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Formaldehyde Disinfection : Experimental Work Done in the Laboratory of Hygiene, Augusta, Maine, 1904

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State Board of Health of Maine

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EXPERIMENTAL WORK DONE IN THE LABORATORY OF HYGIENE, AUGUSTA, MAINE.

HENRY D. EVANS, Chemist,
DR. J. P. RUSSELL, Bacteriologist.

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In the method of room disinfection which the chemist and bacteriologist report in the following pages, formaldehyde gas is evolved by pouring formaldehyde solution upon the relatively right quantity of potassium permanganate in a large enough vessel. This process has the following advantages: All special forms of generators or other apparatus are done away with. There is no lamp or other source of danger from fire. Nearly the whole quantity of formaldehyde available for disinfection is liberated instantaneously so that within a very few minutes the maximum concentration of formaldehyde gas is reached. As a result, extremely efficient results are attained with an exposure of not more than four hours. Of the 1,529 cultures made with the various bacteria exposed, all remained sterile save 27. Of the 27 test organisms which were not killed, 21 were B. subtilis.

A. G. YOUNG, *Secretary.*

FORMALDEHYDE DISINFECTION.

Experimental Work Done in the Laboratory of Hygiene, Augusta, Maine.

By Henry D. Evans, Chemist, and Dr. J. P. Russell, Bacteriologist.

REPORT OF THE CHEMIST.

At the present time formaldehyde is the most popular and efficient of the various substances in use as disinfectants. But among people who have had to do with the practical problems of room and house disinfection the present methods, of either generating the gas or of liberating it from its water solution, fail to give anything like general satisfaction. Three methods of procuring the formaldehyde gas are at present in quite general use: i. e., the lamps which form formaldehyde by oxidation of methyl alcohol, generally through the agency of platinum black; the lamps which evaporate the solution of the gas in water, commercially known as "formalin;" and the "sheet method" as at present used in Chicago, where the solution of "formalin" is sprayed upon suspended sheets from which it evaporates and diffuses throughout the rooms.

The chief objection to all of these methods is that a very long period of time is required before all of the formaldehyde to be used is liberated in the space to be disinfected. This naturally allows a considerable quantity of the gas to escape by leakage, and also prevents the gas reaching its maximum density quickly. In the case of the synthesis of formaldehyde from methyl alcohol there is a very considerable loss of formaldehyde for disinfecting purposes by the further decomposition of the alcohol into carbon dioxide. The first two methods offer the additional objection of using fire in procuring the formaldehyde, thus necessitating bulky apparatus, and very constant attention to prevent the spreading of fire. The long and disagreeable process of spraying fails to commend the "sheet method" to the mapority of practical workers. It has long been thought that, if we could

get an almost instant liberation of the total amount of formaldehyde either from alcohol or formalin, the time of exposure would be much reduced, and probably the amount of the gas required for thorough disinfection would also be much less. Preference has been given by most operators to the solution known as formalin since it carries with the formaldehyde gas a large amount of water, this latter being necessary in order to get the maximum destructive action from the formaldehyde. The work recorded below, which grew out of a chance suggestion, has been performed in an effort to remove from the path of the health officer these following difficulties: bulky apparatus to transport; the danger of fire from the use of an open flame; the necessity of supplying moisture from a source other than the gas generator; and the opportunity for great loss of formaldehyde through leakage. In other words, I have attempted to provide for a *very rapid* liberation of the gas, the source of the necessary heat coming, not from a flame, but from a chemical reaction. How near this has been attained the description given below, together with the bacteriological results will show.

The chemical work was preformed during the spring of 1904 at my Laboratory at Saco, and the bacteriological work at Augusta during the summer of 1904. This last was in conjunction with Dr. J. P. Russell, State Bacteriologist, who reports upon it. Most of the chemical work has been qualitative though all that relates to the formaldehyde produced by the reaction has been quantitative as well. The actual figures of all the long series of tests will not be tabulated individually, nor will the methods of estimation be designated other than by name, as they are all methods familiar to chemists. No particular form of generator is presented for this reaction, as it has been found that any vessel large enough to hold the reagents when in action will give good results. The form of apparatus which has given the best results so far will be briefly spoken of in its proper place. With these words of introduction, I turn to the method of operation to be presented in these pages.

In December, 1903, Dr. Young called my attention to the fact that a mixture of potassium permanganate ($KMnO_4$) and formalin is attended by the formation of considerable heat, and the liberation of a gas which seemed to be formaldehyde. He requested that I would look into the matter and see if this reac-

tion could be turned to practical account. The reaction has been known for a very long time, and has probably been often used by teachers of chemistry to illustrate the formation of an acid from an aldehyde by direct oxidation.

Theoretically formic acid (CH_2O_2) is formed by the oxidation of formic aldehyde (CH_2O), just as the latter results from the oxidation of methyl alcohol (CH_3OH). This reaction can be brought about in a degree by adding to a solution of formaldehyde a substance which readily gives up all, or part of its oxygen, such as the dichromate or permanganate of potassium. In reality, in the presence of an excess of oxygen and heat the reaction proceeds a step farther, and involves the decomposition of the formic acid into water (H_2O) and carbon dioxide (CO_2). The amount of heat liberated by the oxidation of formaldehyde to formic acid is very great, and this large amount of heat is liberated, under proper conditions, in a very short space of time. When proper proportions of the reagents are used the amount of heat liberated is enough to cause the complete evaporation of the formaldehyde not oxidized. This amount of formaldehyde, which is evaporated, is by this process removed from the oxidizing action of the permanganate, and is thus available almost instantly, and in its maximum quantity for disinfecting purposes. The permanganate is itself reduced to a lower oxide of manganese, with the formation of potassium oxide, and liberation of oxygen. The reaction is very vigorous, and attended by great effervescence.

All the work, both chemical and bacteriological, has been performed using the ordinary commercial formalin supplied the State board of health for use in the Novy generators. The permanganate employed has been the fine needle-shaped crystals of commerce—not the large c. p. octohedral crystals. In the quantitative work the c. p. crystals were powdered before using them. All determinations of the amounts of formaldehyde were made by Romjin's potassium cyanide method, as experience showed that this method is capable of yielding better results than the iodine methods. All of the determinations were made in dilute solutions of formaldehyde, the standard solutions being N-10.

