THE INFLUENCE OF CARBON DIOXIDE ON FOOD-POISONING ORGANISMS

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by

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I. INTRODUCTION

With the exception of the disease known as botulism, most cases of food poisoning result from eating meat infected with certain members of the Salmonella group or an enterotoxic substance elaborated by staphylococci. Such meat may come from an animal infected during life, or from a healthy animal, and be contaminated by contact with rats or mice or through handling by human carriers during the course of processing or preparation for eating.

If living organisms are present, infection may result and cause a slow after-development of symptoms. Even though these organisms may be destroyed during the process of cooking, their toxins are frequently thermostable. Therefore, meat which has been the site of bacterial development, may give rise to sharp outbreaks of food poisoning characterized by acute gastrointestinal symptoms.

From the standpoint of prevention, rigid government inspection has been advocated as the only means whereby such outbreaks may be prevented. It must be remembered, however, that since these organisms are found even in healthy animals, it is highly questionable whether any amount of inspection would entirely eliminate the danger. How great the danger is it is rather difficult to determine. Since the great majority of food poisoning cases are generally of a mild character, and of short duration, they are never heard from beyond the immediate family circle.

Only when the attack is more serious than the average or when a large number of persons are affected simultaneously does knowledge of its occurrence become more widespread. A very small proportion of food poisoning cases receives notice in the public press, a still smaller proportion is reported in the scientific journals, while very few are thoroughly investigated as to their origin.

Be this as it may, the safeguarding of the healthfulness of the public food supply constitutes one of the most difficult yet one of the most fundamental problems of public health. So, in spite of the great advances in scientific methods of food preservation, the food technologist is still confronted with one very important question, namely, what is the effect of the particular method of preservation on the viability of pathogenic organisms which may be present in or upon the food?

During recent years, considerable work has been done by English investigators at the Low Temperature Research Station at Cambridge, England, on the application of carbon dioxide gas as a supplement to cold storage in the preservation of meat and meat products. In this country, most of the work thusfar has been carried out with fruits and vegetables, but at present the method is also being applied to meat, poultry, fish, eggs and dairy products.

According to the literature, it has been conclusively demonstrated that carbon dioxide is a vital factor in the growth of practically all bacteria. In addition, a 10 per

cent concentration of this gas is generally recognized as having a stimulating effect on the growth of such disease-producing organisms as the meningococcus, gonococcus, staphylococcus, the tubercle bacillus, Brucella abortus and the Salmonella group.

Moreover, it has been well established that the toxicity of Staphylococcus aureus is increased by growing the organism in the presence of 10 to 25 per cent carbon dioxide. The presence of 10 per cent carbon dioxide has also been known to stimulate the growth and toxin production of Salmonella aertrycke. Finally, carbon dioxide has been reported to have a preservative effect upon the toxins of pathogenic organisms after they have once been formed thus preventing detoxification through oxidation by molecular oxygen.

From the standpoint of public health, all this is very significant, for it would seem to indicate that the application of carbon dioxide to the preservation of foodstuffs would be an exceedingly dangerous practice inasmuch as it would not only tend to promote the growth of pathogenic organisms but increase the toxin production of staphylococci as well. It should be remembered, however, that all of the studies which have been cited relative to this point were conducted at 37° C.

Nevertheless, there has been a fear on the part of those concerned with the processing of meat and meat products that the introduction of carbon dioxide into the meat storage refrigerator would tend to stimulate the growth and toxin

production of food-poisoning organisms commonly found on meat and thus create a serious health hazard. Therefore, a series of tests was made, using various members of the Salmonella group and staphylococci as test organisms, to determine the effect of carbon dioxide at both high and low temperatures on the viability of these organisms. Special attention was directed to moderate concentrations of carbon dioxide at low temperatures.

II. HISTORICAL

The influence of carbon dioxide on the growth of micro-organisms has been exhaustively studied by a great number of investigators, and in 1928, Valley (14) compiled a most excellent and comprehensive, chronological review of the work which had been done on the subject up to that time. During the 12 years which have elapsed, many valuable additions to our knowledge of the subject have been made especially with respect to the practical application of carbon dioxide in the preservation of foodstuffs.. At present, studies relative to the preserving and tenderizing action of this gas on meat are in progress in this laboratory.

As the result of constant research in this field, the existent literature has assumed vast proportions. Yet no systematic endeavour has been made to present an all-inclusive and up-to-date resume of this material. Therefore, for the sake of completeness, and somewhat for the feeling of making this information more readily accessible to future investigators, the whole subject has been unified and brought up to date. With this thought in mind, both the theoretical and practical aspects of the subject are presented in the following historical review.

A. The Bactericidal Effect of Carbon Dioxide

The first attempt to make a practical use of the antiseptic properties of carbon dioxide was made by Barthell, (1) in 1848, when he patented a process for preserving milk

by means of carbonation. Many years later, Pasteur and his co-worker Joubert (9) (1877) claimed that carbon dioxide had a lethal effect on Bacillus anthracis. This was contrary, however, to the observation of Szpilman (13) (1880) who reported that 5 to 8 hours' exposure to pure carbon dioxide did not kill this organism nor alter its pathogenicity. Likewise, Grossmann and Mayerhausen (5) (1881) maintained that carbon dioxide does not kill bacteria but merely inhibits their motility while minimal amounts accelerate it.

Frankle (3) (1889) studied the influence of carbon dioxide on the growth of various organisms in liquid and solid media. He observed that the true beer yeasts were able to grow as well in 100 per cent carbon dioxide as in air but the majority of the parasitic and saprophytic forms failed to survive. Since these organisms again resumed their normal growth upon subsequent exposure to ordinary air, Frankle concluded the effect was not of a lethal nature but merely inhibitory. Like Frankland, (4) (1889) he emphasized the fact that even members in the same culture (aside from spore forms) may vary in their susceptibility to the action of carbon dioxide.

