	11	6
Total	2340	2305	35	25

It was not attempted to carry the work beyond this point as the State board of health adopted the proportions of 500 c. c. to the thousand cubic feet after looking over what had been done. This certainly leaves a wide margin of safety as 300 c. c.

to the thousand cubic feet gives as good results as does 500 c. c., or as does 1,000 c. c. for that matter. Work will be carried on with this method to determine its efficiency at low temperatures and in very dry air, but it may be mentioned that some results already obtained point to but little, if any diminution in the efficiency of the gas thus liberated at a temperature as low as 50 F., and using 300 c. c. to the thousand cubic feet. Also it may be mentioned that some experiments have shown that the time limit is far on the side of safety. The Maine board of health recommend an exposure of the organisms to the gas for four hours. Some experiments have shown that using 300 c. c. of formaldehyde solution to the thousand cubic feet the typhoid bacillus is killed by an exposure of one hour when the temperature is as low as 54 F.

It seems to me that this method of disinfection is at least the equal of others in common use in efficiency and their superior in ease and simplicity of operation and, in view of the experimental work that has been done upon it, the proportions of 500 c. c. to the thousand cubic feet with an exposure period of four hours will give entire safety from contagion.