The first work was to determine the amounts of formalin and permanganate which would secure the greatest yield of the gas

in the shortest time. The results of a long series of experiments led me to adopt the proportion of 10cc of formalin to 3.75 gms. of permanganate, or approximately 6.5 ounces of permanganate to a pint of formalin. This was the proportion used in all laboratory work and in most of the bacteriological work, although a few tests using 4 gms. in place of 3.75 pointed to a slightly increased yield of formaldehyde. The bacteriological results using 3.75 gms. were, in every way, as satisfactory as the ones obtained from using 4 gms., so the first proportion was the one finally adopted.

The next question to engage attention was the method of bringing the reagents together in order to get the quickest and maximum yield of the gas. The results of my experiments led to adopting the very simple method of putting the permanganate into the generator, pouring the formalin upon it, and making as hasty an exit from the room as possible.

Experiments were tried where the permanganate was gradually fed into the measured amount of formalin; and also where the formalin was run into the weighed amounts of permanganate. In each case there was a smaller and slower yield of formaldehyde than in the method adopted, and the amount of permanganate required to complete the reaction was increased. This increase in the amount of permanganate required when it was fed into the formalin, was probably due to the fact that the small amounts of permanganate did not allow of sufficient oxidation of the formalin to liberate the amount of heat necessary to evaporate the solution. Also the increased length of time the reagents were in contact allowed for a greater oxidation of the formalin into formic acid, with a corresponding decrease in the amount of formaldehyde available for disinfecting purposes. It is also proable that the permanganate was to some extent decomposed as it fell into the generator through the gas arising from the latter, since a gas, at the instant of liberation is especially active.

The same difficulties in regard to diminished yield of formaldehyde, increased amount of permanganate, and slowness of action were encountered when the formalin was run upon the permanganate. The best results were always obtained by pouring the requisite amount of the formalin upon the corresponding quantity of permanganate.

Another factor affecting the quickness and quantity of the yield of the formaldehyde is the size of the permanganate crystals. In order to get as rapid and vigorous action as possible the permanganate must be in a powdered form, or in the long needle-shaped crystals which are usually met with in the commercial article. If the large c. p. octohedral crystals of permanganate are to be used they must first be powdered. The reason for using the fine or powdered crystals 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. 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 a crystal is 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 used should always be in a fine crystal or powdered form.

There is little to be said in regard to the kind of generator which has been used in this work, as any dish, with sides high enough to prevent the solutions boiling over, will answer the purpose. So far as our bacteriological results go, no preference is to be given to any particular form or of material for a generator. From a chemical point of view there is a slight difference between the yield of the gas from different shaped generators.

The reaction is so violent, and attended by so much frothing and effervescence that very tall dishes are necessary to prevent the materials from running out. In the actual generation of large quantities of the gas, glass is a very poor substance to use as a generator, since the sudden production of high temperatures on the bottom of the beaker only, combined with the poor conducting power of the glass, results in a too sudden strain within it, and the bottom breaks out. In addition, since glass conducts the heat so poorly, a considerable quantity of the gas coming in contact with the cool sides of the beaker, is converted

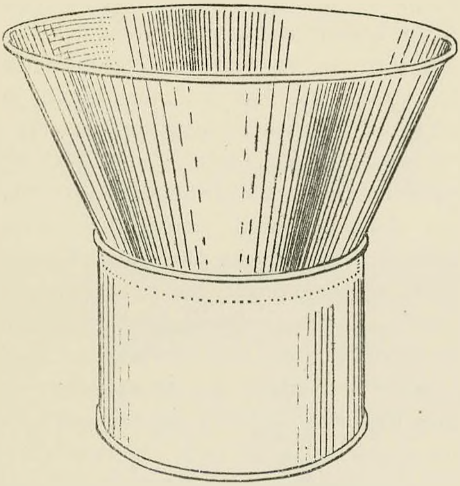
into the solid white para-formaldehyde ($C_3H_6O_3$). This is deposited on the glass and so is lost for disinfecting purposes.

In the disinfecting experiments below mentioned, the generators were 3 and 5 gallon earthen jars—the 3 gallon jars being used in rooms requiring not over a pint and a half of formalin, used in the proportion of a quart to 1000 cu. feet, and the 5 gallon jars in rooms requiring from 1 to 2 quarts of formalin. These gave very good results indeed. There was very little polymerization to be noticed, but a large quantity of heat was lost by absorption by the jars. Tin jars were also used having straight sides. The use of the tin did not remove the tendency of the gas to polymerize any more than did the earthen jars, but in both of these cases the polymerization was less than when using glass. The loss from polymerization is in any case very slight, so that any dish with high enough sides can be used as a generator.

When straight walled jars were used to generate the gas in, this gas rose in a straight column to the ceiling and then spread out, working its way into the corners of the room from the ceiling down. In order that the gas might the quicker distribute itself throughout the room a trial was made with a generator whose top flared out like a funnel. Tests with this showed that, when the funnel shaped part of the apparatus was so near the bottom of the generator as to be partly filled when the reaction was in progress, the resulting gas rose in the form of an inverted cone, spreading out to all the upper parts of the room at once. Examination of the apparatus at the end of the operation showed no evidence of any polymerization whatever.

Acting on this hint a small generator has been constructed which is giving very good laboratory results. It consists of a bright tin dish, about 10 inches in diameter, and whose sides rise to a perpendicular height of 8 inches. These sides are wrapped with asbestos paper so as to retain all possible heat. Above the height mentioned the sides flare out like a funnel at an angle of about 50 or 55 degrees, and rise to a perpendicular height of 9 inches above their junction with the straight walls below. This funnel part of the apparatus can be made to detach from the bottom; and the latter can be packed into the former for transportation. Such an apparatus has a slight advantage over the

other forms, as it distributes the gas quicker, prevents polymerization, and loss of heat by radiation.



FORMALDEHYDE GENERATOR.

The apparatus shown in this cut is not an essential, but is a convenience. The diameter of the lower part is 10 inches and its height 8 inches. The diameter of the funnel-shaped part is 17½ inches at the top, and the height of the whole apparatus is 15½ inches.]

A large number of experiments were made with the object of determining whether or not the reaction between the farmalin and permanganate resulted in the formation of any products which would have an injurious effect on clothes, paper, or metals which would be left in a room to be disinfected. The results showed that there was no danger to be feared from this source. Analysis of the gas thrown out into a room by this reaction showed it to consist of formaldehyde, water vapor, carbon dioxide, a very small amount of formic acid, and a little oxygen. None of these compounds will have any effect on materials left in a room. In the generator were found a lower oxide of manganese, a little formaldehyde, carbon dioxide, potassium hydroxide, and, I think, a little potassium formate resulting from the neutralization of the potassium hydrate by formic acid.