According to Schaffer and Freudenrich, (12) (1891) broth cultures of typhoid and anthrax bacilli were unaffected by 7 atmospheres of carbon dioxide. Moreover, Sabrazès and Bazin (11) (1893) demonstrated that a concentration of 95 to 97 per cent carbon dioxide at 90 atmospheres pressure and beyond did not prove injurious to Staphylococcus aureus, Aerobacter aerogenes and B. anthracis inasmuch as these organisms

grew unhindered in both plain agar and that containing added buffer.

In a study of the effect of carbon dioxide on members of the colon-typhoid group, Hoffman (7) (1906) obtained sterilization of water suspensions of Escherichia coli and the dysentery bacillus with 50 atmospheres of carbon dioxide in from 2 to 3 hours. Berghaus (2) (1907) considered the effect of carbon dioxide upon various organisms which he subjected to carbon dioxide treatment on freshly poured agar plates at 37°C. He found that Vibrio comma was killed in 24 hours at atmospheric pressure, B. anthracis was inhibited but not killed, while Esch. coli and Salmonella enteritidis were able to develop under a pressure of 2 atmospheres of carbon dioxide. Plummer (10) (1916) observed that the ammonifying bacteria were very insensitive to the action of this gas since a concentration of 60 per cent had no appreciable effect on the rate of ammonification. Although carbon dioxide of less than 40 atmospheres had no effect whatsoever on the organisms studied, Larson et al. (8) (1918) found that when they increased the pressure to 50 atmospheres, they were able to destroy Eberthella typhosa, Esch. coli, Mycobacterium tuberculosis, staphylococci, streptococci and pneumococci in 1½ to 2½ hours. Yeast cells, however, remained unaffected. after 48 hours.

According to Valley and Rettger, (15) (1927) such organisms as Staph. aureus, Salmonella paratyphi and Salmonella schottmuelleri were able to grow equally well both in plain agar and phosphate-buffered media in 95 to 97

per cent carbon dioxide. In buffered media, such delicate organisms as V. comma could develop in atmospheres of carbon dioxide as high as 70 to 75 per cent. More recently, in their experiments with Esch. coli and Eberth. typhosa Guillerd and Lieffrig (6) (1935) have shown that carbon dioxide has no appreciable bactericidal action on water-borne bacteria.

B. Carbonated Waters & Beverages

Much of the earlier knowledge concerning the germicidal value of carbon dioxide was derived from a study of the behaviour of micro-organisms in carbonated waters and beverages. Leone (6) (1886) succeeded in reducing bacterial development in the city water supply of Munich by means of carbonation. Likewise, Hochsetter (3) (1887) reported that V. comma was unable to survive in carbonated waters but Eberth. typhosa remained viable after 5 to 7 days. Scalla and Sanfelice, (7) (1891) on the other hand, demonstrated that pathogens like V. comma, B. anthracis, Staph. aureus and Staph. albus and Eberth. typhosa were unaffected by quantities of carbon dioxide naturally dissolved in water at 15°C. Acid-sensitive organisms like V. comma were injured when the water was saturated with carbon dioxide but the others remained unaffected. Carbon dioxide introduced into soda water under pressure proved injurious to Bacillus subtilus and the spores of this organism as well as those of B. anthracis failed to germinate, but Proteus remained unharmed. In his study of

artificial carbonated waters, Slater (8) (1893) found carbon dioxide had a germicidal or inhibitory effect on Esch. coli, Eberth. typhosa, V. comma and Staph. aureus but the effect varied with the organism in question.

Colin (1) (1915) performed a number of experiments with water suspensions of organisms which he subjected to varying pressures of carbon dioxide. Eberth. typhosa was killed in 20 hours at 10 kilos. pressure, while Esch. coli remained viable after 5 days under 25 kilos. pressure and sterilization of Corynebacterium diphtheriae was obtained in 3 hours at 20 atmospheres. In their studies on the behaviour of the colon-typhoid group in carbonated waters and beverages, Koser and Skinner (5) (1922) found that Esch. coli remained viable in non-acid beverages after 7 days under 28 pounds pressure at 24°C., but agar plates were sterile after 3 days. At room temperature, Eberth. typhosa was destroyed in 24 hours under 24 pounds pressure, but at 1°C. it persisted for 4 days. Sal. schottmuelleri was viable after 48 hours at 24°C. and 23 pounds pressure, and was still alive after 10 days at 1°C. Bacillus mesentericus and B. anthracis spores were unaffected in carbonated water even after 1 month. Donald et al. (2) (1924) have pointed out that the decline in bacterial numbers in carbonated beverages is directly proportional to high pressure and storage. More recently, Kliewe and Kindhauser (4) (1933) have shown that the germicidal action of carbonic acid on water vibrio, colon and typhoid bacilli, and on Breslau, suipestifer and Schottmueller types of paratyphoid organisms,

was enhanced by raising the temperature to 37°C. but was almost entirely absent at 18° to 20°C.

