THE EFFECT OF CARBONATION ON BACTERIA IN BEVERAGES*

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IN many of the contributions to the literature on the sanitary condition of soft drinks, there is a lack of scientific discrimination, as is apparent in the failure to make clear the bearing of the many factors that may influence this condition. The bacterial count of a soft drink at the time of examination may depend upon sanitation at the bottling plant, the water supply, the hydrogen-ion concentration and the composition of the drink, the degree of carbonation, and the time that has elapsed between bottling the drink and its examination.

The popular attitude among writers on the subject is to point out existing evils and to decry the high counts which admittedly are found sometimes in examining soft drinks. The writers feel that the broad statements occasionally made along these lines do great injustice to the many bottlers of carbonated beverages who are exerting every effort to produce sanitary products and who are making beverages of excellent quality. The present paper is confined to the effect of carbonation on the sanitary condition of beverages and does not attempt to deal with the other factors. The experimental work reported was suggested by the experience of one of the authors, who had occasion to examine some samples of ginger ale from a Canadian lumber camp where a typhoid epidemic was raging and where the water supply was known to be contaminated.

The fact that carbonation tends to reduce the bacterial count of a water or a beverage was pointed out by Leone[1] in 1885, and many other investigators of the nineteenth century confirmed this observation.[2–8] Elsdon[9] found lower counts in siphon waters than in carbonated bottled waters, and correctly attributed this result to the greater carbon dioxide pressure in the siphons. Further, carbon dioxide has always been regarded as an antiseptic agent by brewers.

Considerable work has been done on the effect of carbon dioxide on various types of bacteria. The present state of our knowledge indicates that carbon dioxide at atmospheric pressure reacts differently on different bacteria, but no case has ever been recorded in the literature in which carbon dioxide under commercial carbonating pressures was found to favor the growth of any microörganism. On the contrary, complete sterilization may be obtained with carbon dioxide by the use of sufficient pressure or of sufficient time. Colin,[10] who worked with a number of pure cultures at rather high pressures, found that 20 hours at 213 pounds p.s.i., or shorter times at higher pressures sterilized cultures of *typhosum*. *B. dysenteriæ* were destroyed in 6 hours at 284 pounds; *B. choleræ* in less than 10 hours at 142 pounds; *B. pyocyaneus* in 48 hours at 256 pounds; *B. diphtheriæ* in 3 hours at 284 pounds, or 24 hours at 142 pounds; but Colin found the spore form of *subtilis* uninjured after 4 hours at 355 pounds. When water from the Seine was held at 355 pounds for a few hours, only the spore form of *subtilis* persisted. However, Colin's longest experi-

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ments ran for only 48 hours and he did not investigate the effect of commercial carbonating pressures at all. Other investigators[11-18] have determined the effect of carbon dioxide on various individual strains, the outstanding feature of the work being the variability of the effect depending upon the organism. In no case is there reported a sustained increase or even a maintenance of count where the carbon dioxide pressure exceeds two atmospheres. Recent work has shown that *B. tuberculosis* is particularly sensitive to moderate percentages of carbon dioxide at atmospheric pressure, and it has been suggested that carbon dioxide deficiency in the body promotes tuberculosis, while an excess retards the disease.[19]

Contributions to the literature which show that carbon dioxide at atmospheric pressure may stimulate certain organisms should be mentioned,[20-21] although they are not of direct interest in considering the effect of carbon dioxide at higher pressures.

Many published articles have been prepared for the purpose of exposing bad and unsanitary conditions in the carbonated beverage industry. Bad conditions undoubtedly have been found in some places, and perhaps may exist today, but these articles often imply a general condemnation of carbonated beverages. It should be pointed out that no paper on the sanitary condition of a beverage is a complete and scientific contribution unless it records the carbon dioxide content in volumes of gas per unit volume of beverage or the bottling pressure, and the time that has elapsed between bottling and examination. Stokes,[22] Gershenfeld,[23] and Klein[24] have written articles which are typical of this class. They purchased and examined commercial beverages, and their results show dangerously unsanitary conditions in many cases. They do not, however, specify the amounts of carbon dioxide present, and Stokes does not even state whether the drinks examined were carbonated beverages or uncarbonated beverages. In some instances it is reasonable to assume that the containers may have leaked and a large percentage of the carbon dioxide escaped.

