DIRECT UNIT PHENOL COEFFICIENT

A NEW METHOD

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THE RESISTANCE of bacteria is a most important issue in the estimation of germicidal values. The other factors concerned in any method of determining this activity can be standardized. The adoption of certain standard measures, as strength of disinfectant, time of action, temperature, media, etc., should be governed by application of knowledge possessed. On the other hand, the resistance of bacteria, although subject to wilful modification, is the most difficult factor to control, and for this reason, any method of determining phenol coefficients should be based on this variable property of organisms.

In order to better understand the factors which affect resistance it is necessary to study first the principles upon which living cells are founded, and second the means of deleteriously disturbing vital function.

A study of facts pertaining to living cells shows that those of both the animal and vegetable kingdom possess many properties in common. Briefly, the vital properties common to all cells are irritability, respiration, growth, metabolism and movement. Other commonly possessed characteristics are the semi-solid state, viscosity, colloidal nature, biosmosis and surface tension. All cells exhibit kinetic energy in some form, as light, heat, mass movement or electrical energy, and in addition possess potential energy. All cells are composed of water, inorganic salts and products of fats, carbohydrates and proteins. All exhibit oxidation, reduction, dehydration and hydration, and all have habit and method of performance. All possess enzymes and apparently antienzymes. All maintain the chemistry characteristic of their type in equilibrium and exhibit instability.

From a consideration of these numerous, commonly possessed properties it is logical to assume that there must be at least one principle upon which all cells are based. An analysis of the facts concerning these properties shows that this basic principle consists of a structural plan identical for all types of cells. Space prohibits a detailed discussion of this conclusion and permits merely a description of this universal structural plan.

It can be proved that the water, inorganic salts, fats, proteins and carbohydrates of the cells cannot exist or function individually, and that they are united in the form of a physico-chemical emulsion controlled and guided in normal channels by neuro-electrical forces.

It is of the utmost importance in the study of resistance to consider a living cell as an unstable physico-chemical emulsion held in equilibrium because the problem then becomes simplified to the study of factors which affect and modify the emulsion. The action of these factors can be conveniently described under three classes: first, a disturbance of chemical equilibrium towards stability; second, alteration of the electrical state; and third, alteration of the state of hydration. Many facts are available to prove these points. At any rate, we have on the one hand factors as classified above, operating in a manner to produce conditions unfavorable for the existence of independent cells; on the other hand there is the attempt on the part of the living emulsion to maintain its equilibrium, and this activity is called resistance. Naturally all of the processes involved and means employed are not yet known. However, their ability to adjust themselves to strange environment depends on many factors, for example, changes in reaction

of their surroundings are combated by the buffer action of the contained proteins; changes in moisture content may produce spores; the tubercle and some other bacilli contain many substances which resist untoward changes. Broadly speaking, the greater the power of adjustment the greater the resistance. Specialization either in function or food requirements leads to lessened power of adaptability, hence, a lower resistance. In accordance with this ruling, the pathogens and especially those of selective pathogenicity as the gonococcus, the typhoid bacillus, and the meningococcus possess slight resistance under unfavorable conditions. In contrast to these, the less selective pathogenic organisms as the staphylococci have greater resistance. Proof of this is to be had in the type of food required and again against the action of carbolic acid. A comparison of the resistance of several kinds of pathogenic organisms including staphylococcus and typhoid bacilli under conditions where all units except resistance are equal, shows that the typhoid bacilli and other selective pathogens are much more easily destroyed. This of course is a test against only the power to resist certain chemical change, but sufficient to show the necessity of selecting the most resistant pathogen as the test organism in adopting a method of estimating the relative strength and value of germicides.

The present methods of determining germicidal value have not given complete satisfaction, and considerable difficulty is encountered by the manufacturer in complying with the various federal and state rulings in regard to germicidal values. The present methods depend too greatly on personal equation and too little on standardized units. For example, the time period and the strength of phenol control are variable and are different for each set of tests. The length of time and strength of phenol to kill a definite number of organisms are constant, however, and should be adopted as a standard.

In any method of analysis it is well to have as many constants as possible. Although the selection of a definite time period seems to be entirely arbitrary, there are reasons for the choice of a definite short interval. Germicides in practical application have a minimum of effectiveness whether this is the result of human impatience or the involved chemical conditions. Experience has proved that the average maximum should be five minutes for reliable determination of germicidal value under actual conditions. The apparent discrepancies to this statement are due entirely to the physical conditions involved and not to any differences of chemical action.

An examination of tables made by present methods containing the results of phenol coefficient tests shows that within a period of five minutes the length of time for a definite strength of phenol to kill a definite number of organisms is constant; and that this time period becomes variable after that period. Under those experimental conditions physical factors were removed, and the chemical units were constant, thereby proving, by process of elimination, that the resistance of the strain of bacteria was the variable. This would undoubtedly prove true regardless of type of organism, except in degree.