C. The Carbonation of Dairy Products

On the whole, the reduction of microflora by the carbonation of such food products as milk, ice cream and butter has been attended with very little success. Still Hoffman (1) (1906) maintained that he was able to obtain a material reduction of members of the colon-typhoid group after 48 hours by employing 50 atmospheres of carbon dioxide. But this is contrary to the observations of Van Slyke and Bosworth (6) (1907) who claimed that the carbonation of milk without added pressure had no preservative value although lactic acid fermentation was slightly delayed. Likewise, Prucha and his associates (3) (1922) found that carbon dioxide had no germicidal action on Esch. coli or Eberth. typhosa in milk subjected to 10 to 30 pounds extra pressure, while at 20 pounds pressure the bacterial count actually rose from 47 to 153 million organisms per cc. They further showed that carbon dioxide does not cause a reduction of bacteria found in ice cream. Moreover, in their experimental work on carbonated raw milk and ice cream, Valley and Rettger (5) (1927) found that atmospheres of 95 to 97 per cent carbon dioxide under ordinary atmospheric pressure had little or no inhibitive or bactericidal effect.

Hunziker (2) (1924) has shown that butter cannot be successfully preserved by carbonation, while Prucha et al. (4) (1925) state that if the process is to be of any value, the

the product should be stored in an air-tight container.

D. Carbon Dioxide as a Meat Preservative

Kolbe (13) (1882) first observed the preservative action of carbon dioxide on meat; he successfully preserved beef for 18 days at 32°C. in an atmosphere of carbon dioxide, and even after 4 or 5 weeks, there was no evidence of putrefaction, but the meat was unpalatable when cooked. Killefer (12) (1930) studied the effect of carbon dioxide on meat and fish and presented bacteriological data on the reduction of bacterial infection by the use of this gas. His work showed that meat and fish could be kept longer in an atmosphere of carbon dioxide, even at relatively high temperatures, than in air. Although pork and lamb kept at 4.5°to 7.2°C. spoiled after 10 days in air, no deterioration occurred after 3 weeks in carbon dioxide. Similarly, Callow (3) (1932) preserved pork in perfect condition for over 2 months at 0°C. in an atmosphere of carbon dioxide. Even after 70 days, it was more palatable than the fresh product while pork kept in air spoiled in 17 days. He more than doubled the storage-life of mild-cured green bacon at 5°C. by employing an atmosphere of carbon dioxide. Culture medium heavily seeded with pork-spoilage organisms showed no growth at -1°C. in the presence of carbon dioxide after 50 days, whereas in nitrogen and air, there were signs of growth after 4 days. Lea (14, 15) (1933) demonstrated that the development of rancidity in beef fat stored at 0°C. was greatly retarded by 10 percent carbon

dioxide, the time required in a saturated atmosphere for the production of a perceptible off flavor being approximately doubled in the presence of 10 per cent carbon dioxide. This preservative effect was more marked when the meat was placed in an atmosphere of carbon dioxide shortly after slaughter and the humidity reduced to 90 per cent. Empey and Vickery (9,10) (1933, 1934) succeeded in extending the storage-life of chilled beef 40 per cent by employing an atmosphere of 10 to 12 per cent carbon dioxide. These workers found that with but slight initial contamination, chilled beef could be held for 53 days in an atmosphere of 12 per cent carbon dioxide, while beef stored in an atmosphere of 11 per cent carbon dioxide showed no deterioration 44 days after slaughter and the bloom remained unimpaired. Likewise, Coyne (6, 7) (1932, 1933) found that various types of fresh fish could be kept in 20 per cent carbon dioxide as long as 28 days without serious spoilage, while controls stored in air were inedible after 12 to 14 days. He also studied the influence of various concentrations of carbon dioxide (5 to 100 per cent) at 0°, 10°, 25°, and 37° C. on micro-organisms responsible for slime formation on chilled beef. Carbon dioxide suppressed the growth of Achromobacter, Flavobacterium, Micrococcus, Bacillus and Pseudomonas, but had no effect on Aerobacter and Proteus. This is in accord with the findings of Chistyakow (5) (1933) who reported that practically all putrefactive bacteria responsible for the spoilage of meat, fish and other food products were inhibited

by carbon dioxide. Moreover, these observations have been confirmed by Haines (11) (1933) and more recently, by Mallmann and Zaikowski (17) (1940) in this laboratory. Callow (4) (1938), however, reports that pork stored in 10 to 15 per cent carbon dioxide at -10°C. for various periods up to 22 weeks showed considerable loss in weight and appreciable bacterial spoilage.

According to Brooks, (1) (1933) the color and bloom of fresh beef is not affected by concentrations of carbon dioxide below 20 per cent. Nevertheless, Brooks and Moran (2) (1933) have pointed out that the gaseous storage of meat may be limited by the rate of absorption of carbon dioxide. They claim that the commercial use of carbon dioxide requires equilibration for at least 5 days with 100 per cent carbon dioxide before storage in the final 20 per cent mixture. This, however, results in a marked discoloration of the product.

Although gaseous storage at 0°C. practically eliminates mold and bacterial growth in chickens, Lea (16) (1934) has shown that autolysis of the tissues by enzymatic action prevents the extension of the storage-life of undrawn birds. Oxidative rancidity also may be a contributing factor towards spoilage over long periods unless the concentration of carbon dioxide closely approaches 100 per cent. Smith (18) (1934) likewise states that at least 70 per cent carbon dioxide is necessary to retard microbial decomposition in chickens during the minimum storage period of 4 months. In one extensive test

he actually obtained better results by storage in air than in carbon dioxide.

