coloured glasses in the combination which matched. I believe Mr. Lovibond has suggested the use of such glasses for special substances, like water-white oils, glycerine, etc.

DISCUSSION.

Mr. J. F. LIVERSEEGE said that the colour of the Welsh water in Birmingham had been regularly determined with the tintometer, and notable seasonable variations had been found. The amount of colour was smallest in the middle of the year. About September there was a decided increase, the maximum being attained about November. The colour then gradually fell till about May. The colour was determined at several stations, and it was, of course, advisable that different workers should get the same results. A sample of water was examined in Wales and the colour given as 2.4 of red, 10.6 of yellow and 0.5 of blue. Mr. Knapp and himself had examined the sample the next day and found the colour to be: red 3.0, yellow 11.2, blue 1.0. With the glasses then in use the first colour could be produced by either using five or eight glasses. The latter was a fair match of the water. They used six glasses in their match, and if, for the two extra glasses, 0.6 was added to the figures of the observer in Wales, the result was very similar to what they obtained. To avoid this cause of disagreement, rules previously drawn up for tintometer observations were amended as follows:—The white reflector must be placed so that the surface is uniformly illuminated. The sun must not shine on it. The right and left fields must have the same appearance. The equality of the fields should be tested occasionally by transferring the trough from one side of the instrument to the other. This reversal should make no difference to the colour of the water as determined by the glasses. The reflector, the glasses, and the trough must be kept clean. The bottle of water should be shaken thoroughly before the trough is filled. If the water is turbid, the contents of the trough should be stirred periodically. Welsh water should be matched if possible with yellow and red glasses only, not less than 0.2 of a colour being added at once. With dark waters the addition may be 0.4 or 1.0. If red and yellow glasses alone give a colour brighter than the water, add blue, and increase the red and yellow proportionately. (The Triple "neutral" glasses may be used as a guide to the amount of increase.) In cases of doubt, record the smaller amount of blue. For instance, if between 0 and 0.2, put down 0; if between 0.2 and 0.4, put down 0.2. Use the smallest possible number of glasses, that is, replace 0.2 and 0.4 of the same colour by 0.6; or replace 0.4 and 0.6 by 1.0. When a match is apparently obtained, give the eyes a little rest, stir the water if turbid, then test the accuracy of the match by adding 0.2 of red (or 0.4 if the water is dark) to the right and left of the instrument, and if the change effects an improvement, modify the glasses accordingly. Then try 0.2 of yellow in the same way, then red and yellow together, and finally 0.2 each of red, yellow, and blue together. If none of these changes make an improvement in the match, the result is recorded. No "neutral" glasses must be present in the final match, and all glasses must be on one side. *Glasses used.*—Red, yellow, and blue, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0, 5.0, 10.0, 15.0, 20.0; neutral (for trial matches only) 0.2, 0.5, 1.0.

Mr. F. H. ALCOCK said it appeared to him that the author's suggestion presented some difficulty. He did not think that it was a question of multiplication, addition, subtraction, or division. They had to look for the difficulty in the reflection, diffusion, refraction and polarisation of light. When he was dealing with turbid waters such considerations, which were constantly cropping up, in the subject of light, were concerned in the variation.

Mr. KNAPP, in reply, observed that Mr. Liversedge had recommended that 0.1 should no longer be used in matching the colour of Birmingham water. He thought that was wise, since a 0.1 glass added 0.1 of colour, and 0.3 of grey to the light passed through; so that, for example, the addition of a 0.1 yellow glass caused a reduction of brightness which was more marked than the increase in yellow. Personally, he had found that where there were more than 5 units of a colour already present, his eye was not always sensitive even to an addition of 0.2 of that

colour. In his own experiments the possibility of refraction, etc., having an influence was eliminated, for he had used no medium, but had compared the standard glasses with one another.

Manchester Section.

Meeting held at the Grand Hotel, Manchester, on Friday, November 4th, 1910.

DR. G. J. FOWLER IN THE CHAIR.

CONTRIBUTION TO THE STUDY OF CHEMICAL DISINFECTANTS.

BY PROFESSOR SHERIDAN DELEPINE, M.B., C.M., M.S.C.

I propose to approach this subject from the bacteriological and hygienic point of view only. I cannot attempt to deal fully with all the facts bearing upon the biological and hygienic aspect of disinfection by chemical disinfectants, all I can hope to do is to indicate by means of records of experiments and observations some of the factors which have to be considered. For this purpose I propose to utilise chiefly the work which has been carried out in my laboratory either by myself or by several workers who have employed under my supervision the same methods as those I have used. During this period I have gradually improved many technical details, but most of my older work has been controlled by more extended experiments conducted in my laboratory in the course of the last ten years by a number of research workers.

As I am not at liberty to refer in detail to the investigations I have carried out on behalf of a number of firms, I will not refer to any patent disinfectant by name (except when published statements are available). I propose, however, as soon as I have been able to complete independent and strictly comparable series of tests with all the most important disinfectants at present on the market, to publish my results. In the time at my disposal it is impossible to refer to the work done by other observers, but as I have given elsewhere* a general account of the subject I may perhaps be excused for this omission.

The problem of disinfection is more complicated than is generally supposed. In principle it is simple enough; we know that infectious diseases and fermentations are due to living microbes, and that by killing or rendering these microbes inactive we may stop the spread of infection. We may therefore say that chemical disinfectants are substances which prevent the spread of infection by bringing about the death of the living organisms causing infection. It is not always easy to distinguish between the results brought about by the death of the infecting microbes and those caused by a suspension or inhibition of their activities. Certain products have the power even in very minute doses to arrest the multiplication and other activities of bacteria so completely that perfect sterilisation is simulated. Substances acting in this way are generally termed antiseptics; they are only inhibitory agents. The same product may in small doses act as an antiseptic and in larger doses as a disinfectant, in fact this is usually the case.

Inhibitory action is very different in its effects from lethal action, for microbes which have been kept in a state of suspended activity by the action of an antiseptic may begin to multiply again when the material containing them is mixed with other products or simply diluted.

The mode of action of antiseptics is understood in very few cases only.

Goppert showed in 1889 that mercuric chloride may be fixed by the living bacteria and inhibit their growth and multiplication without killing them. This is easily proved by transferring the bacteria which have been exposed for a certain length of time to the action of certain

*Standardizing of Disinfectants, J. Royal Inst. of Public Health, 1908.

dilutions of mercuric chloride to fluids containing ammonium sulphide or free sulphuretted hydrogen. When this is done an inert sulphide is produced and the bacteria resume their suspended activity.

The same kind of phenomenon may occur in connection with other substances, but is not so easily demonstrated as in the case of mercuric chloride. I have made several experiments in order to ascertain to what an extent this inhibitory action interfered with estimates of the germicidal actions of mercuric chloride, and found that even non-sporing organisms could be revived after a time considerably exceeding the duration of their exposure to fairly strong solutions of the salt. With the assistance of Dr. H. Osborne I repeated several years ago some of these experiments. Silk threads were impregnated with a 48 hours old culture of *Bacillus coli communis*, and were allowed to remain dry for four days; at the end of that time they were severally immersed for 20 minutes in various watery dilutions of mercuric chloride, after which a first lot was washed for 60 minutes in distilled water, and transferred to peptone bouillon, a second lot was washed in distilled water for 60 minutes, and then treated by saturated watery solution of sulphuretted hydrogen for 20 minutes, and then transferred to peptone bouillon, a third lot was left in distilled water for 35 minutes, then in sulphuretted hydrogen solution, before being transferred to bouillon, the fourth lot was placed in sulphuretted hydrogen solution immediately after removal from the mercuric chloride solution, and after a stay of 20 minutes in the sulphuretted hydrogen was transferred to bouillon. the results of this experiment are summed up in Table I.

at the same temperature, and guinea pigs are inoculated with these threads they contract anthrax and die of the disease almost as rapidly as those inoculated with threads that have not been treated.