The strength of phenol selected should be that which exhibits complete destruction of the entire number of organisms within the five minutes. This action should not be instantaneous, but should be graduated so that the action is complete only after three or four minutes. The strength to be selected by experiment and once determined should become a standard, thereby furnishing a second constant, strength of solution, which can always be duplicated.

Present methods contain no such definite time limit or standard single strength of phenol; and in addition employ the typhoid bacillus for the test organism. The argument in favor of this choice is that B. typhosus is claimed to be the one pathogen which is most constant in characteristics; hence, all laboratories can use

an identical strain. As to its consistency of characteristics, the typhoid bacillus is very prone to change. Not only in the Pennsylvania Department of Health Laboratories, but also in others, it has been known to lose its main characteristic, the lack of power to ferment lactose and to acquire the ability to split this sugar.

Again, the typhoid bacillus has been shown to be less resistant and more variable in resistance under different conditions to the action of phenol. Since it is not constant in characteristics, and since it is not exceptionally resistant, it appears to be a poor choice to represent the pathogens and the filterable viruses of unknown resistance. And disinfection has for its aim the destruction of other infectious agents as well as the typhoid bacillus.

On the other hand, the less specific pathogens staphylococci and diphtheroids represent classes which are much more constant in their resistance to unfavorable conditions; hence, are a better choice to test the germicidal action of a substance, and being most resistant will guarantee destruction of unknown viruses better than the less resistant typhoid bacillus. Strains of staphylococci and diphtheroids from any source show a uniform resistance to the action of phenol within a period of five minutes. The choice of staphylococci and diphtheroids furnishes the least variable test groups, and since their resistance under controlled conditions is so nearly uniform, we may say that they furnish a third constant or standard.

How often in determining phenol coefficients do accidents occur, so that when the test is read there is a break in the continuity of findings. Then comes the question whether the organisms were killed in one tube and not in the next, or the turbidity was caused by a contamination, and this necessitates corroboration. The present methods give only presumptive qualitative results and do not furnish what is needed, which is a definite determinative quantitative evaluation of the germicide. The present methods are involved and difficult to perform, and require a great number of tubes and other materials and frequently assistants in the actual performance.

Although the present methods are rather complicated they are limited in applicability, permitting only the testing of germicides incorporated in water or soap emulsions, and cannot be employed with preparations such as ointments.

Results obtained by present methods by different individuals or by the same individual at different times on the same material will vary several points; hence are unreliable in forming an opinion of the true value of the germicide.

With the above points in view an attempt was made in this laboratory to devise a method which would eliminate all of the undesirable features. The Direct Unit Phenol Coefficient was established in the Pennsylvania Department of Health as a substitute for the old and indirect, inconstant phenol coefficient and will be presented herewith in detail.

This method employs a single unit of control phenol solution, a time period of five minutes, two classes of resistant pathogens, controlled environment of the bacteria, and is performed on plates, which permits a direct determinative and quantitative identification of the cultured test organisms.

PHENOL CONTROL

Phenol is used in a 1.75 watery solution. It is prepared by accurately weighing 1 gram of the melted crystals into a weighed beaker, and adding 74 grams of sterile distilled water.

TEST ORGANISMS

A strain of staphylococcus aureus and one of diphtheroids from any source; these are transplanted every twenty-four hours on nutrient agar, which is made from meat infusion broth, bactopeptone and 1½ per cent agar, adjusted to a pH value of 7.6 and slanted in tubes approximately 150 x 16 mm. Incubation is carried out at 37° C.

To prepare for use separate suspensions of both organisms are made by adding 4–5 c.c. of sterile physiological salt solution to the agar growth, washing off the growth and transferring the concentrated suspension to a tube approximately 150 x 16 mm. containing 3 sterile beads, and shaken gently until all clumps have been broken and the opacity is uniform. Sterile salt solution is added in small quantities with agitation of the tube until the markings on a 1 c.c. pipette become visible, when held within the tube against that part of the tube farthest from the observer. At this degree of opacity the number of bacteria is 2,000,000,000 per c.c. Equal quantities of the separate suspensions are mixed and 0.1 c.c., equal to 200,000,000 bacteria, used as the standard quantity in the test.

PERFORMANCE OF TEST

Melt agar of pH 7.6, cool to 45–50° C. and keep it in lukewarm bath.

Arrange six serially numbered and labelled Petri dishes in consecutive order on the table. Arrange the Petri dish for contact by slanting it on another dish.

Place, by means of a large calibre 10 c.c. graduated pipette, 1 c.c. of the preparation to be tested into the contact dish, so that all of the material is on one side. By means of a heavy platinum wire formed in a 4 mm. loop, transfer a loopful of the germicide to the dish labelled germicide control. Add agar and rotate the plate.