E. Carbon Dioxide as a Mycostatic Agent

Carbon dioxide not only serves as a bactericidal or bacteriostatic agent but it may also function as a mycostat as well. Lopriore (3) (1889) has demonstrated that a 10 per cent concentration of carbon dioxide retarded spore germination of Mucor mucedo, and although pure carbon dioxide caused total inhibition, it had no antibiotic effect even after 3 months. Likewise, Brown (2) (1922) found that carbon dioxide retarded the development of molds responsible for fruit-rot. He observed that the concentration necessary to suppress growth varied with the particular species, that the inhibitive effect was accentuated by lowering the temperature, and with regard to concentrations of carbon dioxide which would be commercially practical, temperature is a more important factor in reducing mold growth than carbon dioxide. At 15°, a moderate concentration of carbon dioxide (10 to 20 per cent), although it does produce an initial retardation, eventually causes an acceleration of fungal growth. According to Moran, Smith and Tompkins, (6) (1932) carbon dioxide in any concentration will suppress the growth of meat-attacking fungi, the rate of growth in 20 per cent carbon dioxide being only 1/2 to 1/5 that in air, inhibition being more marked the lower the temperature. Similar observations were made by Tompkins (7) (1932) who found that the growth rate of various meat-attacking fungi on

artificial media is reduced to approximately 50 per cent of its value in air in 10 per cent carbon dioxide at 0°C. and 100 per cent relative humidity. Andreicha (1) (1936) in studying the combined mycostatic effect of carbon dioxide and high sugar concentrations, found that neither the highest sugar concentrations used in commercial jams and jellies nor an atmosphere of carbon dioxide approaching 100 per cent, alone, can suppress the development of common molds. The combined action of these two preservative agents, however, gave satisfactory results. On 60 per cent glucose media, an atmosphere of 10 per cent carbon dioxide prevented the development of Aspergillus repens and Penicillium glaucum, but lower sugar concentrations required higher carbon dioxide concentrations to arrest growth. More recently, Tompkins (7) (1938) has pointed out that in certain instances, the storing of food in concentrations of carbon dioxide up to 20 per cent may accelerate instead of retard the development of certain molds. Moran (4, 5) (1937, 1939) found that carbon dioxide would retard mold growth on eggs and therefore permit the use of higher humidities in the storage room. At 0°C. and a relative humidity of 85 per cent, 2.5 per cent carbon dioxide eliminates fungal growth, prevents the appearance of a metallic "storage" taste and results in the retention of a firmer yolk, whereas in a saturated atmosphere, the carbon dioxide concentration must not be less than 60 per cent.

F. The Nature of the Inhibitory Action of Carbon Dioxide

Although experimental evidence concerning the inhibitory

effect of carbon dioxide on the growth of micro-organisms has been accumulating for over 90 years, the exact manner in which this gas retards microbial growth still remains unexplained. In this connection Valley and Rettger (7) (1927) observed that bacteria become resistant to the action of carbon dioxide and grow normally if the medium is strongly buffered with phosphates, whereas on ordinary media, growth is suppressed. Therefore, these authors concluded that the antibiotic effect of carbon dioxide must be due primarily to the increased hydrogen-ion concentration of the medium which is deleterious to bacterial growth. Killefer (5) (1930) also concurs with this view and explains the preservative action of this gas on meat on this basis.

This hypothesis has been adversely criticized by Callow (1) (1932) and refuted by Coyne (2) (1932) who found that if the pH of the medium is altered by means other than carbon dioxide to the same amount, bacterial growth is not retarded to the same extent as with carbon dioxide. This is also in agreement with the findings of Tompkins (6) (1932) who obtained a similar result quantitatively with certain fungi. Moreover, Haines (3) (1933) concluded that the change in pH which occurred in nutrient broth sown with Achromobacter comparable with that obtained by using 100 per cent carbon dioxide does not account for the retardation of growth obtained with as low as 10 per cent concentration of this gas.

According to these workers, the inhibitory action of carbon dioxide may possibly be due to the fact that this gas actually penetrates the cell wall thereby causing an alteration

in intracellular pH and consequently has a deleterious effect on the dehydrogenase systems of the cell. This conforms with the view of Jacobs (4) (1920), namely, that since the ability of carbon dioxide to penetrate cell membranes is so great, it owes its physiological effect to hydrogen-ions which act on the interior of the cell. Tompkins (6) (1932) has pointed out that the action of carbon dioxide is like unto that of ammonia which upon dissolving in water produces physiologically active ions. Hence, the presence of this gas affects a living system in two ways: "it influences the uptake of ions and depresses respiration."

G. The Stimulating Effect of Carbon Dioxide

In their natural environment, bacteria are constantly being exposed to carbon dioxide. Even so, ordinary air contains but .03 per cent of this gas and some bacteria require an excess of carbon dioxide above that found in the normal atmosphere for their proper development. So, it has been found that the growth of many fastidious organisms is greatly facilitated by small increments of added carbon dioxide.

Thus, Nowak (10) in 1908, observed that the primary isolation of Bruc. abortus was greatly enhanced when he resorted to the Bacillus subtilis tandem method of cultivation. Although Nowak attributed the success of his technique to the reduced oxygen tension in the cultural environment, subsequent work has shown that it was due to the additional carbon dioxide given off by B. subtilis in the closed system. The partial-tension method was also successfully applied to the primary

cultivation of the gonococcus by Wherry and Oliver, 1916 (17); Reudiger, 1919 (12) and Swartz and Davis, 1920 (16); and to the meningococcus by Cohen and Markle, 1916 (3) and Cohen and Fleming, 1918 (4).

Subsequently, it was found that when the accompanying culture of B. subtilis was replaced by additional carbon dioxide in air the cultures grew quite as well. Therefore, the tandem technique was superseded by direct application of the gas to the cultural environment. Investigators found that the growth of the gonococcus, Chapin, 1918 (2); the meningococcus, Cohen and Fleming, 1918 (4); Kohman, 1919 (7); and Bruc. abortus, Huddleson, 1920, 1921 (5,6); T. Smith, 1924, 1926 (15) and McAlpine and Slanetz, 1926 (9) was stimulated by an atmospheric concentration of 10 per cent carbon dioxide. Likewise, Kulp (8) (1926) observed that the same was true of Lactobacillus acidophilus.