I made the first experiments of this kind in 1895 and I have repeated them since then with the same results.

The same fact may be brought out in a more striking manner by exposing the anthrax spores for 20 hours to the undiluted disinfecting emulsion, washing them for 20 minutes in water, transferring them to peptone bouillon and incubating them for 48 hours. After ascertaining that no apparent growth has taken place, the threads are then removed from the first tube of bouillon, washed and transferred to a second tube of fresh bouillon in which growth generally takes place. It is thus possible by culture methods to demonstrate the same fact which is brought out so clearly by the inoculation method.

These examples are sufficient to show the importance of distinguishing between inhibiting and sterilising action, and the difficulty of proving that a given dose of a substance has actually produced the death of certain bacteria in a given time without making lengthy experiments.

As infection is almost invariably conveyed by some organic product containing the microbe, it is necessary to consider at least three main special factors in the problem of disinfection :—1. The kind, quantity and state of the microbe. 2. The composition, quantity and state of the disinfectant. 3. The composition, quantity and state of associated matter. These three factors are considerably influenced by general factors of which the most important are :—The duration of exposure; the

TABLE I.
Antelethal inhibitory action of mercuric chloride.
Bacillus coli threads 4 days old. Temperature of solutions 21° to 21° 5. C. Duration of exposure, 20 minutes.

		Dilutions of mercuric chloride.								
Duration of stay in distilled water before transfer to nutrient medium—time allowed for delayed action.		Duration of stay in sulphuretted hydrogen water.	$\frac{1}{100}$	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{3000}$	$\frac{1}{4000}$	$\frac{1}{5000}$	$\frac{1}{20000}$
1	60 min.	None	—	—	—	—	—	—	0	0
2	60 "	20 min.	0	0	0	0	0	0	+	—
3	35 "	20 "	0	0	0	+	+	+	+	+
4	0 "	20 "	0	+	+	+	+	+	+	+

— = no observation. 0 = no growth, i.e., bacilli killed. + = growth, bacilli not killed.

From these results it would appear that the first product of the action of the mercuric chloride upon the bacillus coli (probably an albuminate of mercury) remains localised and probably quite superficial when the bacilli are treated with solutions of a strength less than 1:500, but that even the small amount of toxic product thus formed can bring about the death of the whole bacillus in the course of 60 minutes when dilutions ranging between 1:500 and 1:2000 have been used, and in 35 minutes after exposure to 1:500 and 1:300 dilutions.

The relation between the quantity of disinfectant and the duration of what I call the *antelethal inhibitory period* deserves careful study. The amount of mercuric chloride retained by the silk thread must be taken into consideration. Experiments made with bacteria in fluid culture yield equally striking results, although they are not so convenient for demonstrating the relative duration of anteletal inhibition. Phenol and emulsified higher phenols, especially the latter, have also an inhibitory action which is, however, more difficult to study than that of mercuric chloride, and which may possibly be due to the clinging of the insoluble or very slowly soluble disinfectant to the surface of the bacteria. If threads loaded with anthrax spores are left for one hour in emulsions of certain phenoloids, at a temperature of 15° to 20° C., washed in water, transferred to bouillon, and incubated, no growth is usually observed even after 168 hours' incubation. But if similar anthrax spores, prepared at the same time and with the same cultures as the threads used in the previous experiments, are left in the emulsion for 24 hours,

temperature of exposure: the degree of dilution or division of the disinfectant. There are, however, other influences that must be taken into consideration, so that the problem of disinfection is one of very great complexity and not lending itself to simple treatment. I am of opinion that our empirical knowledge of the action of disinfectants is not yet sufficiently advanced to justify our expressing that knowledge in mathematical terms. I must, therefore, discuss successively the three main factors, viz., the microbe, the disinfectant, and the associated matter.

The microbe.—Under the term microbe I cover all minute protophyta and protozoa. As bacteria include the most resisting microbes, I will specially refer to them. The ordinary bacteria multiply by fission, and it might be assumed that when a certain amount of nutrient material is inoculated with one non-sporing bacterium all the descendants of that bacterium should be of the same age and offer the same resistance to various agents. But this is probably never the case, for it is practically impossible for all the bacteria growing in a medium to satisfy equally all their requirements. Moreover, it is probable that the process of fission does not necessarily yield equal units. As a matter of fact, evidence is found in bacterial cultures of differences between individuals, and among the most striking differences I may mention the morphological differences between normal bacteria and bacteria that have undergone involution. Involution forms are most striking features of cultures of the diphtheria bacillus in which one may see, side by side, slender

bacilli of nearly uniform diameter, and other bacilli with considerably swollen extremities and the bulk of which is often several times that of normal bacilli. Differences so easily recognisable are not exhibited by all bacteria, but it may be assumed that analogous changes do take place. Even in cultures in which there is no clear evidence of alteration in the shape of the organisms, one generally observes marked differences in the size of various individuals. This is particularly evident in cultures of the *Bacillus typhosus* in which some of the bacilli are so short as to resemble cocci, while others form long filaments, the mass of which may be 10 or more times that of the shorter elements. These differences must affect the relative resistance of the individual bacilli to chemical agents, for mass is an important factor in chemical reactions. We may, therefore, suppose that even pure cultures of a given bacterium are not composed of individuals having all the same powers of resistance to external agencies. It is therefore necessary to remember that when cultures of microbes are used for the purpose of testing the action of any physical or chemical agents, it is not safe to look upon these cultures as composed of absolutely homologous parts, but we must rather look upon them as mixtures of elements which are heterogeneous as regards their physical and chemical properties. An estimate of the action of a chemical reagent upon such a mixture based upon counting the number of bacteria which have survived after exposures of various duration has not the same meaning as, for instance, an estimate of the amount of acid which has been left unneutralised by the addition of a given amount of alkali. The number of bacteria surviving after the addition of a germicidal agent may indicate either the number of bacteria with which the germicidal agent has not yet combined, because contact has not been possible or sufficiently long, or the number of bacteria which, though they have been exposed to the action have been able to resist on account of their greater resistance or greater mass. It is to avoid this difficulty that I have always rejected Krönig and Paul's method (which has been used only by a few workers), and that I invariably use the lethal point as my guide. When one uses cultures obtained under identical conditions, and of the same age, it is obvious that although the elements are heterogeneous the mixture must on an average be composed of approximately the same number of the various elements. The differences between non-sporing bacteria of the same species, though appreciable, are not considerable, but those between an ordinary vegetative bacterial cell and the spores which it may produce are enormous. It is not always realised that chemical substances that have the reputation of being strong disinfectants, because they easily cause the death of non-sporing bacteria, are under ordinary conditions practically without lethal effect upon the spores of the same bacteria. This is not special to chemical agents; the same phenomenon is observable in relation to physical agents such as heat, and suggests that the difference of resistance is due to the structure and composition of the walls surrounding the sensitive protoplasm.

