Relation of Action of Chlorine to Bacterial Death*

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CHLORINE in the form of gas or of its many compounds has found wide use in public health work. It is perhaps the most popular of the numerous germicides—seemingly an effective agent in causing bacterial death.

This bacterial death, however, is a somewhat misunderstood phenomenon, largely because there are no well defined criteria for it. With bacteria any measure of death is negative, a sort of inverse to life, since one must grow the organisms surviving in order to arrive at the number dead. With animals, any of several phenomena can be used as an index of death—for example, rigor mortis and cessation of heart beat. With higher plants there are criteria, somewhat less well defined, that tell when life is gone, but in the case of bacteria, no such tests exist.

Fulmer and Buchanan [1] have used a vital staining technic which they claim is a satisfactory criterion under certain conditions. According to them, "All cells which take up the stain (methylene blue) are dead, that is, they no longer can reproduce." Five words of this quotation tell the story—"they no longer can reproduce." The reproductive phenomenon in some form is always used in studies of bacterial death; and all our concepts of lethal action are based on the hypothesis that if the bacteria do not reproduce they are dead.

That this basic premise is incorrect has been shown by Rahn and Barnes.[2] Although these authors used yeast as a test organism, their results are applicable to bacteria. They subjected bottom yeast to the action of 4 lethal agencies: heat, mercuric chloride, ultraviolet light, and X-rays. At definite intervals the survivors in the cultures were enumerated by the plate count (to show reproduction), gas production (to show enzyme action), staining reaction (to show dead tissue), and coagulation. The cells lost first their power of reproduction, then their enzyme activity. Considerably after losing these 2 powers, they took the stain; and later they coagulated. Data in this paper indicate that for a given time the plate count showed but 0.0002 per cent survivors, whereas the fermentation and stain test showed 5 per cent and 39 per cent living, respectively. Rahn and Barnes consider that the coagulation of the cell protoplasm is not the fundamental reaction of cellular death.

This concept is at variance with a considerable body of opinion to the effect that coagulation is important. Heilbrunn [3] accepts the latter view and cites many instances of belief in it. Bancroft and Richter [4] have studied the action of germicides under the ultramicroscope, using various lethal agents upon yeast cells. They report seeing a

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visible coagulation that led them to "understand readily why the cells die." The "death" that they described was, unfortunately, not checked by the usual means. Perhaps the most original part of their work was the direct observation that the coagulation within the cell is reversible. This reversibility they noted; and, if there is any correlation between their coagulation and death, the death process too must be reversible within limits. They further postulate the grossly empirical equation—

Living bacteria+disinfectant=dead bacteria.

Given such an equation, they believe, certain reaction velocities would obtain. Increasing the disinfectant increases the velocity to the right (McClintic).[5] Increasing the dead bacteria increases the velocity to the left (Lange).[6] Increasing the living bacteria, however, throws the equation into a "hideous disagreement"—to use their words. It is their idea that the death phenomenon is reversible—a conception not very new in bacteriological literature. A number of authors have reported such findings. Rodewald[7] placed the organism causing fowl cholera in a 0.1 per cent mercuric chloride solution. After several minutes the cells were removed and washed. Mice inoculated with them were killed. As a control, Rodewald treated a similar quantity of cells in like manner and attempted to grow them. He failed; the cells were "dead." Similar experiments are reported by Liesegang,[8] Süpfle and Müller,[9] Müller,[10] and Gegenbauer.[11] All these authors treated cells with various germicides, and by subsequent treatment with antidotes were able to cultivate them, whereas the untreated cells showed no evidence of growth.

The experiment of Süpfle and Dengler[12] is exceedingly interesting, since it apparently indicates that the death phenomenon is a function of the medium used to grow the organisms. Süpfle and Dengler dried anthrax spores upon silk threads, which were then suspended in a steam bath. After various periods of heating, duplicate threads were removed. One was placed in plain broth; the other in broth enriched by sugar and serum. In the richer medium, the spores germinated after exposure to the steam for a longer time—a very significant observation.