Rockwell (13,14)(1921, 1924) pointed out that carbon dioxide facilitates the growth of the gonococcus, the aerobic bacteria and the facultative group as well. Moreover, Nye and Lamb (11) (1936) observed that 3 per cent carbon dioxide produced by burning a candle in a sealed container, enhanced the growth of the pneumococcus type VIII and therefore advised the growing of all routine cultures in this manner. More recently, Auger (1) (1939) found that .5 to 25 per cent carbon dioxide not only stimulated the growth of all types of pneumococci, but other respiratory pathogens such as Hemophilus influenzae and Streptococcus hemolyticus as well.

H. The Influence of Carbon Dioxide on Toxin Production & Preservation

As early as 1918, Larson and his colleagues (7) reported that Esch. coli as well as other Gram negative bacilli became far more toxic after having been killed by carbon dioxide than the living cultures. Parker et al. (9) (1925) also observed that staphylococci grown in the presence of 10 per cent carbon dioxide produced much more skin toxin than in its absence. This in accord with the findings of Burnet (1, 2) (1929, 1930) who attributed the favorable influence of 20 to 25 per cent carbon dioxide on the toxin production of certain strains of Staph. aureus to the increase in intracellular acidity which favors haemolysin production. Parish and Clark (8) (1932) obtained powerful Staphylococcus toxins from cultures grown in an atmosphere of 25 per cent carbon dioxide. Likewise, Woolpert and Dack (14) (1933) secured lethal, hemolytic and skin-necrosing toxins from several cultures of food-poisoning strains of staphylococci grown under the same conditions. Similarly, Dolman (4) (1934), working with more than 200 strains of Staphylococcus from various clinical sources, found that 85 per cent were highly toxigenic when ingested by human volunteers after having been grown for 40 hours in an atmosphere of 30 to 40 per cent carbon dioxide at 37°C.

On the other hand, Burnet (3) (1931) found that carbon dioxide had a diminishing effect on the toxicity of the typhoid bacillus. This is in agreement with the observations

of Hanks and Rettger (5) (1932) who found it impossible to demonstrate that the growth and toxin production of Salmonella pullorum was materially enhanced by an atmosphere of 10 per cent or 20 per cent carbon dioxide. Herter and Rettger (6) (1937), however, reported that the presence of 10 per cent carbon dioxide increased the potentcy of Sal. aertrycke toxin for mice and rabbits. Plastridge and Rettger (10, 11, 12, 13) (1927, 1929) reported that the growth and toxin production of C. diphtheriae was increased and made more regular when the cultures were aerated with 3 to 10 per cent carbon dioxide. Deterioration of the toxin through oxidation was also prevented.

I. Carbon Dioxide as an Essential Growth Factor

Most of the earlier work dealing with the influence of carbon dioxide on micro-organisms had to do with its antibiotic or bacteriostatic effect on bacteria. But now it is very well known that although excessive amounts of this agent may prove injurious to microbial life, a certain minimal amount is just as indispensable for the sustenance of life in bacteria as it is in plants, the higher animals and man.

Thus, Winogradsky (25) (1890) demonstrated that carbon dioxide is essential for the growth of the autotrophic nitrifying bacteria. This was later confirmed by Bonazzi (1) (1921); Godlewsky (4) (1892, 1895, 1896) and Gowda (5) (1924). Likewise, Wakesman and Starkey (21, 22) (1922) and Starkey (17) (1925) recognized the necessity of carbon dioxide for the sulphur-oxidizing bacteria.

Wherry and Ervin (23) (1918) concluded that carbon dioxide was prerequisite for the growth of the of the tubercle bacillus. Although this conclusion has been disputed by Novy and Soule (8) (1925), it was later sustained by Rockwell and Highberger (14) (1926). Huddleson (6,7) (1920, 1921) and T. Smith (16) (1924) recognized that carbon dioxide was also an essential factor in the growth of Bruc. abortus which is in full accord with the findings of Wilson (24) (1931). Moreover, the carbon dioxide studies of Rockwell and McKann, 1921 (10); Rockwell, 1921, 1923, 1924 (11, 12, 13); Rockwell and Highberger, 1926, 1927 (14, 15); Valley and Rettger, 1925-27, (18, 19, 20) and Gladstone, Fildes and Richardson (3) (1935) have shown that carbon dioxide, in minimal amounts, is a vital factor in the growth of all bacteria.

It appears that the presence of carbon dioxide not only plays an essential role in bacterial development, but also is an important factor in the growth of molds and yeasts. Accordingly, Durrell (2) (1924) observed that Basisporum gallarum spores did not germinate in the absence of carbon dioxide and were in turn stimulated by small amounts of this gas. Rippel and Bortels (9) (1927) likewise noted that Aspergillus niger spores germinated very poorly in the absence of carbon dioxide, while Rockwell and Highberger (15) (1927) demonstrated that carbon dioxide was essential for the proper growth and development of Mucor and Saccharomyces.

J. The Role of Carbon Dioxide in Bacterial Metabolism

Although the experimental evidence has been too inadequate

to warrant the formulation of any conclusions, various hypotheses have been proposed in an endeavour to explain how carbon dioxide produces its essential effect. At first, it was thought that the favorable action of carbon dioxide merely influenced bacterial growth indirectly. Consequently, the stimulating action of carbon dioxide under partial-tension conditions was generally attributed to the displacement of oxygen from the environment resulting in a reduced oxygen tension, Nowak, 1908 (7); Cohen and Fleming, 1918 (1) and Reudiger, 1919 (8).