It would be natural to suppose that a large amount of formic acid would be given off in the process of this reaction, but only a small amount is actually liberated. The reason that this state of affairs exists is that formic acid, in the presence of heat and

oxygen, is decomposed into water and carbon dioxide, and also because some of the acid is used up in neutralizing the potassium hydroxide formed at the same time by this reaction. These transformations of formic acid cannot be regarded as detrimental to the principal reaction, as they remove an undesirable compound and also add to the heat produced by the first reaction. The amount of moisture furnished by this secondary reaction is so small that it can be disregarded.

A series of quantitative experiments were undertaken to determine the amount of formaldehyde actually derived from the formalin, and available for disinfecting purposes. The results showed this amount to be quite constant, the yield of gas being, on an average, 81% of that in the original solution of formalin. The remaining 19% represents the fuel necessary to produce the heat to evaporoate the 81% available for disinfecting. This figure—81%—represents the total yield of available gas. In actual work this total is not all furnished at once. Between 80 and 85% of the available gas is set free within 5 minutes from the time the reagents are put together. The remainder is given off in rapidly diminishing amount for 12 hours. In our tests in the rooms of an ordinary French tenement house, using no precautions to prevent leakage save to stop the key holes, the amount of the gas given off in this secondary stage just about offset the natural leakage during the first 3 hours. It acts almost as an automatic device for preserving the amount of gas in a room at its maximum density for the time necessary for thorough disinfection. Since it acts thus, this secondary reaction seems to me to be more in the nature of an advantage than of a disadvantage.

The results of this reaction may be briefly expressed by saying that the maximum amount of formaldehyde is liberated within 5 minutes of the beginning of the action, and the gas is kept at its maximum during the period necessary for disinfection, by this secondary reaction replacing the loss through natural leakage from the room. It may be in order for me to state at this point that a 3-hour exposure has been found just as fatal to bacterial life as an exposure of 16 hours. This is true in the case of both open and buried cultures.

Since potassium permanganate yields much more oxygen in the presence of sulfuric acid than without the addition of this reagent, it was at first decided to use it with the formalin. It

was thought that by its use a lesser quantity of permanganate would be required than without it. The results of my experiments seem to contradict this view, as there was a falling off in the amount of available formaldehyde, amounting to 5% and often more. Also there was a corresponding increase in the amount of the useless carbon dioxide. The reason for this probably is that oxygen is liberated faster than is necessary to produce the required degree of heat, thus forming more formic acid and leaving less formaldehyde for use, although increasing the useless carbon dioxide. The addition of the sulfuric acid also raises the boiling point of the solution, and thus necessitates more heat to cause evaporation.

I may sum up the results of this work in a few words: The oxidation of a little formaldehyde by permanganate results in the evaporation of 81% of the original weight of aldehyde within 5 minutes of the beginning of the reaction. The chemical reaction furnishes all the heat necessary to vaporize the formalin and its water of solution, doing away with all need of fire. The ordinary house can furnish all material necessary for a generator, while the reagents can be cheaply purchased at any drug store. The method of operation is so simple that anyone can attend to it who can tell the difference between a solid and a liquid, requiring only the pouring of the measured amount of formalin upon the corresponding quantity of permanganate. Absolutely no care is required after mixing the reagents, the reaction starting and dying out of itself.

I wish to make no large or unwarranted claims for the above method of procuring formaldehyde for disinfecting purposes. Neither is it put forward with any claims of originality of invention or research. It will stand for what it is worth, and that worth will be judged by its actual usefulness as a disinfectant. To determine this worth, Dr. Russell and myself have carried through a series of 1529 bacterial tests, which I may say have given results which will stand with any, both as concerns the destructive power of the gas, thus prepared, upon bacterial life, and as concerns the length of exposure necessary to destroy such life. I think that I may say that the idea that an instantaneous liberation of the total necessary amount of foraldehyde, would cut down the time of exposure, is proved.

Below, Dr. Russell reports upon our bacteriological work with formaldehyde obtained as above described.

H. D. EVANS, *Chemist, Laboratory of Hygiene.*

REPORT OF THE BACTERIOLOGIST.

In the work of testing the efficiency of formaldehyde gas as a disinfectant when liberated by a process which is new in the work of practical disinfection, an extended series of experiments have been made. In this work formaldehyde gas has been liberated by pouring the strong solution of formaldehyde upon potassium permanganate. Mr. H. D. Evans, the chemist of the Hygienic Laboratory, who has co-operated with me in this work has reported upon the preliminary chemical work which was required before the bacteriological tests were undertaken.

The tests have been made under widely varying climatic conditions, having extended from January to the late summer of 1904. They have been made in rooms which differ greatly as to location, size, finish, and tightness as regards windows, doors, cracks in the walls and ceilings, and the amount of exposure to sunlight. Some of the exposures were made in a suite of offices in Masonic Temple on Water street, and the rest in the different rooms in a tenement on Bridge street. This tenement is in the so-called "Bridge Street Block" which includes seven other tenements. The one used in these experiments is at the east end of the block. On the north which is the rear of the tenement there is a vacant lot, on the east a space of about five feet separates it from another dwelling house, on the south is the street on which the block fronts, and the next adjoining tenement of the block is on the west. The results of the exposure of many cultures I have attempted to arrange so that the effect upon them after different lengths of exposure and in different parts of the various rooms may easily be found.

The test organisms used were diphtheria bacillus, typhoid bacillus, B. anthracis, B. sublitis, some of which were old cultures, S. pyogenes albus, S. pyogenes aurens, B. coli com., B. pyocyaneus, M. tetragenus, streptococci, and mixed cultures mostly derived from swabs which had been applied to throats supposed to be diphtheritic. These were exposed on pieces of

sterilized filter paper and pieces of ordinary writing paper which were put up on the wall or suspended from wires stretched across the rooms at various heights and at various distances from the walls.

As will be seen in the last column of the tabulation, some of the pieces of infected paper were exposed uncovered, while others were placed between layers of cotton flannel, ticking, or silk. The pieces of cloth were held tightly together at their edges by clamping outside of them pieces of tin in the centre of each of which was a hole three inches in diameter. The infected pieces of paper were so placed that they were in the center of that part of the cloth which was uncovered by the tin.