Still another argument can be used in indicate the possibility that the death reaction is reversible. Not only Chick,[13] but Madsen[14] and Nyman, showed that the action of a lethal substance in a bacterial population follows a rather orderly and predictable course: the death rate in such a population depends on the number of cells living at any given time. Expressed mathematically,

$$\frac{d\,b}{d\,t} = K\,b$$

This, on integrating and evaluating the constant of integration, gives

$$K = \frac{1}{t}\,\text{ln.}\,\frac{b}{a}$$

In these equations "a" represents the initial number of cells, "b" the number of cells living after time involved, "t" the time, and "K" the rate of death. The second expression is easily recognized as the monomolecular equation so much used by the chemist for the rate of certain reactions. In this case it is an over-all equation reflecting the action of the germicide on the countless individual cells that make up the population. Some contend[15] that the lethal curve does not follow the monomolecular reaction exactly. There is little doubt, however, that the death phenomenon is chemical in nature, and so it must follow chemical laws in other respects and must be reversible or at least must reach an equilibrium somewhat short of actual death.

There is still another possible view regarding the death phenomenon.

Richet,[16] Gegenbauer,[11] and later Winslow,[15] and his coworkers [17] have all shown that for most substances, lethal or not, there are concentrations which first stimulate, then inhibit, and later in higher concentrations kill. Gegenbauer postulates zones of life, of dormancy, and of death, but dormancy is not death, though the criterion is likely to be the same—failure to reproduce.

Is it possible in much of the work where "death" has been studied that dormancy alone has been observed and that the cells were indeed alive? If so, most concepts of the action of certain germicides need revision. The work of Leonard [18] apparently leads to the same conclusion. Studying the phenol coefficients of certain compounds, he pointed out that the Hygienic Laboratory makes no claim for this method unless the germicide is closely related to phenol. Those with which he worked were not related. He attempted to determine whether the effect of his compounds was really lethal or merely bacteriostatic—that is, whether he had death or dormancy. To do this he took the 15 minute tubes of the usual test and transferred a loopful of the contents to tubes of fresh broth. In this way the germicide was further diluted. When the transfer was from the phenol tubes there was no growth, whereas the tubes made from his unknown showed growth.

We are especially interested in chlorine compounds, particularly the hypochlorites. These compounds are extensively used in dairy plants and on producing farms. In many instances, particularly on dairy farms, chlorine compounds are the sole means of sterilization. This situation is seemingly justifiable, since the literature is filled with studies made on such compounds. Always the results are reported to be satisfactory. Hypochlorites are supposed to have an exceedingly high phenol coefficient. Zoller [19] reports 300 for instance; Tilley [20] 80 to 90. In the light of Leonard's work, one wonders whether such high phenol coefficients are reliable. Possibly the reaction may be reversible. Furthermore, dormancy or bacteriostasis may have been the pitfall for the unwary in such studies.

We report the results of several experiments on the germicidal action of chlorine compounds. Our procedure was essentially the same as that of many others found in the literature, and the results are in entire accord with theirs. We went just one step farther, however, made observations that to us seem highly significant.

METHODS

For organisms we used milk in which bacteria had grown. This milk had a population well into the millions but contained no clots in which bacteria might be trapped. It was diluted with distilled water in various ratios. The presence of milk in our active mixtures has an excellent precedent in the work of Chick and Martin,[21] who postulate that the action of lethal agencies on bacteria is best carried out in an environment where bacteria can live. The bacteria in such milk were assumed, furthermore, to represent those forms that the disinfectant must combat in actual practice. Although the flora of our various experiments varied, the constancy of our results left no doubt that the practice was valid. The hypochlorite used was the liquid bleach of commerce, containing about 16 per cent available chlorine. It was strongly alkaline, for there was residual alkali in the container. In order to stop the action of chlorine and thus prevent a bacteriostatic action in the plate, 0.5 c.c. of a sterile 1 per cent solution of sodium thiosulphate was added to each plate which had previously been shown to have no effect on colony counts. Being interested in trends and not in

absolute values, we used only 2 dilutions. Some of the colony counts were exceedingly high. In order to count such crowded plates, we used a dissecting microscope, calibrating the field with a given magnification in terms of the petri dish area. Whenever resort was made to the microscope, 8 to 10 fields were counted, and averaged.