Chemists and bacteriologists generally recognize the ability of carbon dioxide under pressure to inhibit the growth of bacteria and to reduce the count of beverages under practical conditions. This fact is apparent even in statements such as the following conclusion to a report which gave the results of an extensive investigation:[25] "Were it not for carbonation, the count apparently would be enormously high." The inquiries of Haenle,[26] and of Young and Sherwood[27] have also pointed out the reduction in count. The comprehensively valuable report of Koser and Skinner,[28] which came to the writers' attention after their work had been started, shows the shorter viability of *typhosum* and *B. coli* in carbonated than in plain water and attributes the effect of CO_2 to the change in hydrogen-ion concentration. However, their work does not extend to the higher commercial pressures used in the present investigation.

EXPERIMENTAL PART

Some samples of carbonated beverages which, it was known, were produced under very unsanitary conditions, were purchased and bacteriological counts taken thereon. It was found that in almost every instance *B. coli* were absent and the count was remarkably low, taking into consideration the fact that these beverages were of the sweet type and afforded an excellent medium for bacterial growth.

At this time the writers became interested in determining what factors in carbonation produced the reduction in count. A study of the literature showed that other observers had noted the same phenomenon, and that work had been done which indicated that carbonation destroyed bacteria, but that some types were more resistant than others. The present writers also found in the literature patents claiming sterilization by the application of gas under pressure, independent of the

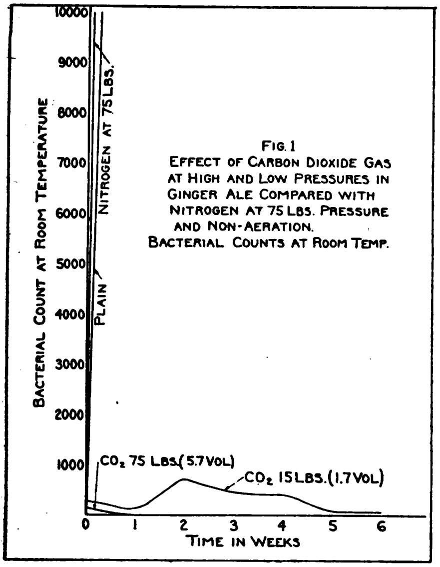

Fig. 1
Effect of Carbon Dioxide Gas at High and Low Pressures in Ginger Ale Compared with Nitrogen at 75 Lbs. Pressure and Non-Aeration. Bacterial Counts at Room Temp.

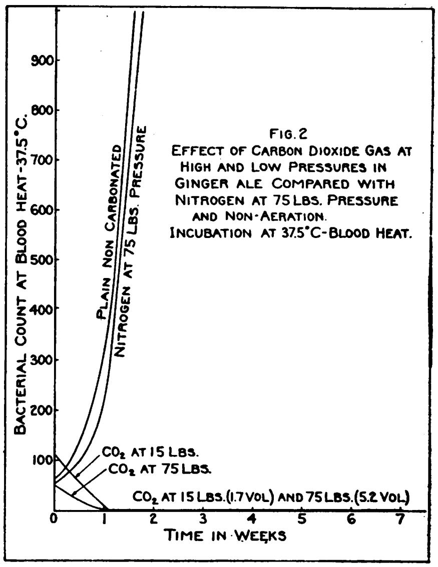

Fig. 2
Effect of Carbon Dioxide Gas at High and Low Pressures in Ginger Ale Compared with Nitrogen at 75 Lbs. Pressure and Non-Aeration. Incubation at 37.5°C-Blood Heat.

type of gas used. This raised the question as to whether the effect was due to the chemical influence of the gas, the pressure, or the combined effect of the pressure and the gas. After considering these points, it was decided to carry out the investigation along practical lines.

Through the courtesy of a manufacturer of carbonated beverages, it was arranged to bottle an ordinary ginger ale under the following conditions: (1) plain without gas; (2) under a high pressure of nitrogen; (3) under a moderate pressure of carbon dioxide; (4) under a higher pressure of carbon dioxide.

No attempt was made to inoculate the syrup, which had been prepared very carefully with filtered and sterilized water in a sanitary plant, according to usual plant procedure. The samples were stored at room temperature of about 70° F., and to prevent leakage were placed head downward in a vertical position. Pressures were taken before the samples were opened, and any sample showing leakage was discarded. The results of counts taken after storage and incubation at room temperature are shown graphically in Fig. 1, while similar experiments incubated at blood heat are recorded in Fig. 2. These results indicate at once that, although the count in the plain beverage was low at the beginning of the tests, the bacteria multiplied rapidly at room temperature of 70° F. and soon became innumerable, the resulting beverage being far from sanitary.