As an example of this difference between the resistance of a bacillus and of its spores I may take the anthrax bacillus. Non-sporing anthrax bacilli in the moist state are usually killed in 10 minutes at 54° C., and in 1 or 2 minutes at 85° C. Sporing anthrax bacilli in the moist state are not killed for hours at 54° C., are not killed in 15 minutes at 85° C., but are killed in about 1 minute at 100° C. Spores of the *Bacillus anthracis* may be kept for many hours in strong carbolic acid at ordinary room temperature without being killed or losing their virulence. It is necessary to raise the temperature of the disinfectant to 30° or 40° C. to obtain effects within a reasonable time.

If it is not safe to argue from the degree of resistance of the various individuals composing cultures of the same organism, it is still less safe to apply to all bacteria deductions based upon the study of any single organism, for various bacteria differ very widely with regard to their resistance to physical and chemical agents. Account has therefore to be taken of the action of several physical agents which influence the action of chemical agents.

The resistance of various bacteria to drying has been carefully studied in my laboratory by Dr. G. G. Buckley, who has recorded his results in a Thesis (1907). It will be sufficient for my purpose to refer to the results obtained by exposing cotton wool infected with various bacteria to air dried over sulphuric acid. The results of these experiments show that when dried under the above conditions the *Spirillum cholerae* lived only 3 hours and died in 4 hours. *Bacillus pestis* lived 25 days and died in 34 days. *Bacillus typhosus* lived 50 days and died in 57 days. *B. coli communis* lived 50 days and died in 64 days. *B. diphtheriae* lived 81 days and died in 103 days. *Staphylococcus pyogenes aureus* lived 134 days and died in 145 days.

Although some other experiments gave slightly different results, they all indicated the same kinds of differences.

The action of heat upon bacteria is of special importance in connection with the study of the bactericidal action of disinfectants, which is also considerably influenced by temperature. The optimum as well as the lower and higher inhibitory temperatures vary according to bacteria, and the following examples may be taken to indicate the range of these variations.

TABLE II.

Influence of temperature upon the growth of six kinds of bacteria.

	Lower inhibitory temper- ature.	Optimum.	Higher inhibitory temper- ature.
	° C.	° C.	° C.
<i>Bacillus phosphorescens</i> (Fischer)	about 0	5 to 10	about 35
<i>Bacillus typhosus</i>	+ 4	25 to 30	" 45
<i>Bacillus anthracis</i>	+ 12	about 30	" 45
<i>Pneumococcus</i>	+ 20	35	42
<i>Bacillus tuberculosis</i>	+ 25	38	42
<i>Bacillus thermophilus</i> (Miquel)	+ 40	65	70 to 72

It is obvious that the influence of temperature upon the germicidal power of a disinfectant may be considerably modified by the action of temperature alone upon the bacteria exposed to the action of that disinfectant. Equally marked differences are observed with regard to the resistance of spores to heat and to chemical disinfectants. Thus spores of the *Bacillus anthracis* are killed by current steam in less than 1 minute, while the spores of the *Bacillus mesentericus ruber* generally require 180 minutes.

Exposed to the action of a thin layer of a 1 in 10 solution of chlorinated lime (3 per cent. available chlorine), the spores of the *Bacillus anthracis* were generally killed in 3 minutes, while those of *Bacillus mesentericus* were killed in 60 minutes. When the solution was reduced in strength to 1 in 100 the spores of the *Bacillus anthracis* were killed in 15 minutes, but those of *B. mesentericus* resisted for several hours.

Non-sporing bacteria resist also very unequally the same chemical disinfectant, as is shown by exposing the *Bacillus coli*, the *Bacillus typhosus*, and the *Bacillus pestis* to the action of different watery dilutions of phenol at the same time and under the same conditions.

The following table shows the results of 2 sets of experiments made respectively with the thread and suspension methods which I will describe in the second part of this paper. In this, as in the following tables, I have indicated the strength of the solutions in parts per 100,000 (i.e., in mgrms. per 100 c.c.), and recorded the highest dose found insufficient to kill and the lowest lethal dose observed.

These experiments show that the *Bacillus pestis* is much less resistant to phenol than the *Bacillus typhosus*, which itself is less resistant than the *Bacillus coli*.

TABLE III.
Resistance of three bacilli to phenol.

	Bacilli suspended in fluid medium. Exposure 5 minutes.		Bacilli dried on threads. Exposure 20 minutes.	
	Lowest lethal dose observed.	Highest non-lethal dose observed.	Lowest lethal dose observed.	Highest non-lethal dose observed.
<i>Bacillus coli communis</i>	$\frac{1}{100}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$
<i>Bacillus typhosus</i> ...	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$
			Exposure 5 minutes.	
<i>Bacillus pestis</i>	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{10}$	$\frac{1}{10}$

$\frac{1}{100}$ = 1000 parts per 100,000; $\frac{1}{10}$ = 1428; $\frac{1}{100}$ = 1250; $\frac{1}{10}$ = 1000;
 $\frac{1}{100}$ = 833; $\frac{1}{10}$ = 666; $\frac{1}{100}$ = 500.

It has been generally assumed that the age of bacterial cultures had probably an influence upon their resistance to germicidal agents; it would, however, be desirable to differentiate between the effects which the number of bacteria and the age of the cultures might have. Up to

some 10 years ago I was under the impression that cultures and more especially potato cultures of the *Bacillus coli*, were more resistant at the end of 8 or 9 days than when younger, and I used such cultures for testing disinfectants. I have since realised that young agar or bouillon cultures are more resistant than older cultures, and to obtain full data on this point I asked Dr. Howarth to compare agar and bouillon cultures of 3 different bacilli. Each set of cultures was obtained from the same original stock and kept for various lengths of time. Equal quantities of these various cultures were at intervals used to prepare cultures, the resistance of which to phenol was tested within 24 hours. This method gave a distinct chance of rejuvenation, and does not indicate quite correctly the state of the bacilli in the original cultures: the results are, however, very interesting, and show that as cultures of non-sporing bacilli get older their resistance diminishes, though this occurs very slowly.

Table IV. shows the effect of the age of cultures on the resistance of the *bacillus coli communis* to phenol. Both cultures in fluid and cultures on solid media show evidence of diminished resistance after the first few days, when in fluid cultures the resistance remains almost constant for over two months. That of agar cultures is altered even more slowly.

TABLE IV.
Effect of the age of cultures of Bacillus coli communis on the resistance of the bacillus to phenol.

Cultures in peptone bouillon (+ 5) incubated at 37° C. in closed tubes.
Cultures on peptone bouillon agar (+ 5) " " " "