Now, at a definite minute, add from a pipette 0.1 c.c. of the mixed bacterial suspensions to the 1 c.c. of germicide. Heat loop and cool either in sterile salt or the cooled agar, and thoroughly mix the germicide and bacteria. At the end of one minute remove a loopful; hold it in the centre of a Petri dish and add cooled agar. Stir vigorously with the wire and rotate the plate. Sterilize the wire, and repeat the transplantation at intervals of one minute for five minutes. Set plates aside to cool.

Repeat this technique, using phenol 1:75 water solution, as the germicide.

Make a control plate of the bacterial suspension by transferring a loopful of the suspension to a dish and adding agar. Make a control of the unmodified agar in order to determine its sterility.

When all plates have hardened, invert and place them in an incubator at 37° C. for twenty-four hours.

READING

Agar control should be sterile. Phenol control should be sterile. Phenol plate 1 should exhibit marked inhibition of growth. The two minute plate should show a still greater amount of inhibition. Plate 3 should be sterile, but may exhibit some colonies. Plates 4 and 5 should be sterile, thereby showing that the bacteria are killed in three to four minutes of contact. The tested germicide plates are compared directly with those of phenol.

In order to obtain some idea of the coefficient of an unknown germicide, several dilutions are made. Each dilution is placed in its contact dish, and seeded at the proper time with the standard quantity of organisms. Instead of making 5 plates at one minute intervals, only 1 plate after three minutes of contact is made for each dilution. The entire five minute plates are run on phenol to serve as a check on the procedure. The strength equalling the three minute phenol plate is then selected for the test of comparative strength. When this is done, the Direct Unit Coefficient is determined by simply dividing the strength of disinfectant by 75, the strength of phenol.

This method furnishes a direct estimation of the number of organisms destroyed, and permits immediate identification of type, as contaminations are easily determined. The symbols employed are: — No growth; + — from 1 to 50 colonies; + from 50 to 200 colonies; ++ from 200 to ∞ colonies; +++ = ∞ colonies. The method has been used for several years in the Pennsylvania Department of Health Laboratory in testing the germicidal value of material incorporated in ointments, as well as those in

water vehicles and has proved to be very economical, easy to perform, reliable and consistent in results, and is adaptable to the testing of materials with organic matter as well as those without.

In the examination of materials selective for gram negative organisms, as acriflavine, the colon bacilli can be very properly substituted as a test organism, since its resistance is equally high and constant.

At present the tests are performed at room temperature, since staphylococci and diphtheroids are not as variable to slight changes of temperature as the less constant typhoid bacilli. But if a constant temperature is required, it probably can be secured very easily by making a simple water bath, heated by electric lamps and regulated by an easily made mercury contact system.

OBSERVATIONS ON DIPHTHERIA TOXOID AS AN IMMUNIZING AGENT

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BOTH diphtheria toxoid and diphtheria toxin-antitoxin derive their immunizing potency from altered diphtheria toxin. The freshly prepared toxin has such an instant action on the human tissues that only minute amounts can be used, and immunization with it is therefore a long drawn out process.

Diphtheria toxoid was used for animal immunization in the first experimental work in producing antitoxin and it has continued to be used to some degree ever since.

The utilization of a toxin-antitoxin mixture by von Behring led to the use by us and others of toxin-antitoxin rather than toxoid. During the past three years Glenny and Hopkins[1] in London and Ramon[2] in Paris have been suggesting the substitution of toxoid for toxin-antitoxin. Independently of them we[3] had begun to test the value of toxoid preparations.

Diphtheria toxin can be changed to toxoid by various means, such as storage at moderate temperatures, heating to 50 to 54° C. for a few hours, or exposure to chemicals.

The accepted method at the present time is to add 0.2 to 0.3 per cent of formalin to the toxin, the amount depending on the protein content and the duration of storage in the incubator.

The advantages claimed for the toxoid are that it is simpler to prepare; is more stable; is somewhat more potent, and it contains no horse serum globulin. In our opinion toxoid is no easier to prepare than toxin-antitoxin. Experience and the following out carefully of every detail of a proper method are necessary in both cases. To the inexperienced a toxoid is easier to prepare because it is a single substance instead of two substances and the difficulty of making a proper mixture is avoided.

The question of stability is more debatable. The longer a toxin is stored, and to a certain extent the greater the percentage of change from toxin to toxoid, the less rapid will be any further change.

The toxin used for toxin-antitoxin is really a mixture of toxin and toxoid. If the toxin-antitoxin mixture is made so that when mixed it has just the right toxicity, it will drop off in potency faster than a toxoid preparation, but if it is made with some excess of toxicity and stored for several months before it be-