St. John (11) (1919) and Torrey and Buckell (12) (1924), however, ascribed the beneficial results to the increased moisture content of the closed system. Others were of the opinion that carbon dioxide affected a favorable adjustment in the hydrogen-ion concentration of the medium by the establishment of a buffer system which prevented extreme deviations in pH due to excessive alkalization, Gates, 1919 (2); Kohman, 1919 (6); Sierkowski and Zajdel, 1924 (10).

Later on, however, it became apparent that the function of carbon dioxide was not that of an added stimulus, but that it was directly concerned with the very existence of the bacterial cell itself. Thus, Huddleson (3, 4) (1920, 1921) recognized its importance in the cultivation of Bruc. abortus, while Valley and Rettger (13) (1927) regarded it as satisfying a definite requirement which ordinary air is incapable of meeting. Rockwell and Highberger (9) (1927) concluded that carbon dioxide served as a source of carbon and was the only source which could be so utilized. More recently, Koebs

(5) (1937) has suggested that carbon dioxide probably acts as a respiratory catalyst (hydrogen carrier) in Esch. coli.

III. HISTORY OF CULTURES

All the paratyphoid cultures were originally received from Dr. P. R. Edwards of the Kentucky Agricultural Experiment Station, Lexington, Kentucky. Three of the Staphylococcus cultures, namely, Nos. 169, 168 and 85 were obtained from Dr. G. M. Dack of the Department of Bacteriology, University of Chicago. These strains were all of the hemolytic variety and proved toxin-producers. A fourth culture, a non-toxic strain, was acquired from the department stock culture collection in this laboratory.

IV. EXPERIMENTAL

In order to make a quantitative study of the effect of carbon dioxide on the viability of the organisms under investigation, the following technique was employed throughout all the experiments: Fifty milliliter quantities of sterile Bacto-tryptose, dextrose, phosphate-buffered broth in an Erlenmeyer flask were planted with a known number of organisms. Duplicate cultures of each organism were prepared, and then stored in a Frigidaire ice cream cabinet at temperatures of 3°, 5° and 20° C. respectively. One culture was placed in a compartment and subjected to treatment with carbon dioxide, while the other was held at the same temperature in the absence of the gas to serve as a control.

At the time the cultures were placed in storage, the cotton plugs were carefully removed from the flasks so that the carbon dioxide might have free access to the surface of the medium.

All counts were made on Bacto-tryptose agar after being incubated for 48 hours at 37°C. The cultures were plated out at various intervals for 4 days to 3 weeks.

The carbon dioxide concentrations were automatically maintained and controlled by means of a Carbostat unit manufactured by the White Mfg. Co., St. Paul, Minn. This device automatically checked the gas concentration every 45 seconds and maintained the concentration within a range of plus or minus .3 per cent.

TABLE I

A Comparison of the Effect of 5 Per Cent and 10 Per Cent Carbon Dioxide on the Growth of Staphylococci at 3 to 5 C.

Name of Organism	Treatment	Initial No. of Orgs.	Per Cent Decrease in Numbers 5 Days	12 Days	21 Days
		5% Carbon Dioxide			
Staph. aureus #85	CO_2	118,000	1.70	72.88	88.73
	None	118,000	40.68	79.66	93.73
		10% Carbon Dioxide			
	CO_2	122,000	54.26		92.17
	None	122,000	77.05	91.64	97.62
		5% Carbon Dioxide			
Staph. aureus (Lab.)	CO_2	45,000	74.04	86.66	100.00
	None	45,000	15.55	90.00	100.00
		10% Carbon Dioxide			
	CO_2	157,000	70.06	91.78	97.07
	None	157,000	75.86	94.27	98.06

TABLE II

A Comparison of the Effect of an Atmosphere of 5 Per Cent and 10 Per Cent Carbon Dioxide on Members of the Salmonella Group at 3° to 5° C.

Name of Organism	Treatment	Initial No. of Orgs.	Per Cent Decrease in Numbers 5 Days	12 Days	21 Days
		5% Carbon Dioxide			
Sal. abortus	CO_2	41,000	87.80	*85.37	19.51
	None	41,000	00.00	4.39	51.22
		10% Carbon Dioxide			
	CO_2	29,000	*86.20	*107.00	*70.00
	None	29,000	00.00	*80.00	*80.00
		5% Carbon Dioxide			
Sal. bredeney	CO_2	235,000	20.85	65.96	91.92
	None	235,000	10.64	*21.70	88.94
		10% Carbon Dioxide			
	CO_2	540,000	44.44	20.37	86.85
	None	540,000	50.00	16.70	*831.00
		5% Carbon Dioxide			
Sal. derbey	CO_2	114,000	83.86	87.72	99.04
	None	114,000	62.28	87.72	93.86
		10% Carbon Dioxide			
	CO_2	450,000	94.22	95.55	98.10
	None	450,000	94.67	90.67	49.11
		5% Carbon Dioxide			
Sal. enteritidis	CO_2	221,000	35.75	44.34	76.47
	None	221,000	33.04	59.27	100.00
		10% Carbon Dioxide			
	CO_2	310,000	55.00	13.00	80.97
	None	310,000	32.22	49.36	61.30
		5% Carbon Dioxide			
Sal. gallinarum	CO_2	91,000	3.30	48.35	94.39
	None	91,000	17.58	72.53	80.68
		10% Carbon Dioxide			
	CO_2	99,000	77.77	81.82	79.80
	None	99,000	39.39	77.98	97.38