Plain bouillon and Loeffler's blood serum were used in making the cultures. Each lot of media was tested with the various kinds of bacteria and controls were also made by inoculating the tubes after they failed to show growth from the exposed cultures. All the cultures made from the exposed bacteria were kept in the incubator at least forty-eight hours at 37° C.

In tabulating the results the rooms are here designated by "R" followed by Roman numerals.

R. I. indicates the room which was numbered one and was on the first floor of the tenement house fronting the south. It has three windows, two on the south side and one on the east, a mantel on the north side of the room and one door. It had a volume of 1,521 cubic feet.

R. II. with a volume of 968 cubic feet is on the second floor of the tenement and has two windows looking to the north, a closet and one door.

R. III. is on the second floor of the tenement and faces the south. There are two windows on the south side, one on the east, and two doors, one opening into the hall and the other leading to the attic stairway. The size was 1,850 cubic feet.

R. IV. situated on the northeast corner of the second floor has one north window, one door, and a volume of 525 cubic feet.

R. V. is an office in Masonic Temple and has three windows, two doors, and a volume of about 1,000 cubic feet.

The doors and windows in all the rooms were unsealed except in the case of very wide cracks which were covered with adhesive plaster.

In the following tabulation of results the character of the test organisms used is shown in the first column. Each horizontal line indicates the results obtained with a series or group of cultures ranging in number from one to twenty-five as is shown in the column of totals in column number seven. The figures at the left of the first column are simply for facilitating reference to any particular group or series. Their number—the number of the series of cultures—is 279, but the total number exposed in these experiments is 1529.

The second column gives the time of exposure in hours. The third column indicates the temperature of the room at the beginning of the period of exposure; and the fourth column shows the humidity of the external atmosphere when the exposures were made.

An exhibit of the results is given in columns 5, 6, and 7, and the last column shows the particular room and the location in the room where each culture was exposed.

It was the aim in these experiments to have the conditions approach as nearly as possible those usually existing in the actual work of disinfection. The rooms, as has already been stated, varied in capacity from 525 to 1,850 cubic feet. The closeness of construction of some of the rooms at least was considerably inferior to that of the average room which the health officer is called upon to disinfect. The care exercised in closing cracks and crevices was probably less than that usually exercised, and no artificial methods were put into operation to secure a uniform diffusion of the gas.

TABULATION OF TESTS.

TABULATION OF TESTS.

		Time of exposure in hours.	Temperature.	Humidity.	Results.			Where exposed.
					growth.	No Growth.	Total.	
1	Diphtheria	16	75	70	0	4	4	R. I. Over window and door.
2	Mixed	16	75	70	0	5	5	R. I. On mantel.
3	Mixed	16	75	70	0	5	5	R. I. West base-board.
4	Mixed	16	75	70	0	2	2	R. I. N. W. base-board.
5	Mixed	16	75	70	0	2	2	R. I. N. E. base-board.
6	Mixed	16	75	70	0	2	2	R. I. West floor.
7	Mixed	16	75	70	0	1	1	R. I. N. W. floor.
8	Mixed	16	75	70	0	8	8	R. I. East window.
9	Mixed	16	75	70	0	1	1	R. II. Over door.
10	Mixed	16	75	70	0	1	1	R. II. Over closet door.
11	Mixed	16	75	70	0	1	1	R. II. Floor.
12	Diphtheria	16	75	70	0	3	3	R. II. N. W. window.
13	Typhoid	16	75	70	0	3	3	R. II. N. E. window.
14	Mixed	16	75	70	0	7	7	R. II. N. W. base-board and floor.
15	Subtilis	16	75	70	0	3	3	R. II. East base-board.
16	Subtilis	16	75	70	0	2	2	R. II. East floor.
17	Subtilis	16	75	70	0	2	2	R. II. South base-board.
18	Diphtheria	16	75	70	0	4	4	R. II. Closet door.
19	Subtilis	16	75	70	0	3	3	R. II. In closet.
20	Typhoid	15	70	69	0	5	5	R. III. N W. base-board.
21	Diphtheria	15	70	69	0	5	5	R. III. N. E. base-board.
22	Diphtheria	15	70	69	0	5	5	R. III. Mantel.
23	Mixed	15	70	69	0	5	5	R. III. S. E. base-board
24	Typhoid	15	70	69	0	5	5	R. III. S. W. window.
25	Mixed	15	70	69	0	5	5	R. III. Floor by door.
26	Mixed	15	70	69	3*	2	5	R. IV. Window.
27	Typhoid	15	70	69	0	5	5	R. IV. N. E. corner base-board.
28	Diphtheria	15	70	69	0	5	5	R. IV. S. W. corner floor.
29	Diphtheria	15	70	69	0	5	5	R. IV. East floor.
30	Typhoid	15	70	69	0	5	5	R. IV. Window; upper sash.