We cannot submit all our experiments for lack of space. Our results, furthermore, do not lend themselves to the process of averaging, dangerous in itself; so we submit a protocol (Table I). The higher numbers of colonies in plates with thiosulphate are to be noted. Particularly important is the fact that the 1–10 dilution gives a higher count than the direct plate (1–1).

A very satisfactory reduction in bacteria was caused by the action of the hypochlorite—a result in accord with the usual findings (Table I). The protocol also shows, as might be expected, that in most cases the counts are higher in the plate where the thiosulphate is present than where it is not. It is strange that more workers have not used thiosulphate to offset the effect of the germicide itself. The most interesting observation in our work is splendidly exemplified in the protocol: the 1–10 dilution made in addition to the usual direct plate is always the higher of the two. One may reasonably assume that if the plate made direct from the active mixture shows 5,000 colonies, then the 1–10 dilution should have but 500, but instead of 500, there are 28,000. We found this "jump up" throughout our work, regardless of the amount of milk present, the strength of the chlorine, the time of action, or the presence of thiosulphate.

Diligent study has been made of the "jump up," which is doubtless a phenomenon similar to that described by Leonard. The next experiment follows naturally the conclusions of the one above. A mixture of bacteria with water was taken. It was our plan to plate this mixture both before and after

TABLE I

PROTOCOL OF EXPERIMENTS SHOWING THE EFFECT OF CHLORINE ON BACTERIA

Initial bacterial count: 100,000,000
Ratio of milk to water: 1–10

Time of Action of Chlorine, Minutes	*Plate Dilution*	*Concentration of Chlorine p.p.m.*	*Liquid Bleach*	
			No Thiosulphate	*With Thiosulphate*
3	1–1	50	5,000	294,000
3	1–10	50	280,000	224,000
5	1–1	50	33,000	56,000
5	1–10	50	640,000	160,000
10	1–1	50	48,000	67,000
10	1–10	50	196,000	640,000
3	1–1	100	28,000	5,600
3	1–10	100	600,000	144,000
5	1–1	100	33,000	33,000
5	1–10	100	960,000	960,000
10	1–1	100	33,000	28,000
10	1–10	100	1,312,000	224,000
3	1–1	200	210	2,000
3	1–10	200	4,300	5,800
5	1–1	200	324	2,100
5	1–10	200	1,000	5,800
10	1–1	200	762	3,240
10	1–10	200	3,600	5,040

the action of chlorine. To check the plate count which—according to Rahn and Barnes—gives the first evidence of death, we also ran serial dilutions on the same mixture at the time the plates were made. We expected close agreement betwen the plate and the dilution tube before the action of chlorine. For instance, a mixture showing a colony count of 1,000,000 would also show growth in tubes out to at least the 10^{-6} dilution. After the action of chlorine we thought that there might be a discrepancy between the plate and the tube. The 1,000,000 bacteria (of the previous example) might be reduced to 1,000, as evidenced by the plate count. The serial dilution tubes should not show growth in dilutions greater than 10^{-3}. The experiment indicates the correctness of our reasoning. There is close agreement between the plate and tube before the action of chlorine (Table II, columns 2 and 3), but after no such agreement is noted (columns 4 and 5). The plate count shows the expected decrease up to 99 per cent. The serial dilution tubes, however, show growth in as great dilutions after the action of chlorine as before. There is, therefore, virtually no reduction as measured by this method (column 7).

By one method there is evidence of death; by the other, no such evidence. Both cannot be right.

Admittedly, errors are inherent in the dilution technic. However, a dilution procedure is also an integral part of the plate method, so that the inherent errors are more or less common in both cases. Furthermore, we obtained these results with such regularity that there is little doubt as to their essential truth. In the light of all the evidence cited, together with that which we submit, there are apparently few tests that can be relied upon where bacterial death is concerned.