Age of cultures before rejuvenation by subculture for 24 hours.	Bouillon cultures tested by the suspension method. Exposures, 5'. Average Temp., 17.5° C.		Agar cultures tested by the thread method. Exposures, 20'. Average Temp., 17.5° C.	
	Minimal lethal dilution observed.	Maximal non-lethal dilution observed.	Minimal lethal dilution observed.	Maximal non-lethal dilution observed.
	parts per 100,000	parts per 100,000	parts per 100,000	parts per 100,000
24 hours	$\frac{1}{100}$ = 1000	$\frac{1}{10}$ = 1428	—	—
48 "	$\frac{1}{100}$ = "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = 1428	$\frac{1}{10}$ = 1250
3 days	$\frac{1}{10}$ = 1428	$\frac{1}{100}$ = 1250	—	—
4 "	$\frac{1}{10}$ = "	$\frac{1}{100}$ = "	—	—
5 "	$\frac{1}{10}$ = "	$\frac{1}{100}$ = "	—	—
6 "	—	—	$\frac{1}{10}$ = 1428	$\frac{1}{10}$ = 1250
7 "	$\frac{1}{10}$ = "	$\frac{1}{100}$ = "	—	—
8 "	$\frac{1}{100}$ = 1250	$\frac{1}{100}$ = 1111	—	—
9 "	$\frac{1}{100}$ = "	$\frac{1}{10}$ = "	$\frac{1}{100}$ = 1250	$\frac{1}{100}$ = 1111
10 "	$\frac{1}{10}$ = 1428	$\frac{1}{100}$ = 1250	—	—
14 "	$\frac{1}{10}$ = "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = 1250	$\frac{1}{100}$ = 1111
21 "	$\frac{1}{10}$ = "	$\frac{1}{100}$ = "	—	—
28 "	$\frac{1}{10}$ = "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1000
42 "	$\frac{1}{10}$ = "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = 1000
49 "	$\frac{1}{10}$ = "	$\frac{1}{100}$ = "	—	—
50 "	$\frac{1}{100}$ = 1250	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1250	$\frac{1}{100}$ = 1111
63 "	$\frac{1}{10}$ = 1428	$\frac{1}{100}$ = 1250	—	—
70 "	$\frac{1}{100}$ = 1250	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1000
77 "	$\frac{1}{100}$ = 1000	$\frac{1}{100}$ = 900	—	—
84 "	$\frac{1}{100}$ = 1250	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1000	$\frac{1}{100}$ = 900
99 "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = 1250	$\frac{1}{100}$ = 1111
113 "	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1000	$\frac{1}{100}$ = 1000	$\frac{1}{100}$ = 900
120 "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1000
140 "	—	—	$\frac{1}{100}$ = 1250	$\frac{1}{100}$ = 1111
141 "	$\frac{1}{100}$ = 900	$\frac{1}{100}$ = 833	—	—
168 "	—	—	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1000
190 "	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1000	—	—
192 "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1000
190 "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = "	$\frac{1}{100}$ = 1111	$\frac{1}{100}$ = 1000
211 "	$\frac{1}{100}$ = 1000	$\frac{1}{100}$ = 900	$\frac{1}{100}$ = 1000	$\frac{1}{100}$ = 900
224 "	$\frac{1}{100}$ = 1000	$\frac{1}{100}$ = 900	$\frac{1}{100}$ = 1000	$\frac{1}{100}$ = 900

= Means no observation. The figures given are based on five simultaneous tests made with successive dilutions. Under "Parts per 100,000" the figures indicate the number of milligramms of phenol in 100 c.c. of solution.

I will mention only two other sets of observations bearing upon the effects of drying. These observations are important in relation to the disinfection of dust and other dry material, and also to the use of dried bacteria for the purpose of testing disinfectants.

A large number of silk threads of the same size, and loaded with a mixture of cultures of the *Bacillus coli communis* in such a way as to secure uniformity, were prepared, dried, and kept in the dark at a temperature of 14° to 18° C., for three months, and at frequent intervals during that period the resistance of these threads to phenol was tested. One-half of the threads was tested by Dr. Haworth and the other half by myself in a different place.

The results of this double experiment are given in Table V., and show that, with the exception of some

divergent results attributable to experimental errors, the resistance of the dry bacilli remained almost constant up to the 17th day, after which more than half of the threads had distinctly lost some of their resistance.

These differences were brought out more clearly when the temperature of the disinfectant was raised by 2° or 3° C. The results recorded under A and B, Table V., indicate clearly the effects which slight changes of temperature have upon the germicidal action of phenol.

The Disinfectant.

Products that have been used as disinfectants.—Out of the large number of products, which have been used as disinfectants since Sir John Pringle attempted in 1750 to estimate the "Comparative powers of salts in resisting

TABLE V.

Effect of drying for various lengths of time upon the resistance of the Bacillus coli communis to phenol.

48 hours old agar culture of *Bacillus coli communis* dried rapidly on silk threads. Duration of exposure to solutions of phenol, 20 minutes in every case.

Time the bacillus was kept dry.	A.		B.	
	Temperature, 14° to 18° C.		Temperature, 18° to 22° C.	
	Minimal lethal dilution observed.	Maximal non-lethal dilution observed.	Minimal lethal dilution observed.	Maximal non-lethal dilution observed.
	parts per 100,000	parts per 100,000	parts per 100,000	parts per 100,000
1 day	$\frac{1}{10}$ = 1428	$\frac{1}{10}$ = 1250	—	—
2 days	—	—	$\frac{1}{10}$ = 1250	$\frac{1}{10}$ = 1111
3 "	$\frac{1}{10}$ = 1428	$\frac{1}{10}$ = 1250	—	—
4 "	—	—	$\frac{1}{10}$ = 1250	$\frac{1}{10}$ = 1111
5 "	$\frac{1}{10}$ = 1250	$\frac{1}{10}$ = 1111	—	—
6 "	—	—	$\frac{1}{10}$ = 1111	$\frac{1}{10}$ = 1000
7 "	$\frac{1}{10}$ = 1428	$\frac{1}{10}$ = 1250	—	—
8 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = 1111	$\frac{1}{10}$ = 1000
9 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	—	—
10 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = 1111	$\frac{1}{10}$ = 1000
11 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	—	—
12 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	—	—
13 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = 1111	$\frac{1}{10}$ = 1000
15 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	—	—
16 "	—	—	$\frac{1}{10}$ = 1000	$\frac{1}{10}$ = 900
17 "	$\frac{1}{10}$ = 1250	$\frac{1}{10}$ = 1111	—	—
20 "	—	—	$\frac{1}{10}$ = 900	$\frac{1}{10}$ = 833
21 "	$\frac{1}{10}$ = 1111	$\frac{1}{10}$ = 1000	—	—
22 "	—	—	$\frac{1}{10}$ = 900	$\frac{1}{10}$ = 833
24 "	—	—	$\frac{1}{10}$ = 1000	$\frac{1}{10}$ = 900
25 "	$\frac{1}{10}$ = 1111	$\frac{1}{10}$ = 1000	—	—
28 "	—	—	$\frac{1}{10}$ = 1000	$\frac{1}{10}$ = 900
30 "	$\frac{1}{10}$ = 1250	$\frac{1}{10}$ = 1111	—	—
37 "	$\frac{1}{10}$ = 1428	$\frac{1}{10}$ = 1250	—	—
40 "	$\frac{1}{10}$ = 1250	$\frac{1}{10}$ = 1111	—	—
50 "	$\frac{1}{10}$ = 1428	$\frac{1}{10}$ = 1250	—	—
53 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	—	—
61 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	—	—
67 "	$\frac{1}{10}$ = 1250	$\frac{1}{10}$ = 1111	—	—
74 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	—	—
80 "	$\frac{1}{10}$ = 1111	$\frac{1}{10}$ = 1000	—	—
86 "	$\frac{1}{10}$ = "	$\frac{1}{10}$ = "	—	—
95 "	$\frac{1}{10}$ = 1250	$\frac{1}{10}$ = 1111	—	—
105 "	$\frac{1}{10}$ = 1250	$\frac{1}{10}$ = 1250	—	—
115 "	$\frac{1}{10}$ = 1253	$\frac{1}{10}$ = 1111	—	—

N.B.—The lethal point was determined in each case by five simultaneous tests made with five consecutive dilutions.

putrefaction," very few have retained the confidence of hygienists. Dr. Baxter's investigations (Report of the Medical Officer of the Privy Council and Local Government Board), published in 1875, have perhaps done more than any other investigation in indicating the relative value of some of the disinfectants used at the time and some of the most important factors influencing their germicidal power.