The samples bottled under pressure with nitrogen showed approximately identical results to the plain beverage, thus indicating that the pressure alone had no effect on the bacterial count.

The carbonated beverages, on the other hand, showed no increase in count. On the contrary, their examination revealed a decrease in count, the samples under the higher pressure reacting more rapidly to the effect of the carbon dioxide. Inasmuch as the carbonic acid in solution is proportional to the pressure, the effect on bacteria may be said to be proportional to the amount of carbon dioxide in solution. Comparing Figs. 1 and 2, it will be noted that the effect of the carbon dioxide shows the same general trend at both temperatures of incubation, but the reduction in count is more effective at 37.5° C. It will be noted that at the low pressure at room temperature there was a temporary increase in count followed by a decrease. The practical significance of this point lies in the fact that the effectiveness of the

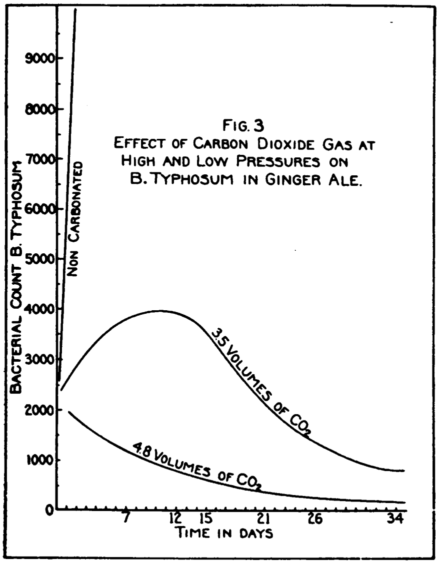

FIG. 3
EFFECT OF CARBON DIOXIDE GAS AT HIGH AND LOW PRESSURES ON B. TYPHOSUM IN GINGER ALE.

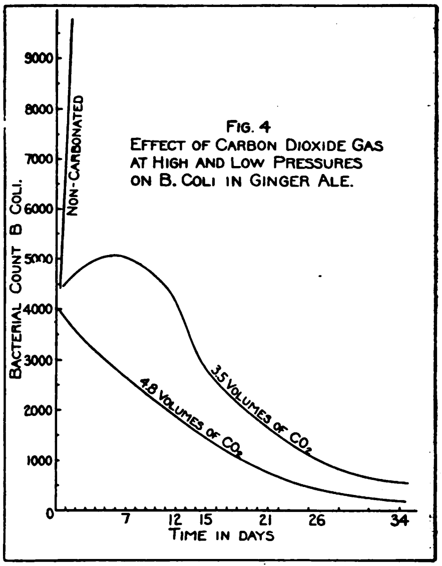

FIG. 4
EFFECT OF CARBON DIOXIDE GAS AT HIGH AND LOW PRESSURES ON B. COLI IN GINGER ALE.

carbonation in reducing bacterial count is greater on the types of bacteria which grow in the human body.

Having thus established that the effect of carbonation in reducing bacterial count was due to the carbon dioxide, and not merely a pressure effect, it was decided to determine the effect of carbonation on certain dangerous types of bacteria which are frequently present in water supplies or find their way into beverages through unsanitary plant conditions. Previous work along similar lines did not deal with commercial beverages, at the higher pressures used in this work.

For this purpose it was arranged to inoculate a ginger ale, similar to that used in the first series of experiments, with bacilli of the colon-typhoid group. The following series of samples were made up:

1. Two dozen bottles of noncarbonated ginger ale inoculated with *B. typhosum.*
2. Two dozen bottles of noncarbonated ginger ale inoculated with *B. coli.*
3. Two dozen bottles of ginger ale carbonated at 70 pounds pressure, equivalent to 4.8 volumes of gas, and inoculated with *B. typhosum.*
4. Two dozen bottles of ginger ale carbonated at 45 pounds pressure, equivalent to 3.5 volumes of gas, and inoculated with *B. typhosum.*
5. Two dozen bottles of ginger ale carbonated at 70 pounds pressure, equivalent to 4.8 volumes of gas, and inoculated with *B. coli.*

The samples as in the first instance were stored at room temperature of about 70° F., a suitable temperature for bacterial growth of the type which would take place in storing the beverages. The bacterial count at blood heat on these samples was taken every week for a period of 5 weeks and a final bacterial count was made at the end of 17 weeks. These results, which appear in Figs. 3 and 4, show that a noncarbonated beverage inoculated with *B. typhosum* or *B. coli* multiplies rapidly in count; and that, if the beverage contains any of these organisms on leaving the plant of the manufacturer, it will rapidly become worse and worse, eventually becoming highly dangerous, if not already so.