TABLE II Continued

Name of Organism	Treatment	Initial No. of Orgs.	Per Cent Decrease in Numbers 5 Days	12 Days	21 Days
		5% Carbon Dioxide			
Sal. muenchen	CO_2	400,000	49.75	74.25	99.12
	None	400,000	40.00	71.50	98.70
		10% Carbon Dioxide			
	CO_2	1,170,000	60.00	84.62	90.00
	None	1,170,000	67.52	*131.00	*308.00
		5% Carbon Dioxide			
Sal. suipestifer	CO_2	65,000	41.54	58.50	95.85
	None	65,000	*42.45	49.23	73.85
		10% Carbon Dioxide			
	CO_2	330,000	89.40	91,82	95.76
	None	330,000	66.66	48.50	65.45
		5% Carbon Dioxide			
Sal. typhimurium	CO_2	230,000	57.39	76.52	89.51
	None	230,000	41.74	25.65	42.18
		10% Carbon Dioxide			
	CO_2	410,000	66.00	70.73	83.66
	None	410,000	00.00	47.32	93.17

* Denotes per cent increase in numbers.

TABLE IIa

A Comparison of the Effect of an Atmosphere of 5 Per Cent & 10 Per Cent Carbon Dioxide on Salmonella pullorum at 3° to 5° C.

Name of Organism	Treatment	Initial No. of Orgs.	Per Cent Decrease in Numbers 7 Days	14 Days	21 Days
		5% Carbon Dioxide			
Sal. pullorum	CO_2	97,000	54.64	91.96	100.00
	None	97,000	67.01	95.05	100.00
		10% Carbon Dioxide			
	CO_2	64,000	57.81	91.87	94.06
	None	64,000	73.44	95.78	100.00

TABLE III

The Effect of 10 Per Cent Carbon Dioxide on the Growth of Toxin-Producing Staphylococci at 5°C. **

Name of Organism	Treatment	Initial No. of Orgs.	Per Cent Decrease in Numbers 7 Days	14 Days	21 Days
Staph. aureus #169	CO_2	86,000	58.14	56.98	56.98
	None	86,000	41.86	83.72	83.72
Staph. aureus #168	CO_2	350,000	81.14	67.43	*717.00
	None	350,000	60.00	73.43	100.00

* Denotes per cent increase in numbers.
** The cultures listed were tested at 10% CO_2 only.

TABLE IV

The Effect of 10 Per Cent Carbon Dioxide on Members of the Salmonella Group at 5°C. **

Name of Organism	Treatment	Initial No. of Orgs.	Per Cent Decrease in Numbers 7 Days	14 Days	21 Days
Sal. give	CO_2	330,000	39.12	36.36	54.24
	None	330,000	*35.30	24.24	39.12
Sal. kentucky	CO_2	121,000	60.33	70.25	15.70
	None	121,000	42.15	62.81	53.71
Sal. london	CO_2	190,000	31.58	60.53	70.00
	None	190,000	71.05	79.47	90.00
Sal. minnesota	CO_2	150,000	*20.00	*13.33	84.66
	None	150,000	*13.33	56.70	75.00
Sal. montevideo	CO_2	300,000	36.66	86.66	90.00
	None	300,000	30.00	16.66	43.33
Sal. newbrunswick	CO_2	280,000	14.29	*3.57	*232.00
	None	280,000	7.14	*71.86	*1356.00
Sal. newport	CO_2	590,000	*9.08	87.29	96.27
	None	590,000	61.00	80.51	91.87
Sal. panama	CO_2	140,000	64.29	75.75	75.75
	None	140,000	*51.73	37.14	75.73
Sal. senftenberg	CO_2	400,000	60.00	85.25	91.50
	None	400,000	70.00	80.00	83.75
Sal. worthington	CO_2	180,000	83.89	100.00	93.11
	None	180,000	83.11	83.33	95.51

* Denotes per cent increase in numbers.
**The cultures listed were tested at 10% CO_2 only.

TABLE IVa

The Effect of 10 Per Cent Carbon Dioxide on Members of the Salmonella Group at 5°C. **

Name of Organism	Treatment	Initial No. of Orgs.	Per Cent Decrease in Numbers 5 Days	12 Days	21 Days
Sal. bareilly	CO_2	270,000	33.33	48.14	96.00
	None	270,000	55.00	74.82	84.08
Sal. california	CO_2	720,000	12.50	27.77	95.70
	None	720,000	30.00	36.00	50.00
Sal. cerratum	CO_2	540,000	57.41	78.52	92.22
	None	540,000	76.00	76.00	51.85
Sal. choleraesuis	CO_2	140,000	61.43	80.72	97.72
	None	140,000	13.00	62.86	92.14
Sal. newington	CO_2	450,000	64.44	80.44	51.11
	None	450,000	57.78	80.21	84.22
Sal. oranienberg	CO_2	320,000	25.00	95.93	90.94
	None	320,000	62.50	43.75	65.63
Sal. paratyphi A	CO_2	310,000	25.80	58.71	86.13
	None	310,000	61.30	81.62	43.90
Sal. schottmuelleri	CO_2	320,000	92.50	95.63	100.00
	None	320,000	83.73	63.44	65.63

** The cultures listed were tested at 10% CO_2 only.

TABLE V

The Effect of a 10 Per Cent Concentration of Carbon Dioxide on the Growth of Staphylococci and Members of the Salmonella Group at 18°-20° C. after 4 Days.