In the classical paper of Nicolai Jalan de la Croix published in 1881, the results of comparative experiments made with 20 antiseptic substances are given in a table, part of which I reproduce in this paper chiefly for the purpose of indicating the nature of the products which had attracted the attention of hygienists up to that date. The table is, however, also interesting as a record of very good work which may still be consulted with advantage.

The bactericidal action was tested by adding carefully measured quantities of the products under investigation to raw meat juice diluted with 40 parts of distilled water, which after exposure to air had become infected with various sporing and non-sporing organisms carried into the fluid by the air. The table shows (a) the smallest proportion of each disinfectant capable of rendering the infected fluid sterile, and (b) the largest proportion that failed to sterilise although it might arrest for a time the growth of bacteria.

TABLE VI.

Some experiments by Jalan de la Croix, with raw meat juice diluted with 40 parts of water and infected by aerial bacteria.

Disinfectant.	Dilutions of the disinfectant.		
	(a.) Sterilizing.		(b.) Failing to sterilize.
		Parts per 100,000.*	
Mercuric chloride	1 in 2525	30	1 in 3358
Chlorine	1 " 1061	94	1 " 1364
Bromine	1 " 875	114	1 " 995
Iodine	1 " 843	118	1 " 919
Aluminium acetate	1 " 478	200	1 " 584
Salicylic acid	1 " 343	291	1 " 450
Sulphurous acid	1 " 135	748	1 " 223
Picric acid	1 " 100	1000	1 " 117
Benzole acid	1 " 77	1290	1 " 121
Sulphuric acid	1 " 72	1388	1 " 116
Potassium permanganate	1 " 35	2857	1 " 50
Oil of mustard	1 " 40?	2500?	1 " 60?
Thymol	1 " 20	5000	1 " 36
Alcohol			1 " 142
Chloroform			1 " 122
Sodium borate			1 " 37
Eucalyptol			1 " 30
Phenol			1 " 10

* I have added these figures to make comparison with other tables easier.

This table does not include some of the compounds tested by Jalan de la Croix and others which have been extensively used or have been the subject of scientific investigation, among these I may mention :—

Ozone, peroxide of hydrogen, hypochlorites, hydrochloric acid, oxides of nitrogen, arsenious acid, chromic acid, osmic acid, salts of iron, zinc, copper, silver, etc., and various organic compounds such as hydrocyanic and formic acids, allyl alcohol, quinine, various tar products, etc.

Among the disinfectants which have gained reputé in latter years I may specially mention formaldehyde and the emulsions prepared with the higher phenols or phenoloids. Creolin was, I believe, the first of these preparations, having been introduced before 1880.

Of the higher phenols, *ortho*-, *meta*-, and *para*-cresol have been most extensively used, as well as another product or mixture having a higher boiling point than cresol and xylol, and which is known under the name of Izal oil.

All these products are more or less insoluble and are much less poisonous than phenol; they form the basis of a great number of patent disinfectants which, on account of the insolubility of their chief constituents, have to be used as emulsions. A list of the best known of these preparations was given in the *Lancet* twelve months ago (*Lancet*, 1909, p. 1457) with the results of various tests carried out by Professor Woodhead and Mr. Vasey. Table VII. is taken from their report and includes besides the names of the disinfectants some of the figures obtained by Mr. Vasey, and which indicate roughly some of the constituents of these preparations.

Properties of disinfectants.—A study of the general physical and chemical properties of the various products included in the previous lists of disinfectants reveals the fact that they have only one property in common, viz., their antiseptic power, and that they possess that property in widely different degrees.

Their antiseptic or germicidal action is obviously due to reactions widely different. The nature of these reactions can only be inferred from what we know of the action of various poisons upon the larger cells of which the higher animals and plants are composed, but even that knowledge is most incomplete and unsatisfactory. Perhaps the most scientific attempt which has been made to interpret the action of poisons upon protoplasm was that made by Loew, who divided poisons into :—

A.—*General poisons.* 1. *Oxidising poisons:* Chronic acid potassium permanganate, ozone, hydrogen peroxide hypochlorites, chlorine, bromine, iodine, phosphorus, arsenious acid.

2. *Poisons acting by the production of salts:* Acids, bases, salts of the heavy metals.

TABLE VII.

Analysis of various Disinfectants on the Market by S. A. Vasey, F.I.C.

Disinfectant.	Phenol and phenoloids.	Resins and fatty acids.	Water.	Soda.	Potash.	Gelatine.	Dextrine.	Inert bodies (hydrocarbons) by difference.
Cook's Cofectant fluid	66.27	24.66	6.40	—	2.07	—	—	—
MacDougall's M.O.H. disinfectant fluid	47.13	33.54	5.60	—	2.10	—	—	11.63
Kerol	40.66	19.14	2.40	1.41	—	—	—	36.78
Okol (Santitas)	48.60	—	38.50	0.20	—	—	5.00	0.00
Grude Carbolic	82.65	—	2.80	—	—	—	—	14.55
Bactox (Santitas)	39.70	16.70	6.00	—	1.82	—	—	35.72
Cyllin (medicinal) (Jeyes)	32.08	35.11	6.40	—	1.48	—	—	24.93
Calvert's No. 5 Carbolic A.	93.26	—	6.00	—	—	—	—	0.74
Cyllin (bulk sample)	40.41	31.23	10.40	2.24	—	—	—	15.72
Izal	41.35	—	47.14	0.10	—	11.41	—	Traces
Lysol	50.08	28.12	18.40	—	2.52	—	—	—
Lawes' Fluid	28.20	25.73	13.00	2.00	—	—	—	29.87
Pearsons Fluid	20.70	17.35	8.00	3.87	—	—	—	50.08
Jeyes' Fluid (Chemists)	17.80	20.10	0.40	1.60	—	—	—	54.01
Jeyes' Fluid (Grocers)	14.16	15.22	12.00	2.20	—	—	—	56.42
Zotul	10.00	35.42	21.60	2.40	—	—	—	30.49
Jeyes' No. 2 (Grocers)	5.13	23.21	22.40	1.38	—	—	—	47.88

3. *Substitution poisons*: Ammonia, carbolic acid, hydrocyanic acid, etc. (interfering, even when highly diluted, with the aldehyde- or amido- groups).

4. *Poisons having a catalytic action*: Ethyl ether, chloroform, chloral, etc.

B. *Special poisons*. 1. *Toxic proteids, toxins, anti-toxins, enzymes, etc.*

2. *Organic bases*: Strychnine, atropine, etc.

3. *Poisons working indirectly*: Carbon monoxide, sulphites, etc.

This classification is unsatisfactory in many respects, but has at least the advantage of placing into two fairly natural groups the most powerful germicidal substances, and of suggesting a possible explanation of the action of these disinfectants. But if this explanation is correct it would appear that these poisons act upon living protoplasm in the same way as they do on dead proteid substances. Thus the poisons grouped among the oxidising poisons are also capable of oxidising dead proteid matter,

composed of various elements, and it is not easy to distinguish among the finest particles which can be detected whether they consist of active or inactive ingredients.

When these emulsions are examined under the high powers of the microscope by transmitted light, or better, illuminated by means of the paraboloid condenser, one observes that between the larger globules, many of which are barely larger than ordinary bacteria, there is a large number of finer globules smaller than bacteria and in a state of active Brownian motion. In the spaces left between these small globules there are very much smaller particles, difficult to see and which often appear to form nebula-like clusters.

It is reasonable to suppose that some of these granules may remain attached to the surface of bacteria, but when I mixed active typhoid bacilli with an emulsion, diluted sufficiently not to kill the bacilli rapidly, I was not able to observe clearly what happened to the finest particles. The small globules were frequently hit by the moving bacilli but did not appear to adhere to them.