SUMMARY

The literature shows that—

1. Bacterial death is to a certain extent reversible.
2. There are "zones" of life dormancy and death.
3. The action of some germicides result in dormancy and not death.
4. The measure of death is sometimes a function of the media used to grow the survivors.

TABLE II

COMPARISON OF THE PLATE COUNT WITH THE SERIAL DILUTION BEFORE AND AFTER THE ADDITION OF HYPOCHLORITE

Time of Action of Chlorine in Minutes	*Before the Addition of Chlorine: Numbers of Bacteria as Indicated by Colony Count*	*Before the Addition of Chlorine: Numbers of Bacteria as Indicated by Serial Tubes*	*After the Addition of Chlorine: Numbers of Bacteria as Indicated by Colony Count*	*After the Addition of Chlorine: Numbers of Bacteria as Indicated by Serial Tubes*	*Present Reduction as Indicated by Colony Count*	*Reduction Indicated by Serial Tubes*
1 — 3	9.2×10^5	1×10^7	8.5×10^3	1×10^6	98.9	+
1 — 5	8.0×10^5	1×10^7	7.2×10^3	1×10^7	98.8	0
1 — 10	3.0×10^6	1×10^6	1.5×10^4	1×10^7	99.3	0
2 — 3	1.24×10^4	1×10^5	7.8×10^3	1×10^6	37.0	0
2 — 5	8.9×10^3	1×10^6	7.8×10^3	1×10^6	12.0	0
2 — 10	2.0×10^4	1×10^6	7.8×10^3	1×10^6	61.0	0
3 — 3	4.9×10^5	1×10^7	4.9×10^5	1×10^7	0.0	0
3 — 5	3.8×10^5	1×10^7	3.6×10^5	1×10^7	4.0	0
3 — 10	3.0×10^5	1×10^7	4.8×10^3	1×10^7	99.0	0
4 — 3	2.5×10^5	1×10^6	1.2×10^4	1×10^6	95.0	0
4 — 5	1.2×10^5	1×10^6	3.1×10	1×10^6	99.0	0
4 — 10	1.9×10^5	1×10^6	1.9×10	1×10^5	99.0	0
5 — 3	2.3×10^7	1×10^6	1.2×10^6	1×10^6	95.0	0
5 — 5	2.5×10^7	1×10^7	7.8×10^5	1×10^5	95.0	+
5 — 10	2.1×10^7	1×10^6	4.1×10^5	1×10^5	95.0	+

Our investigations show that—

1. The colony counts increase as the dilutions increase.

2. There is no accord between colony counts and serial dilutions.

3. Growth was observed in as high serial dilutions after chlorination as before.

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Organized Medical Care

IT would appear that if medical care is provided on some organized basis, it should be possible to define the two fields so that there will be a complete curative and preventive service without any overlapping. In order to attain the fullest coördination, it would be desirable to have the provincial department of health responsible for the administration of all medical services, including hospitals, for which the province assumes responsibility. . . . The public health authority should have in mind that administrative responsibility does not mean a dictatorship. In England, health insurance is administered centrally by the Ministry of Health, which also appoints the regional officers, who act as medical referees and inspectors. The Ministry, in the exercise of its administrative powers, turns to the British Medical Association for advice and assistance. The strength of health insurance in England is largely due to placing control for the professional side of the medical benefit with the medical profession. This is something for public health authorities to keep in mind, if they are ever called upon to administer some form of organized medical care.

A fair criticism of health insurance is that it has not been preventive in practice and but little in outlook. It is not enough to render lip service to the idea of the practice of preventive medicine by the general practitioner, and then to disregard him in planning public health services. Under whatever name organized medical care is provided, it should make possible the systematic practice of preventive medicine, including periodic health examinations, by the general practitioner. . . .—The Relationship of Public Health to Medical Care, Grant Fleming, M.D., McGill University, Montreal. *Canad. Pub. Health J.*, Oct., 1934, p. 465.