Name of Organism	Treatment	Initial No. of Orgs.	Fold Increase in Thousands
Staph. aureus #169	CO_2	22,000	36.4
	None	22,000	5.9
Staph. aureus #168	CO_2	17,000	15.1
	None	17,000	5.9
Staph. aureus #85	CO_2	11,000	28.6
	None	11,000	63.6
Staph. aureus (Lab.)	CO_2	1,300	66.2
	None	1,300	461.5
Sal. aertrycke	CO_2	98,000	9.0
	None	98,000	3.9
Sal. enteritidis	CO_2	291,000	45.7
	None	291,000	23.4
Sal. muenchen	CO_2	135,000	31.6
	None	135,000	3.6
Sal. oranienberg	CO_2	94,000	45.8
	None	94,000	37.2
Sal. paratyphi A	CO_2	171,000	21.1
	None	171,000	24.6
Sal. schottmuelleri	CO_2	22,000	86.4
	None	22,000	295.5
Sal. senftenberg	CO_2	9,000	211.1
	None	9,000	68.9
Sal. suipestifer	CO_2	35,000	15.7
	None	35,000	27.7

V. DISCUSSION & CONCLUSIONS

Table I shows the results obtained by exposing 2 strains of Staph. aureus to atmospheres of 5 per cent and 10 per cent carbon dioxide at 3° to 5°C. It will be observed that the bacterial count of both strains was approximately the same in the presence and in the absence of carbon dioxide at both concentrations of this gas. At the end of 5 days' exposure there was a reduction of 2 to 77 per cent. After 12 days, the number of viable organisms had reduced 73 to 90 per cent, while at the end of 3 weeks, this reduction approached 100 per cent.

The effect of concentrations of 5 per cent and 10 per cent carbon dioxide on various species of Salmonella at 3° to 5°C. is presented in Tables II and IIa. In general, both the air and the carbon dioxide cultures showed a progressive decrease in numbers throughout the period of examination. The trend of development at the end of 3 weeks showed a reduction of approximately 80 to 90 per cent.

The influence of a 10 per cent concentration of carbon dioxide on 3 toxin-producing strains of Staph. aureus at 5°C. is represented in Table III. These results show that the organisms decrease in numbers at the same rate regardless of the presence or absence of carbon dioxide.

Tables IV and IVa show the trend of development of other representative members of the Salmonella group in an atmosphere of 10 per cent carbon dioxide at 5°C. Although on the whole, the trend shows a progressive decrease in

numbers both in the presence and absence of carbon dioxide, here again, the reduction in count shown by the carbon dioxide cultures, parallels that of the controls.

In analyzing these data, it should be remembered that each individual sample or strain cannot serve as an ultimate criterion as to the behaviour of a particular organism under the conditions which obtain. Nevertheless, when these data are examined as a whole, it is evident that in general all the organisms investigated showed a marked reduction in count irrespective of the presence or absence of carbon dioxide. Neither is this all, but this decrease becomes progressively greater the longer the period of exposure. From this it may be concluded that low temperatures not only have a bacteriostatic effect upon bacteria but produce an antibiotic effect as well. On the other hand, since there was no essential difference between the reduction in numbers which occurred in the presence of carbon dioxide and that which took place in its absence, it may also be inferred that carbon dioxide has no supplementary effect on low temperatures in either reducing or increasing the development of food-poisoning organisms.

Furthermore, if the bacterial population of Salmonella and Staphylococcus tends to reduce in numbers during storage at 3° to 5° C., even though the added presence of carbon dioxide had a tendency to enhance toxin production or prevent detoxification of the toxin once it had been formed, it would have no significance as far as creating a health

hazard is concerned. For, the very essence of toxin production consists in an increase in the number of viable organisms, so that toxin production and reproduction, roughly at least, parallel each other. Therefore, we may conclude that if food is kept at 5°C. or less the danger from food poisoning is relatively slight since the causative agents will not grow in food at such low temperatures. Finally, these data indicate that the application of carbon dioxide atmospheres as a supplement to refrigeration does not create any additional health hazard in foods stored under these conditions.

Table V represents the effect of an atmosphere of 10 per cent carbon dioxide on various members of the Salmonella group and several strains of Staphylococcus at 18° to 20° C. In contrast to the results obtained at low temperatures, from this table it is evident that all species showed a marked increase in numbers both in the presence and in the absence of carbon dioxide. This was in accordance with anticipation based on the fact that the rapidity of microbial development is determined among other things by temperature. On the whole, it is also apparent that proliferation occurred more readily in an atmosphere of 10 per cent carbon dioxide than in its absence.

In this connection it is interesting to recall the observations of other workers already cited pertaining to the effect of carbon dioxide at higher temperatures on organisms commonly incriminated in food poisoning. As early as

1893, Sabrazès and Bazin (1) demonstrated that Staph. aureus grew well in the presence of carbon dioxide in both plain agar and that containing added buffer. This was later confirmed by Valley and Rettger (2) (1927) who also showed that Sal. paratyphi and schottmuelleri were able to grow equally well in both plain agar and phosphate-buffered media in an atmosphere of 95 to 97 per cent carbon dioxide. Although these workers used solid media and employed a much higher gas concentration than was used in this investigation, the trend in either case was the same.

If carbon dioxide has a general tendency to stimulate the growth and reproduction of food-poisoning organisms at higher temperatures, then it must follow that it enhances their toxicity as well. From this we may conclude that it would be inexpedient to introduce carbon dioxide into food storage containers under conditions where high temperatures prevailed, for it would be bound to create a serious health hazard. The main conclusion arising from this phase of the investigation, however, is that it is wise to keep all food, especially animal food from the time of slaughter until its final preparation at a relatively low temperature.

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