Carbonated beverages at the lower pressures showed at first an increase in count, followed by a reduction in count until the beverage approached complete sterilization. It is interesting to note, however, that when the difference between the uncarbonated and low pressure

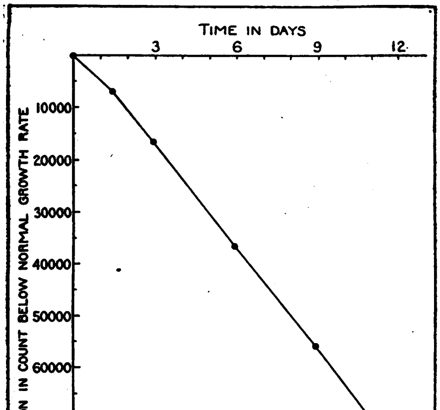

FIG. 5
B. TYPHOSUM IN GINGER ALE BACTERIAL COUNT WITH 3.5 VOLUMES CARBON DIOXIDE COMPARED WITH COUNT IN SAME BEVERAGE UNCARBONATED. (DATA FROM FIG. 2)

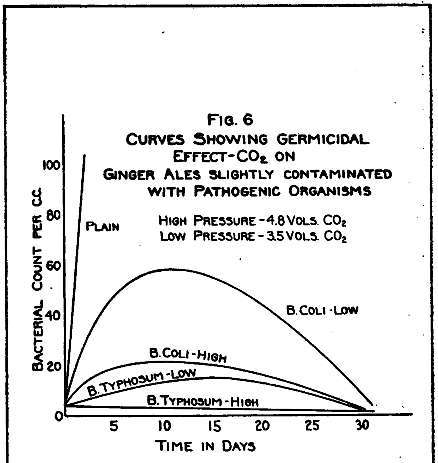

FIG. 6
CURVES SHOWING GERMICIDAL EFFECT-CO_2 ON GINGER ALES SLIGHTLY CONTAMINATED WITH PATHOGENIC ORGANISMS

carbonation curves are plotted, extrapolating the noncarbonated curve, there is obtained a curve the slope of which does not change sign. In other words, while the actual number of bacteria increases for a few days, the lowering of count by the carbon dioxide from the values which would have been reached without carbonation commenced immediately and progressed steadily (Fig. 5).

On the other hand, samples bottled at the higher pressure showed an immediate reduction in bacterial count and a rapid approach to sterilization in agreement with results of previous investigators.[28] *B. coli* showed itself to be more resistant to the effect of carbonation than *B. typhosum.*

The results of the experiments just described were obtained on samples of ginger ale which had been very heavily inoculated, the bacterial count at the beginning of the experiments being very much in excess of any contamination likely to take place under practical bottling conditions. It therefore seemed advisable to corroborate these first results with another series of experiments on beverages inoculated with much lower counts, comparable to normal water contamination. For this purpose another series of samples was made up, including:

1. Noncarbonated ginger ale inoculated with *B. typhosum.*

2. Noncarbonated ginger ale inoculated with *B. coli.*

3. Ginger ale carbonated at 70 pounds pressure, equivalent to 4.8 volumes of carbon dioxide, and inoculated with *B. typhosum.*

4. Ginger ale carbonated at 45 pounds pressure, equivalent to 3.5 volumes of gas, and inoculated with *B. typhosum.*

5. Same as (3), but inoculated with *B. coli.*

6. Same as (4), but inoculated with *B. coli.*

The samples as in previous experiments were stored at room temperature. The results of bacterial counts at blood heat on these samples are shown on the curves in Fig. 6, and confirm the results of earlier experiments. At the lower pressure there is an increase in bacterial count before the decrease commences, and the beverage then gradually approaches sterilization. At the higher pressures there is a slight increase in the *B. coli* before the reduction in count. In the case of *B. typhosum,* however, no increase is found, the decrease commencing immediately. These results confirm the greater resistance of *B. coli* to carbonization, as compared with *B. typhosum.* The very rapid rise in count of the noncarbonated beverages, even at the low inoculation, is especially striking.