31	Typhoid	15	70	69	0	1	1	R. IV. N. W. floor.
32	Albus	12	72	....	0	5	5	R. V. Door.
33	Aureus	12	72	....	0	5	5	R. V. Door.
34	Colon	12	72	....	0	5	5	R. V. Door.
35	Subtilis	12	72	....	0	5	5	R. V. Door.
36	Colon	12	72	....	0	5	5	R. V. Window between 2 layers of cotton.
37	Aureus	12	72	....	0	5	5	R. V. Window between 2 layers of cotton flannel.
38	Diphtheria	12	72	....	0	1	1	R. V. Floor between 2 layers of cotton.
39	Diphtheria	12	74	....	0	5	5	R. V. Door.
40	Aureus	12	74	....	0	5	5	R. V. Door.
41	Pyocyaneus	12	74	....	0	5	5	R. V. Door.
42	Albus	12	74	....	0	5	5	R. V. Door.
43	Diphtheria	12	74	....	0	5	5	R. V. S. E. floor between 4 layers of flannel.
44	Aureus	12	74	....	0	5	5	R. V. S. W. corner between 4 layers of flannel.
45	Diphtheria	12	74	....	0	5	5	R. V. Between 4 layers of flannel.
46	Typhoid	12	80	....	0	5	5	R. V. Door.
47	Mixed	12	80	....	0	8	8	R. V. Door.
48	Colon	6	68	....	0	10	10	R. V. Door.
49	Pyocyaneus	6	68	....	0	10	10	R. V. Door.
50	Subtilis	6	68	....	0	10	10	R. V. Door.
51	Diphtheria	6	68	....	0	10	10	R. V. Door.
52	Typhoid	5¾	72	....	0	15	15	R. V. Door.
53	Colon	5¾	78	58–63	0	5	5	R. IV. Four feet high. West wall.
54	Colon	5¾	78	58–63	0	5	5	R. IV. Floor. N. W. corner.
55	Subtilis. Old	5¾	78	58–63	1	4	5	R. IV. Window.
56	Subtilis	5¾	78	58–63	1	4	5	R. IV. North wall. Five feet high.
57	Aureus	5¾	78	58–63	0	5	5	R. IV. N. E. corner floor.
58	Typhoid	5¾	78	58–63	0	5	5	R. IV. East wall. Eight feet.
59	Diphtheria	5¾	78	58–63	0	5	5	R. IV. S. E. corner. Seven feet.
60	Colon	5¾	78	58–63	0	5	5	R. III. Four feet. Mantel.
61	Subtilis	5¾	78	58–63	0	5	5	R. III. North wall. Six and one-half feet high.
62	Diphtheria	5¾	78	58–63	0	5	5	R. III. East wall. Seven feet.
63	Colon	5¾	78	58–63	0	5	5	R. III. S. E. corner. Seven feet.
64	Typhoid	5¾	78	58–63	0	5	5	R. III. South wall. Eight feet.
65	Aureus	5¾	78	58–63	0	5	5	R. III. Attic door. Seven feet.
66	Subtilis	5¾	78	58–63	0	5	5	R. III. West wall. Two feet.
67	Diphtheria	5¾	78	58–63	1	4	5	R. II. West wall. Five feet.
68	Typhoid	5¾	78	58–63	0	5	5	R. II. Floor. North west.
69	Subtilis	5¾	78	58–63	3	2	5	R. II. North wall. Four feet.
70	Colon	5¾	78	58–63	0	5	5	R. II. N. E. corner. Eight feet.
71	Subtilis	5¾	78	58–63	0	5	5	R. II. East wall. Two feet.
72	Typhoid	5¾	78	58–63	0	5	5	R. II. South wall. Five feet.
73	Aureus	5¾	78	58–63	0	5	5	R. II. Closet door. Eight feet.
74	Diphtheria	5¾	78	58–63	0	5	5	R. I. West wall. Six feet.

TABULATION OF TESTS—Continued.

		Time of exposure in hours.	Temperature.	Humidity.	Results.			Where exposed.
					No growth.	No Growth.	Total.	
75	Colon	$5\frac{3}{4}$	78	58-63	0	5	5	R. I. N. W. corner. Five feet.
76	Typhoid	$5\frac{3}{4}$	78	58-63	0	5	5	R. I. Mantel.
77	Diphtheria	$5\frac{3}{4}$	78	58-63	0	5	5	R. I. North wall. Three feet.
78	Subtilis. New	$5\frac{3}{4}$	78	58-63	0	5	5	R. I. N. E. wall. Eight feet.
79	Subtilis. Old	$5\frac{3}{4}$	78	58-63	0	5	5	R. I. East window. Seven feet.
80	Aureus	$5\frac{3}{4}$	78	58-63	0	5	5	R. I. East wall. Eight feet.
81	Typhoid	$5\frac{3}{4}$	78	58-63	0	5	5	R. I. South wall. Eight feet.
82	Typhoid	$5\frac{1}{3}$	80	77	0	5	5	R. II. N. W. floor.
83	Albus	$5\frac{1}{3}$	80	77	0	5	5	R. II. N. E. window.
84	Diphtheria	$5\frac{1}{3}$	80	77	0	5	5	R. II. South.
85	Diphtheria	$5\frac{1}{3}$	80	70	0	5	5	R. II. Closet.
86	Typhoid	$5\frac{1}{3}$	80	70	0	5	5	R. II. S. W. corner floor.
87	Albus	$5\frac{1}{3}$	80	70	0	5	5	R. II. Closet door.
88	Albus	$5\frac{1}{3}$	80	70	0	5	5	R. II. Door. One half up.
89	Diphtheria	$5\frac{1}{3}$	80	70	0	5	5	R. I. Mantel.
90	Typhoid	$5\frac{1}{3}$	80	70	0	5	5	R. I. N. W. corner.
91	Diphtheria	$5\frac{1}{3}$	80	70	0	5	5	R. I. East window.
92	Typhoid	$5\frac{1}{3}$	80	70	0	5	5	R. I. S. E. window.
93	Albus	$5\frac{1}{3}$	80	70	0	5	5	R. I. Floor by door.
94	Colon	$4\frac{1}{3}$	77	61	0	5	5	R. II. West wall. Five feet.
95	Aureus	$4\frac{1}{3}$	77	61	0	5	5	R. II. North wall. Four feet.
96	Throat	$4\frac{1}{3}$	77	61	0	5	5	R. II. N. E. corner. Eight feet
97	Diphtheria	$4\frac{1}{3}$	77	61	0	5	5	R. II. East wall. Two feet.
98	Mixed	$4\frac{1}{3}$	77	61	0	5	5	R. II. South wall. Five feet.
99	Subtilis. Old	$4\frac{1}{3}$	77	61	0	5	5	R. II. Corner floor.
100	Subtilis	$4\frac{1}{3}$	77	61	4	1	5	R. II. Closet, door closed.
101	Mixed	$4\frac{1}{3}$	77	61	0	5	5	R. IV. S. W. corner floor.
102	Diphtheria	$4\frac{1}{3}$	77	61	0	5	5	R. IV. North wall. Four feet
103	Throat	$4\frac{1}{3}$	77	61	0	5	5	R. IV. Window.
104	Subtilis. New	$4\frac{1}{3}$	77	61	5	0	5	R. IV. North wall. Five feet.