TABLE VIII.

Results of tests applied simultaneously to seven disinfectants.

Disinfectants.	Thread method. Test microbe = <i>Bacillus typhosus</i> grown for 48 hours on peptone bouillon agar (+5) at 37° C. Solutions of phenol prepared with distilled water two threads 2 cm. long in 10 c.c. of phenol solution. Temperature, 17° C. Time of exposure, 20 minutes.		Suspension method. Test microbe = <i>Bacillus typhosus</i> grown for 24 hours on peptone bouillon (+5) at 37° C. Solutions of phenol prepared with distilled water 5 c.c. of culture added to 10 c.c. of phenol solution. Temperature, 17° C. Time of exposure, 10 minutes.	
	Minimal lethal dilution observed.	Maximal non-lethal dilution observed.	Minimal lethal dilution observed.	Maximal non-lethal dilution observed.
Boric acid	—	parts per 100,000 2 ₀ = 5000	—	parts per 100,000 2 ₀ = 5000
Phenol	7 ₀ = 1428	2 ₀ = 1250	5 ₀ = 1250	2 ₀ = 1111
Lysol	3 ₀ = 1111	1 ₀ = 1000	2 ₀ = 500	2 ₀ = 333
Phenoloid A	1 ₀ = 600	2 ₀ = 500	5 ₀ = 200	2 ₀ = 125
Phenoloid B	1 ₀ = 600	2 ₀ = 500	5 ₀ = 200	2 ₀ = 125
Chlorinated lime (32 per cent. avail. Cl.)	1 ₀ = 62	2 ₀ = 50	2 ₀ = 50	2 ₀ = 31
Mercuric chloride	1 ₀ = 15	10 ₀₀ = 10	32 ₀₀ = 31	32 ₀₀ = 15

The figures given in this table are each based upon the results of five simultaneous tests made with five consecutive dilutions.

* Beyond thorough washing and shaking, and use of large quantity of nutrient fluid, no attempt was made to neutralise the action of any of the disinfectants at the end of exposure. (See previous remarks upon antecethal inhibitory period.)

so that it is possible that after all it may only be the action which these products have on the dead cells that the classification indicates and not the action which causes death.

The mode of action of emulsions of almost insoluble phenoloids is among the most difficult to understand. If it is only when in solution that they act upon bacteria the toxicity of the soluble parts must be very considerable, and it is remarkable that higher cells should be so little affected by them, for bacteria are almost invariably more resistant to poisons than the more differentiated cells.

If, on the other hand, they act owing to the high state of division of some of their constituents, behaving like colloidal solutions, it would appear that the molecular structure of bacteria is looser than that of the more differentiated cells, or that the constituents of bacterial protoplasm are very different from those of higher cell protoplasm.

The application of ultramicroscopy to these emulsions is not as helpful as one might have hoped, because they are

After immobilising the bacteria and the globules I was not able to recognise any suggestive alteration in the appearance of the bacilli. These observations are, however, unsatisfactory, because it is difficult to appreciate accurately slight changes in objects that are in a state of active motion, and when they have been rendered motionless it is not easy to interpret the meaning of the appearances observed.

Differences in germicidal power.—Baxter, Jahan de la Croix, Koeh, and many other observers have shown in various ways how the germicidal powers of various disinfectants differ.

In order to obtain a fairly accurate idea of the relative power of disinfectants belonging to various types I conducted some years ago an experiment with the assistance of 14 students who had been made familiar with the two methods of testing which I use, viz., the *thread method* and the *suspension method*. The solutions, the test cultures and threads had all been prepared accurately for them at the same time and were therefore of the same composition and age, all the tests were conducted simul-

taneously, at the same temperature and in the same room, so that all the conditions were identical.

The results obtained are shown in Table VIII. The disinfectants selected include a metallic salt forming combinations with proteid matter, an oxidising hypochlorite, two emulsions of different types and containing phenoloids, a soluble soap containing cresols, phenol, and boric acid, the last representing one of the weakest mineral antiseptics.

The results show that the germicidal power of soluble disinfectants remains very much in the same ratio whether the bacteria are suspended in a fluid or partly protected by the fibres of a thread, but that the action of insoluble disinfectants is very much impeded by this protection.

Mr. Burgess published in 1900 the results of very complete tests carried out entirely by himself, by the thread method used at the time in my laboratory where he had recently

studied it. The figures in Table IX. show the relative value of 14 disinfectants, as estimated by my method, for exposures of one minute to one hour. They also show the maximal non-lethal and the minimal lethal doses observed. It will be noticed that various disinfectants are very unequally affected by the duration of the exposure, a fact which is of great importance as affecting the meaning of "coefficients" so-called.

Effect of dilution.—The degree of dilution of the disinfectant taken in conjunction with the duration of exposures is another important factor. By prolonging exposures it is possible to obtain a germicidal action with doses of a disinfectant which are quite inefficient in shorter exposures, but there is a limit to this, and after a certain degree of dilution has been reached, the duration of the exposure becomes immaterial, for no germicidal action is observed. (See Table X.)

TABLE IX.

Results of five series of tests applied to 14 disinfectants by the thread method (Arthur H. Burgess, M.B., M.Sc., F.R.C.S.). (adapted from the original article in "The Lancet," June 23, 1900).

Test microbe = *Bacillus coli communis* grown for 10 days at 37° C. on potato.

Disinfectants.	Duration of the exposure to the action of the disinfectant.									
	1 minute.		5 minutes.		10 minutes.		30 minutes.		60 minutes.	
	lethal.	non-lethal	lethal.	non-lethal	lethal.	non-lethal	lethal.	non-lethal.	lethal.	non-lethal.
Mercuric iodide*	1 in 1,000	1 in 1,500	1 in 5,000	1 in 7,500	1 in 20,000	1 in 25,000	1 in 80,000	1 in 100,000	1 in 200,000	1 in 250,000
Mercuric chloride*	" 750	" 1,000	" 2,000	" 3,000	" 7,500	" 10,000	" 15,000	" 20,000	" 40,000	" 50,000
Chlorinated lime	" 80	" 100	" 150	" 200	" 500	" 600	" 1,500	" 2,000	" 3,000	" 4,000
Formaldehyde	" 30	" 40	" 50	" 60	" 100	" 150	" 150	" 200	" 250	" 300
Lysol	" 10	" 20	" 30	" 40	" 100	" 150	" 250	" 300	" 300	" 400
Carbolic acid	" 10	" 20	" 40	" 60	" 80	" 100	" 100	" 150	" 150	" 200
Izal	—	undiluted	" 10	" 20	" 80	" 100	" 250	" 300	" 300	" 400
Medical izal	—	undiluted	" 10	" 20	" 20	" 100	" 250	" 300	" 300	" 400
Creolin	—	undiluted	" 5	" 10	" 60	" 80	" 150	" 200	" 200	" 300
Jeyes' fluid	—	undiluted	" 3	" 5	" 60	" 80	" 100	" 150	" 200	" 300
Walker's IXL disinfectant	—	undiluted	undiluted	" 2	" 30	" 40	" 80	" 100	" 100	" 150
Condy's fluid	—	undiluted	—	undiluted	" 2	" 3	" 10	" 20	" 40	" 60
"Sanitas" fluid	—	undiluted	—	undiluted	undiluted	" 2	" 3	" 3	" 3	" 4
Boric acid	—	saturated	—	saturated	—	saturated	—	saturated	—	saturated

* N.B.—The results recorded have to be interpreted in the light of the experiments relating to the ante-lethal inhibitory period, recorded at p. 1345 of this communication.