The results of these experiments clearly show the marked inhibitive and germicidal properties of carbon dioxide. It is worthy of note that the higher pressure of carbon dioxide had the more rapid and valuable effect.

DISCUSSION OF RESULTS

In the manufacture of carbonated beverages, it is practically impossible to secure a sterile product without heat treatment after the bottle has been sealed. However, in plants operated under rigid supervision and the best sanitary conditions, it is possible to produce a bottled beverage of low bacterial count. Unfortunately, when not carbonated, the average beverage is a particularly suitable medium for accelerated bacterial growth. Consequently there is a rapid growth of bacteria, as indicated by these experimental findings and, as a result, the beverage becomes highly contaminated. If colon-typhoid bacteria are present, as is frequently the case, owing to contaminated water supplies or unsanitary conditions in a bottling plant, the drink becomes highly dangerous due to the multiplication of this type of bacteria. On the other hand, carbonation prevents multiplication of the bacteria and actuallv causes a reduction in count.

The result is that a beverage carbonated and bottled under sanitary conditions, and with sufficient pressure, remains in a sanitary condition and actually improves with storage. If, through some unknown condition, the beverage becomes contaminated with bacteria of a dangerous type or otherwise, there is no multiplication of these bacteria but a gradual sterilization is effected with storage, even under adverse temperature conditions.

It has been pointed out that carbonation at high pressure produces a decrease in count more rapidly than carbonation at a low pressure. The higher pressure used in the experiments described in this paper, namely, 70 pounds, is a practical bottling pressure and is employed in bottling certain high class carbonated beverages. These experimental findings indicate that the effect on bacteria is proportional to the amount used, and therefore it is believed that it would be an advantage to the bottler, as well as an additional safeguard to the public, if this higher carbonating pressure were generally adopted.

As an alternative, beverages may be pasteurized after bottling, but this is not looked upon favorably in the majority of cases because of the effect on flavor and appearance.

It is the opinion of the writers that the bottler should not rely wholly on the effect of carbonation to insure the sanitary condition of his products. On the contrary, carbonation with insufficient gas may result in a dangerous beverage being marketed. Moreover, even where a high degree of carbonation is used, an initially dangerous concentration may still be too high when the beverage is consumed, if it is consumed immediately after bottling.

It is conservative to state that a bottler who bottles his beverages under sanitary plant conditions, using not less than 4.8 volumes of carbon dioxide, can rely upon his products reaching the consumer in even better condition than when they left his plant.

From the standpoint of the individual consumer, especially when one is in a strange vicinity with a doubtful or admittedly dangerous water supply, the chances of immunity from disease are better if one drinks only highly carbonated waters and beverages. The presumptive indication of safety is enhanced in such an emergency if it is known that the beverages have been stored for some time before being consumed.

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A TUBERCULOSIS SURVEY OF PHILADELPHIA

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(*Continued from page 30, January*)

NUTRITION WORK IN THE SCHOOLS

One of the most important measures for the prevention of tuberculosis has been introduced into the anti-tuberculosis campaign only during the last few years. Realizing that tuberculous infection occurs most often in childhood, and that the health of the child in a measure determines the health of the adult, public health workers have emphasized the need of maintaining the child in a state of good health. Not only have these efforts aimed to detect early cases of the disease and to prevent the spread of communicable diseases from one child to another, but more recently an active campaign has been waged to maintain the body resistance at a point where tuberculous infection, particularly, might be warded off. This has led to the establishment of nutrition classes for undernourished children, composed of those children who are found to be 10 per cent or more underweight for their respective age and height. The children are physically examined, and special efforts are made to free them from all physical defects. The children in the nutrition classes usually receive a milk luncheon both morning and afternoon. Sometimes graham crackers are provided, and in other cases, children are requested to bring bread and butter from their homes. Each child is weighed once every week, and the weight is plotted on a special nutrition chart. Attempts are made to interest the mothers, and through meetings and demonstrations, much valuable information has been disseminated concerning the principles of healthy living, and the essentials of an adequate and satisfactory diet. Children are also asked to take extra rest periods in order to build up the resistance of the body.

There has been a large amount of excellent nutrition work in Philadelphia during the last two years, but credit must go to the Philadelphia Health