105	Subtilis. Old	4½	77	61	0	5	5	R. IV. N. E. corner floor.
106	Aureus	4½	77	61	0	5	5	R. IV. East wall. Eight feet.
107	Colon	4½	77	61	0	5	5	R. IV. S. E. corner. Seven feet.
108	Subtilis	4½	77	61	0	5	5	R. III. Mantel.
109	Diphtheria	4½	77	61	0	5	5	R. III. North wall. Seven feet.
110	Aureus	4½	77	61	2	3	5	R. III. East window. Seven feet.
111	Mixed	4½	77	61	0	5	5	R. III. S. E. corner. Six feet.
112	Colon	4½	77	61	0	5	5	R. III. South wall. Eight feet.
113	Throat	4½	77	61	0	5	5	R. III. Attic door. seven feet.
114	Subtilis	4½	77	61	0	5	5	R. III. West wall. Two feet.
115	Colon	4½	77	61	0	5	5	R. I. West wall. Six feet.
116	Subtilis	4½	77	61	0	5	5	R. I. Mantel.
117	Subtilis	4½	77	61	0	5	5	R. I. North wall. Three feet.
118	Aureus	4½	77	61	0	5	5	R. I. N. E. wall. Eight feet.
119	Diphtheria	4½	77	61	0	5	5	R. I. East floor.
120	Mixed	4½	77	61	0	5	5	R. I. South wall. Seven feet.
121	Throat	4½	77	61	0	5	5	R. I. S. W. corner floor.
122	Typhoid	4	72	76	0	5	5	R. IV. Door. Four feet.
123	Albus	4	72	76	0	5	5	R. IV. West wall. Four feet.
124	Subtilis	4	72	76	2	3	5	R. IV. N. W. corner floor.
125	Pyocyaneus	4	72	76	0	5	5	R. IV. North wall. Four feet.
126	Throat	4	72	76	0	5	5	R. IV. Middle floor.
127	Tetragenus	4	72	76	0	5	5	R. IV. East wall. Three feet.
128	Subtilis	4	72	76	0	5	5	R. IV. S. E. corner. Eight feet
129	Colon	4	72	76	0	5	5	R. IV. South floor.
130	Throat	4	72	76	0	5	5	R. III. Mantel.
131	Subtilis	4	72	76	0	5	5	R. III. N. E. corner floor.
132	Tetragenus	4	72	76	0	5	5	R. III. S. E. corner. Eight feet
133	Colon	4	72	76	0	5	5	R. III. South wall.
134	Albus	4	72	76	0	5	5	R. III. Attic door. Eight feet.
135	Subtilis	4	72	76	0	5	5	R. III. West wall. Two feet.
136	Typhoid	4	72	76	0	5	5	R. III. N. W. corner floor.
137	Subtilis	4	72	76	0	5	5	R. II. West floor.
138	Subtilis	4	72	76	0	5	5	R. II. Middle floor.
139	Colon	4	72	76	0	5	5	R. II. N. E. window. Six feet.
140	Throat	4	72	76	0	5	5	R. II. N. E. wall. Seven feet.
141	Pyocyaneus	4	72	73	0	5	5	R. II. East wall. Two feet.
142	Tetragenus	4	72	76	0	5	5	R. II. South wall. Five feet.
143	Albus	4	72	76	0	5	5	R. II. Closet door. Eight feet.
144	Typhoid	4	72	76	0	5	5	R. II. Closet. Seven feet.
145	Albus	4	72	76	0	5	5	R. II. West wall. Six feet.
146	Tetragenus	4	72	76	0	5	5	R. I. N. W. corner. Six feet.
147	Colon	4	72	76	0	5	5	R. I. Mantel.
148	Pyocyaneus	4	72	76	0	5	5	R. I. North west. Two feet.

TABULATION OF TESTS—Continued.

		Time of exposure in hours.	Temperature.	Humidity.	Results. No growth.	Results. No Growth.	Results. Total.	Where exposed.
149	Typhoid	4	72	76	0	5	5	R. I. North wall. Eight feet.
150	Subtilis	4	72	76	0	5	5	R. I. North wall floor.
151	Subtilis	4	72	76	0	5	5	R. I. East wall. Eight feet.
152	Throat	4	72	76	0	5	5	R. I. South floor.
153	Pyocyaneus	4	72	76	0	5	5	R. I. S. W. window. Seven feet.
154	Subtilis	4	78	76	0	5	5	R. IV. Door. Four feet.
155	Albus	4	78	76	0	5	5	R. IV. Window. Four feet.
156	Aureus	4	78	76	0	5	5	R. IV. North floor.
157	Diphtheria	4	78	76	0	5	5	R. IV. North wall. Five feet.
158	Diphtheria	4	78	76	0	5	5	R. IV. N. E. corner. Eight feet.
159	Typhoid	4	78	76	0	5	5	R. IV. East wall. Four feet.
160	Subtilis	4	78	76	0	5	5	R. IV. S. E. corner floor.
161	Albus	4	78	76	0	5	5	R. IV. South wall. Five feet.
162	Diphtheria	4	78	76	0	5	5	R. IV. West wall. Five feet.
163	Aureus	4	78	76	0	5	5	R. IV. N. W. corner floor.
164	Diphtheria	4	78	76	0	5	5	R. IV. North wall. Four feet.
165	Albus	4	78	76	0	5	5	R. IV. N. E. corner floor.
166	Typhoid	4	78	76	0	5	5	R. IV. East wall. Five feet.
167	Aureus	4	78	76	0	5	5	R. IV. South floor.
168	Subtilis	4	78	76	0	5	5	R. IV. Middle of floor.
169	Subtilis	4	78	76	0	5	5	R. IV. S. E. wall. Eight feet.
170	Subtilis	$3\frac{1}{3}$	78	76	0	5	5	R. I. West wall. Six feet.
171	Albus	$3\frac{1}{3}$	78	76	0	5	5	R. I. N. W. corner floor.
172	Aureus	$3\frac{1}{3}$	78	76	0	5	5	R. I. Mantel.
173	Aureus	$3\frac{1}{3}$	78	76	0	5	5	R. I. N. E. corner. Six feet.
174	Diphtheria	$3\frac{1}{3}$	78	76	0	5	5	R. I. East window. Four feet.
175	Typhoid	$3\frac{1}{3}$	78	76	0	5	5	R. I. East wall. Four feet.
176	Anthrax	4	78	55	0	5	5	R. I. Mantel.
177	Diphtheria	4	78	55	0	25	25	R. I. Walls and window.
178	Diphtheria	4	78	55	0	5	5	R. I. In closed box on floor.