TABLE X.

Combined effects of dilution of disinfectants and duration of exposure. (Ultimate lethal dilutions.)

All the tests were conducted simultaneously by the thread method, all the threads had been prepared at the same time from the same cultures, peptone bouillon agar (+ 5, incubated for 48 hours at 37° C.

Duration of exposures. Temperature, 14° to 18° C.	Test microbe = <i>Bacillus coli communis</i> .						Test microbe = <i>Bacillus typhosus</i> .					
	Disinfectants.						Disinfectants.					
	Phenol.		Phenoloid A.		Phenoloid B.		Phenol.		Phenoloid A.		Phenoloid B.	
	Lethal.	Non-lethal.	Lethal.	Non-lethal.	Lethal.	Non-lethal.	Lethal.	Non-lethal.	Lethal.	Non-lethal.	Lethal.	Non-lethal.
1 hour	—	100	—	500	—	500	100	200	—	500	—	500
2 hours	100	200	—	500	—	500	100	200	—	500	500	500
4 "	100	200	500	500	500	500	200	200	500	1000	500	100
6 "	100	200	500	1000	500	1000	200	200	500	1000	1000	2000
24 "	200	500	1000	2000	1000	2000	500	500	1000?	2000	2000?	3000
18 "	200	500	1000	2000	2000	3000	500?	500	2000?	2000	2000?	3000
75 "	500?	500	—	5000	—	5000	500?	500	2000?	5000	2000?	5000

— Means no observation, i.e., a suitable dilution was not included in the series tested.

? Means that out of a series of observations, some results were positive and some negative.

According to these results the ultimate lethal dilutions observed in 75 hours were:

When tested against the *Bacillus coli communis*—Phenol between 500 and 1000 parts per 100,000
 Phenoloid A " 1000 and 2000 " " "
 Phenoloid B " 2000 and 3000 " " "
 Phenol " 500 and 500 " " "
 Phenoloid A " 1000 and 5000 " " "
 Phenoloid B " 1000 and 1000 " " "

When tested against the *Bacillus typhosus*—

The *minimal lethal dilutions* vary according to the disinfectant, and the ratio between the lethal doses of two disinfectants is not the same for exposures of various durations. Thus the ratio between the lethal dilutions of phenol and of a certain phenoloid, which is on an average as 70 to 150 when typhoid threads are exposed for 20 minutes, becomes 200 to 1,000 after six hours, 300 to 2,000 after 24 hours, and 400 to about 3,000 in 75 hours.

In the case of phenol the minimal lethal dilution for exposures of great duration is about $\frac{1}{1000}$, a dilution of $\frac{1}{3000}$ having no appreciable effect even after 75 hours upon the *Bacillus typhosus*; for the *Bacillus coli communis* the minimal lethal dose for threads is about $\frac{1}{3000}$. The smallest dilution effective after prolonged exposures may be termed *ultimate lethal dilution*.

If dilution is pressed beyond the ultimate lethal dose a degree is reached when disinfectants, instead of inhibiting the growth of bacteria, actually excite it. This can be

phenomenon is produced when pieces of certain metals such as copper and silver are placed on the media.

Dr. H. G. Ward has for the last six months been engaged in estimating accurately the influence which this stimulating effect had upon the number and physiological characters of bacteria.

The results of one of his sets of experiments are summed up in the accompanying diagram which shows that dilution of phenol of the strength of $\frac{1}{1000}$ actually stimulates the multiplication and growth of the bacilli, and as Dr. Ward can find no evidence of degenerative changes in the bacilli, it may be assumed that part of the phenol is utilised as food. My supposition receives support from some observations made recently by Dr. Fowler, who has succeeded in breaking up products of the phenol series contained in certain effluents by means of a bacillus which has been studied by Dr. Sidebotham, who found that it was the *Bacillus Helvolicus* (Zimmerman).

Associated material.—Much of what I have said indicates the importance of associated material. It is obvious that

TABLE XI.

Influence of certain associated products upon the resistance of the Bacillus Coli Communis to phenol.

Cultures used for *thread method* grown on peptone bouillon agar (+ 5) for 48 hours at 37° C.
" " *suspension method* " " peptone bouillon (+ 5) for 24 hours at 37° C.

Associated matter.				Phenol.			
Products added to the dilutions of phenol.	Quantity added.	Effect without phenol.		Thread method. Exposure, 20 minutes. Average temp., 17° C.		Suspension method. Exposure, 5 minutes. Average temp., 17° C.	
		Threads in 20'.	Suspension in 5'.	Phenol dilutions.		Phenol dilutions.	
				Lethal.	Non-lethal.	Lethal.	Non-lethal.
None	parts per 100,000 —	—	—	parts per 100,000 $\frac{7}{10}$ = 1428	parts per 100,000 $\frac{8}{10}$ = 1250	parts per 100,000 $\frac{7}{10}$ = 1428	parts per 100,000 $\frac{8}{10}$ = 1250
Acetic acid.....	$\frac{7}{10}$ = 250	not lethal	not lethal	$\frac{14}{10}$ = 714	$\frac{16}{10}$ = 600	$\frac{14}{10}$ = 714	$\frac{16}{10}$ = 600
Hydrochloric acid...	$\frac{82}{100}$ = 12.5	"	"	$\frac{13}{10}$ = 700	$\frac{14}{10}$ = 714	$\frac{8}{10}$ = 1250	$\frac{9}{10}$ = 1111
Caustic soda	$\frac{90}{100}$ = 50	"	"	$\frac{7}{10}$ = 1428	$\frac{8}{10}$ = 1250	$\frac{7}{10}$ = 1428	$\frac{8}{10}$ = 1250
Ammonium carbonate	$\frac{50}{100}$ = 200	"	"	$\frac{7}{10}$ = "	$\frac{8}{10}$ = "	$\frac{7}{10}$ = "	$\frac{8}{10}$ = "
Glucose	$\frac{8}{10}$ = 2000	"	"	$\frac{7}{10}$ = "	$\frac{8}{10}$ = "	$\frac{7}{10}$ = "	$\frac{8}{10}$ = "
Lactose	$\frac{8}{10}$ = 2000	"	"	$\frac{7}{10}$ = "	$\frac{8}{10}$ = "	$\frac{7}{10}$ = "	$\frac{8}{10}$ = "
Sputum	$\frac{1}{2}$ = 50000	"	"	$\frac{7}{10}$ = "	$\frac{8}{10}$ = "	—	—
Phaees.....	$\frac{1}{2}$ = 50000	"	"	$\frac{8}{10}$ = 1000	$\frac{7}{10}$ = 1428	—	—
Blood serum	$\frac{3}{10}$ = 3000	"	"	$\frac{8}{10}$ = "	$\frac{7}{10}$ = "	$\frac{8}{10}$ = 1000	$\frac{7}{10}$ = 1428

N.B.—Each figure indicating a lethal or a non-lethal dilution is based upon three sets of experiments, consisting each of five tests with five consecutive dilutions.