179	Typhoid	4	78	55	0	10	10	R.	I.	West wall. Six feet. Between two layers ticking.
180	Typhoid	4	78	55	0	10	10	R.	I.	N. W. corner floor between two layers ticking.
181	Typhoid	4	78	55	0	5	5	R.	I.	West wall between four layers ticking.
182	Typhoid	4	78	55	0	15	15	R.	I.	N. W. corner floor between four layers ticking.
183	Typhoid	4	78	55	0	5	5	R.	I.	N. W. corner between six layers ticking.
184	Diphtheria	4	78	55	0	10	10	R.	I.	N. W. wall. Eight feet. Between two layers ticking.
185	Diphtheria	4	78	55	0	5	5	R.	I.	N. W. wall. Eight feet. Between four layers ticking.
186	Diphtheria	4	78	55	0	10	10	R.	I.	S. E. corner floor between two layers ticking.
187	Diphtheria	4	78	55	0	10	10	R.	I.	S. E. corner floor between four layers ticking.
188	Diphtheria	4	78	55	0	5	5	R.	I.	S. W. corner floor between six layers ticking.
189	Diphtheria	4	78	55	0	10	10	R.	I.	Mantel, two layers cotton flannel.
190	Diphtheria	4	78	55	0	5	5	R.	I.	Mantel, four layers cotton flannel.
191	Diphtheria	4	78	55	0	10	10	R.	I.	East window, two layers cotton flannel.
192	Diphtheria	4	78	55	0	15	15	R.	I.	East window, four layers cotton flannel.
193	Typhoid	4	78	55	0	20	20	R.	I.	Floor, two layers cotton flannel.
194	Typhoid	4	78	55	0	15	15	R.	I.	Floor, four layers cotton flannel.
195	Typhoid	4	78	55	0	5	5	R.	I.	Floor, six layers cotton flannel.
196	Anthrax	4	78	55	0	5	5	R.	I.	Mantel.
197	Anthrax	4	78	55	0	5	5	R.	I.	Floor.
198	Subtilis	4	78	55	1	4	5	R.	I.	West wall. Six feet. Six layers cotton flannel.
199	Subtilis	4	78	55	0	5	5	R.	I.	Under mantel, four layers flannel.
200	Mixed	4	78	55	0	5	5	R.	I.	N. E. wall. Six feet. Six layers flannel.
201	Subtilis	4	78	55	0	5	5	R.	I.	N. E. corner, four layers flannel.
202	Mixed	4	78	55	0	5	5	R.	I.	East window. Four feet. Six layers flannel.
203	Mixed	4	78	55	0	5	5	R.	I.	East wall. Eight feet. Six layers flannel.
204	Typhoid	4	78	55	0	5	5	R.	I.	S. E. corner floor, six layers flannel.
205	Typhoid	4	78	55	0	5	5	R.	I.	S. E. window top. Six layers flannel.
206	Typhoid	4	78	55	0	5	5	R.	I.	South wall. Seven feet. Six layers flannel.
207	Typhoid	4	78	55	0	5	5	R.	I.	South floor between eight layers flannel.
208	Colon	4	78	55	0	5	5	R.	I.	South wall. Four feet. Six layers cotton flannel.
209	Colon	4	78	55	0	5	5	R.	I.	S. W. window, six layers cotton flannel.
210	Subtilis. Old	4	78	55	4	1	5	R.	I.	Middle floor, six layers flannel.
211	Typhoid	4	78	55	0	5	5	R.	I.	Middle floor, eight layers flannel.
212	Colon	4	78	55	0	5	5	R.	I.	West floor, six layers ticking.
213	Colon	4	78	55	0	5	5	R.	I.	Mantel, six layers ticking.
214	Typhoid	4	78	55	0	5	5	R.	I.	N. W. corner. Seven feet. Six layers ticking.
215	Typhoid	4	78	55	0	5	5	R.	I.	North wall. Three feet. Six layers ticking.
216	Typhoid	4	78	55	0	5	5	R.	I.	North wall. Eight feet. Six layers ticking.
217	Diphtheria	4	78	55	0	5	5	R.	I.	N. E. floor, six layers ticking.
218	Colon	4	78	55	0	5	5	R.	I.	Middle of floor, six layers ticking.
219	Colon	1	80	68	0	5	5	R.	I.	Mantle, six layers cotton flannel.
220	Diphtheria	4	80	68	0	5	5	R.	III.	North wall. Three feet. Six layers flannel.
221	Diphtheria	4	80	68	0	5	5	R.	III.	North wall. Seven feet. Six layers flannel.
222	Diphtheria	4	80	68	0	5	5	R.	III.	Top east window, six layers flannel.

TABULATION OF TESTS—Concluded.

		Time of exposure in hours.	Temperature.	Humidity.	Results.			Where exposed.
					~~No~~ growth.	No Growth.	Total.	
223	Diphtheria	4	80	68	0	5	5	R. III. Middle east window. Six layers flannel.
224	Diphtheria	4	80	68	0	5	5	R. III. S. E. corner. Seven feet. Six layers flannel.
225	Mixed	4	80	68	0	5	5	R. III. S. E. window. Seven feet. Six layers flannel.
226	Mixed	4	80	68	0	5	5	R. III. S. W. window. Four feet. Six layers flannel.
227	Colon	4	80	68	0	5	5	R. III. North floor, six layers flannel.
228	Typhoid	4	80	68	0	10	10	R. III. Floor, eight layers flannel.
229	Mixed	4	80	68	0	5	5	R. III. Mantel, six layers ticking.
230	Diphtheria	4	80	68	0	10	10	R. III. Corners of floor, six layers ticking.
231	Colon	4	80	68	0	5	5	R. III. South floor, six layers ticking.
232	Diphtheria	4	80	68	0	15	15	R. III. Attic door, six layers ticking.
233	Mixed	4	80	68	0	5	5	R. III. Wall. Two feet. Six layers ticking.
234	Colon	4	80	68	0	5	5	R. III. West floor, six layers ticking.
235	Typhoid	$3\frac{1}{3}$	78	76	0	5	5	R. I. S. E. corner floor.
236	Typhoid	$3\frac{1}{3}$	78	76	0	5	5	R. I. South wall. Ten feet.
237	Colon	3	76	55	0	5	5	R. II. N. W. corner, eight layers ticking.
238	Typhoid	3	76	55	0	5	5	R. II. West wall. Four feet. Six layers ticking.
239	Colon	3	76	55	0	5	5	R. II. N. W. window, eight layers ticking.
240	Streptococci	3	76	55	0	5	5	R. II. N. W. wall. Five feet. Six layers ticking.
241	Diphtheria	3	76	55	0	10	10	R. II. Wall, six layers ticking.
242	Streptococci	3	76	55	0	10	10	R. II. Corners, six layers ticking.
243	Typhoid	3	76	55	0	5	5	R. II. Closet. Six feet. Six layers ticking.
244	Diphtheria	3	76	55	0	5	5	R. II. N. W. corner, six layers flannel.
245	Diphtheria	3	76	55	0	5	5	R. II. Top door, six layers flannel.
246	Diphtheria	3	76	55	0	10	10	R. II. Windows, six layers flannel.
247	Streptococci	3	76	55	0	5	5	R. II. North wall. Four feet. Six layers flannel.
248	Typhoid	3	76	55	0	5	5	R. II. N. E. wall. Eight feet. Six layers flannel.
249	Diphtheria	3	76	55	0	5	5	R. II. N. E. corner floor, six layers flannel.
250	Typhoid	3	76	55	0	5	5	R. II. East wall. Four feet. Six layers flannel.
251	Diphtheria	3	76	55	0	5	5	R. II. S. W. floor, six layers flannel.
252	Colon	3	76	55	0	10	10	R. II. Floor, eight layers flannel.

253	Diphtheria	3	76	55	0	5	5	R. II.	Closet door, six layers flannel.
254	Typhoid	3	67	59	0	5	5	R. IV.	Door between six layers silk.
255	Typhoid	3	67	59	0	5	5	R. IV.	Window between six layers silk.
256	Typhoid	3	67	59	0	5	5	R. IV.	North wall. Four feet. Six layers silk.
257	Typhoid	3	67	59	0	5	5	R. IV.	N. E. corner floor, six layers silk.
258	Typhoid	3	67	59	0	5	5	R. IV.	S. E. corner. Eight feet. Six layers silk.
259	Typhoid	3	67	59	0	5	5	R. IV.	West wall. Four feet. Six layers Silk.
260	Colon	3	67	59	0	5	5	R. IV.	North wall. Four feet. Six layers ticking.
261	Mixed	3	67	59	0	5	5	R. IV.	North floor. Six layers ticking.
262	Colon	3	67	59	0	5	5	R. IV.	N. E. corner, six layers ticking.
263	Mixed	3	67	59	0	5	5	R. IV.	East wall. Six feet. Six layers ticking.
264	Typhoid	3	67	59	0	5	5	R. IV.	East floor. Six layers ticking.
265	Mixed	3	67	59	0	5	5	R. IV.	Middle floor, six layers ticking.
266	Colon	3	67	59	0	5	5	R. IV.	Door. Seven feet. Six layers flannel.
267	Mixed	3	67	59	0	5	5	R. IV.	S. W. corner floor, six layers flannel.
268	Diphtheria	3	67	59	0	5	5	R. IV.	West wall. Six feet. Six layers flannel.
269	Mixed	3	67	59	0	5	5	R. IV.	N. W. corner. Seven feet. Six layers flannel.
270	Diphtheria	3	67	59	0	5	5	R. IV.	N. W. wall. Six feet. Eight layers flannel.
271	Diphtheria	3	67	59	0	5	5	R. IV.	Window, six layers flannel.
272	Diphtheria	3	67	59	0	5	5	R. IV.	Middle floor, eight layers flannel.
273	Typhoid	3	67	59	0	5	5	R. IV.	Corner, six layers flannel.
274	Diphtheria	3	67	59	0	5	5	R. IV.	N. E. corner. Seven feet. Six layers flannel.
275	Typhoid	3	67	59	0	10	10	R. IV.	East wall, six layers flannel.
276	Diphtheria	3	67	59	0	5	5	R. IV.	S. E. corner floor, eight layers flannel.
277	Colon	3	67	59	0	5	5	R. IV.	South wall. Five feet. Six layers flannel.
278	Diphtheria	3	67	59	0	5	5	R. IV.	South floor, six layers flannel.
279	Diphtheria	3	67	59	0	5	5	R. IV.	Over door. Eight feet. Six layers flannel.

* Near a wide crack in window.

The second column in the preceding tabulation shows that the work began with an exposure of 16 hours and this time was gradually reduced to three hours with no diminution of effectiveness.

An examination of the "results" columns will show that among the 1529 test objects exposed, growth developed from only 27 of them after incubation. In series No. 26 it may be noticed that positive results, that is, a growth, was obtained in three of the five tubes in this series. The place of exposure had been near a wide crack in the window. Again in Nos. 55 and 56, with the exceedingly resistant hay bacillus as the test organism, and the time of exposure 5½ hours, there was growth in one tube out of each group of five. In series No. 67, after incubation, there was growth in one tube, not of diphtheria bacillus, but presumably from faulty handling. In No. 69, three out of the five cultures from subtilis retained their power of growth after five and three-fourths hours' exposure. Subtilis again retained its vitality in Nos. 100 and 104, four cultures out of five in the former and five out of five in the latter giving a growth after 4¼ hours exposure. No. 104 was in the same position as No. 26, near the wide crack by the window.

In No. 110 aureus exposed near the not tightly fitting window showed a growth in two out of five cultures. In No. 124 subtilis, 2 out of 5, again survived an exposure of 4 hours.

Subtilis, 5 out of 10 cultures from Nos. 198 and 210, gave a growth after 4 hours exposure, but in both these series it was protected by three thicknesses of cotton flannel.

Of the 27 test objects which were not sterilized, 21 were subtilis, 3 were mixed, 2 were aureus, and 1 was an accidental growth in a tube in which the diphtheria bacilli were all destroyed. The result, therefore, is that, in all these tests, none of the ordinary pathogenic bacteria survived.

In the last column it may be seen that to No. 179 most, but not all, of the series exposed to the action of formaldehyde were uncovered. From No. 179 on, nearly all were covered on both sides with from 1 to 4 layers of flannel or other cloth, and even then, with the time of exposure reduced to only 3 or 4 hours, there was no diminution in the death-rate of the exposed bacteria. These experiments seem to indicate that formaldehyde

liberated by this process and in the quantity here used, has considerable power of penetration.

Three hours' exposure may probably be considered a satisfactory minimum of time. In the future it is hoped to do some work in the line of determining whether the quantity of formaldehyde per 1,000 cubic feet of space may be lessened without at the same time lessening the penetrative power of the formaldehyde and the general efficiency of this process.

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