proved in various ways. One of the simplest is to place a thread soaked in a solution of mercuric chloride on the surface of a gelatine plate immediately after insemminating it with a suitable micro-organism, such as the *Bacillus coli*. After two days' incubation it will be found that there is a clear zone round the thread where bacteria have not grown; beyond that zone the gelatine is crowded with colonies, and between the sterile part of the gelatine and that full of colonies, there is a narrow zone where the colonies are more crowded and larger and which appears as a dense marginal zone (see photograph). The same

agents which are used up by entering into combination with proteid or other matter, or who are capable of oxidising it, are in great part used up in doing that work when proteid or other matters, acted upon by the disinfectant, are present in large quantities. This fact was pointed out long ago by Baxter. One of the most important features of phenol and phenoloids is that their action is but little affected by proteid and allied matters. During the years 1906 and 1907 Dr. Haworth investigated under my direction the effects of the association of some compounds with phenol, with the results recorded in Table XI. In each

case the amount of the material added to phenol had been proved to have no material effect upon the *Bacillus coli communis* in the time allowed for the exposures.

ground upon which vegetables are cultivated; part of these excreta may be washed by the rain into a shallow well or into a stream, and this water may be used

It is generally understood that infection is almost invariably conveyed by some organic product containing the microbes which are the essential cause of infection, but the importance of the nature of the material is not

to wash utensils; which are afterwards employed to hold milk or various eatables. Flies may alight on the excreta and carry with them some of the material with which they may infect various articles of food in which,

Photograph of a part of a gelatine plate inoculated with bacilli and treated with mercuric chloride thread.

always appreciated. For example, the excreta, and more specially the faeces and urine of patients suffering from typhoid fever, may be thrown upon a piece of

under suitable conditions of temperatures, the typhoid bacillus may multiply. The excreta may rapidly dry, and when reduced to dust may be carried to neighbouring

places and infect various articles of food. The persons attending the patients, if not careful, may, while handling the patients, or carrying vessels containing their excreta, infect their hands and clothing, and in this way convey infection to milk, broth, jellies, ice creams, etc. The patients themselves may act as carriers when able to go about. The soiled clothing and bedding of the patient may be sent to a laundry where they may convey infection to many people in some of the ways already mentioned.

All this is not special to typhoid fever and may be said with equal truth of other infectious diseases, though the most infectious products are not always excreta, and the infective microbe is not always capable of multiplying in articles of food as the *B. typhosus* and allied organisms may. When the problem of disinfection is considered from the preventive medicine point of view, it is therefore necessary to consider how the infective bacillus can be destroyed when present:—*a*, in excreta, *b*, on the skin of persons, *c*, on clothing and various other fabrics and bedding, *d*, in various animal and vegetable articles of food, *e*, in water, *f*, in soil, *g*, in dust, *h*, in insects and vermin generally, to mention only the most important sources and carriers of infection.

It will be obvious at once that if the infection is allowed to be carried out of the sick room the problem becomes very complicated, if not insoluble. Thus, when a patch of ground has become infected it is practically impossible to deal with certainty with all the infective material, part of which may have already passed into water or been scattered as dust, or by means of insects, and, possibly, higher animals.

All articles of food may be sterilised by heat; chemical disinfectants are not applicable to them.

Chemical disinfectants are specially applicable to the disinfection of premises. Water may be sterilised by chemical products, as for instance ozone, chlorine or hypochlorites, or by the ultra violet light, but it is always safer to use unpolluted water.

With regard to disinfectants in the sick room, the problem, though still difficult, is not beyond solution. By sick room, I do not necessarily mean a single room, but the part of the house or hospital where infectious products must be kept for a time. Perhaps the term—infected area—might be more suitable. It is clear that if all the excreta, all the fabrics or articles exposed to infection were sterilised before leaving the infected area, and if the attendants coming in contact with the sick and their excreta removed any possible contamination by frequent ablutions and disinfection, there would be very little chance of the extension of infection beyond the infected area.

Although heat is the most reliable disinfecting agent, it is only applicable to bedding, fabrics, and vessels. The disinfection of excreta by heat in a private house is often impracticable.

From what has been previously stated it is obvious that what has chiefly to be effected is the disinfection of faeces, vomit, urine, blood, sputa, pus, and other morbid discharges, fresh or dry, either in bulk or spread on clothing, sheets, walls, floors, furniture in the sick room.

It is therefore not the bacteria alone that have to be considered, but the bacteria plus the moist or dry products with which they are associated, and which, specially when they have undergone desiccation, may offer a serious barrier between the disinfectant and the bacteria. Moreover these associated products may produce more or less inert compounds with disinfectants, and thus reduce considerably the amount available for actual disinfection.

Among the substances found in various morbid products, which affect in different manners the action of disinfectants, the following may be specially mentioned:—Various proteid substances and their derivatives, fats, urea, uric acid, various organic and inorganic salts, alkalis, acids, masses of non-pathogenic bacteria and cells, etc. The relative value of disinfectants is determined by their action not on pathogenic bacteria alone, but on the bacteria plus these various associated products:

Yorkshire Section.

Meeting held at Queen's Hotel, Leeds, on Monday, October 24th, 1910.

MR. F. W. RICHARDSON IN THE CHAIR.

ACIDS IN TAN LIQUORS.

BY PROF. H. R. PROCTER AND A. SEYMOUR-JONES.

The present great interest in the subject of this paper, as shown in the recent conference of the I.A.L.T.C., has rendered its early publication desirable, a fact which we somewhat regret since the recent important paper of Sørensen on "Enzymes" bears largely on a similar subject. In the present paper we have devoted much attention to indicators, and have had necessarily to accept certain of the views of other chemists who have worked on this subject; and Sørensen's work promises to throw fresh light on many points that we had regarded as settled. As we have as yet had insufficient time to digest his work and none to perform further experiments, much that follows must be regarded as more or less provisional.

For the past twenty-five years the question of the acidity of tan-liquors has been prominent in the chemistry of leather manufacture, and during that period upwards of twelve methods have been devised to estimate it, none of which have proved wholly satisfactory. On the other hand, the effect of the acids on the absorption of tannin by the skin and the rôle which they play in the production of sound leather, has only been studied empirically by practical tanners, and has received no attention from the research chemist.

It therefore seemed advisable to study both these questions; but in the present paper, we only propose to give an account of our work on the estimation of the acidity of tan liquors, leaving the study of its effects to a later publication. As the whole question largely depends on facts in physical chemistry which are of somewhat recent elucidation, it may be well to preface the account of experimental work by some remarks of a theoretical character.

The distinction has long been made in practice between "strong" and "weak" acids, without any very clear conception of the nature of the difference. Equal molecular quantities of either will neutralise or saturate equal quantities of base; but if a "strong" acid, such as hydrochloric, is added in excess to the salt of a weak acid such as acetic or carbonic, the weak acid is liberated and the strong takes its place in the salt. Corresponding to this, salts of strong bases with weak acids often exhibit in solution marked alkaline properties, though chemically neutral (e.g., sodium carbonate). The acid "strong" in this sense does not become "weak" by dilution with water, but rather the "weak" acid when diluted approaches the properties of the "strong" acid of the same concentration.

While the alkali-saturating power of all acids of equivalent molecular concentration is the same, their other effects are often widely different, and though their swelling effect on hide cannot be said to vary directly with their "strength," it is yet closely connected with it, and acids of a very weak character have no appreciable swelling power, though they still neutralise lime. As to the nature of "strength" and "weakness," the following may be stated. All acids may be regarded as compounds of the hydron H^+ and an anion or acid forming group, and, and all alkalis as hydroxyl, OH , and a kation or basic group. When the two are brought together the H and OH unite to form water, while the anion and kation constitute the salt. The more loosely the hydrogen is held and the more readily it reacts, the stronger the acid is; and the same holds of a base with regard to its hydroxyl. In modern chemical theory the strong acid is supposed in dilute solution to be already almost wholly separated (ionized) into hydron (the single H^+) and anion, while in the weak, only a small