1950

Storage of meat in carbon dioxide atmospheres at temperatures above freezing

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STORAGE OF MEAT IN CARBON DIOXIDE ATMOSPHERES AT TEMPERATURES ABOVE FREEZING

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Food Technology-Bacteriology

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Iowa State College
1950

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I. INTRODUCTION

The problem of maintaining quality of meat from the time that the animal is killed until the meat is eaten by the consumer is one that only partially has been solved. This problem arises largely from the fact that meat serves as an excellent medium for the growth of many kinds of microorganisms. Meat preservation, then, is based primarily on the control of microbial growth. This concept has been stated forcefully by Jensen (52, p. 4):

> Reduced to its very fundamentals, the manufacture and subsequent handling of food products may be considered as a race between man and microbes to see which will be the first to consume such materials. Practically every process employed in the packing industry hinges upon the reduction of microbial growth and is within the province of the bacteriologist--refrigeration, pickling, smoking, curing, certain condiment treating, heat processing, canning, rapid transportation, "prompt handling", and aseptic procedures.

Such processes as freezing, canning and extreme desiccation, although they effectively restrict development of microorganisms, are expensive and lower acceptability of the products. Consequently, the bulk of production is marketed as fresh or cured meat. The recent trend toward pre-packaged, self-service meat seems likely to accentuate the spoilage problem by increasing possibilities of mishandling.

Satisfactory preservation involves factors in addition to microbial control. The ideal preservative would extend the storage life of meat

without introducing health hazards or detracting from acceptability; it should not serve as a substitute for quality. Furthermore, it should be economical and adaptable to commercial application. The likelihood of finding a "perfect" preservative is probably slight. However, it appears that carbon dioxide, when applied in the storage atmosphere as an adjunct to refrigeration, can delay microbial proliferation and also conform reasonably well to the other criteria mentioned above.

The effectiveness of carbon dioxide in prolonging keeping time of meat has received some attention in the past. Considerable experimental work has been carried out on a variety of meat products with favorable results. In addition, there has been a notable practical application. During a five-year period just prior to World War II, large quantities of unfrozen beef were shipped from Australia and New Zealand to England under atmospheres containing CO_2. This beef arrived at its destination in excellent condition (76). Previous to that time, such shipments of meat without freezing had not been feasible.

The principal objective of the present work is to study the influence of carbon dioxide on rate of growth of spoilage organisms on certain meats as evidenced by changes in organoleptic properties and numbers of microorganisms present. The effect of CO_2 on the acceptability of meat also receives some attention. In addition, the effect of temperature, relative humidity and initial microbial load in keeping time are considered as well as the effect of temperature and initial load on the inhibitory properties of CO_2.

Cut-up chicken and frankfurters were used in the work reported here primarily because of their availability. It is believed that findings obtained for these products generally will be applicable to other meats. Chicken represents fresh meat while the frankfurter represents a cured, comminuted meat. Because of the considerable differences in composition, organoleptic properties and microbial flora of these two products, results are presented in separate sections.

Although the work does not contain a specific study of methods of applying carbon dioxide to meat storage, some of the points brought out may be helpful in that connection.

II. REVIEW OF LITERATURE

A. General

Early studies on applications of carbon dioxide storage and the effect of CO_2 on microorganisms seem to have been carried out largely by German investigators. The first work found on the storage of meat in CO_2 atmospheres was that of Kolbe (59) in 1882. Later, very thorough investigations were undertaken by the Food Investigation Board of the Department of Scientific and Industrial Research of Great Britain in the decade between 1930 and 1940, and outstanding work was carried out in Australia at about the same time. Very little work of this nature has been done in the United States.

The only sizeable review which applies specifically to this problem was that of Fisk (25), and he omitted or gave very limited treatment to several important aspects of the problem. It would seem, then, that a reasonably comprehensive literature review is indicated at this time.

B. Microbiology of Stored Meats

The importance of the role of microorganisms growing on the surfaces of meat stored at refrigerator temperatures above freezing was recognized many years ago. For instance, Glage (31) in 1901, described the rapid growth of closely related species of bacteria on the surfaces

of meat held at low temperature. In 1908, Richardson and Scherubel (85) noted that pieces of meat began to decay or mold on the surface rather quickly, whereas the progress of interior decomposition was slow.

1. Microorganisms of fresh meats

a. Bacteria. Glage (31) isolated a number of species of "Aromobakterien" from the surfaces of meat stored at low temperature and high humidity. He found that the surface of the meat became covered with tiny drop-like colonies which grew in size and finally coalesced to form a slimy coating. Accompanying the growth of these organisms was a characteristic aromatic odor which he considered rather pleasant in the early stages. Growth was best on the cut muscle, although it occurred to some extent on the fat and connective tissue; the surface of the meat was not peptonized and the bacteria did not penetrate into the meat. Slimy meat fed to rats and mice did not produce sickness.

Glage considered that he had seven species, one of which predominated. The bacteria were oval to rod shaped with rounded ends and occurred occasionally in chains. They were motile, very aerobic, liquefied gelatin slowly, and turned litmus milk alkaline; they grew well at 2° C. but poorly at 37° C., and the optimum temperature was thought to be at 10 to 12° C. On fresh meat these organisms produced a grey coating which later became yellow. Judging by Glage's description, it seems likely that the "Aromobakterien" were closely related to bacteria reported as Achromobacter by Haines (35) and several other investigators and as Pseudomonas by Ayres et al. (1).

Brewer (4) studied the bacteriological flora of various types of meat purchased from small markets. He ground cuts of meat and made plate counts from dilutions of the ground meat. Only a few samples of each type of meat were tested; and the counts varied tremendously. Brewer concluded that prepared meats usually contained more bacteria than fresh meats, that smoked meats contained a much smaller number of bacteria than fresh meats and that the colon group predominated among the types of bacteria encountered. The last conclusion seems questionable in view of the fact that numbers of coliform bacteria were determined solely on the basis of colony appearance. He could not account for the fact that numbers of colonies on plates incubated at 20° C. were usually much larger than the numbers on plates incubated at 37° C.

Prescott et al. (83) reported that the formation of a slimy coating with disagreeable odor on beef in cold storage was due to an almost pure culture of a bacterium capable of growth at temperatures slightly above freezing. Apparently these bacteria were not studied further.

Haines (34) described the rather rapid spoilage of a quarter of beef stored at 0° C. by an organism of the Pseudomonas group. Haines (36), in a study of the bacterial flora of some slaughterhouses, found that somewhat less than half of the bacteria were cocci and the remainder were rods, the majority of the latter being soil types. Of the bacteria found in the air, 9 per cent from a "good" slaughterhouse were intestinal types as compared with 19 per cent in a "poor" one.

A detailed study of bacteria forming slime on the surface of chilled

beef was made by Haines (35). The organisms which made up the slime were almost entirely members of the Achromobacter group. A number of organisms belonging to the genera Pseudomonas and Proteus were also found. About 120 strains of Achromobacter were studied and grouped on the basis of cultural morphological and physiological characteristics. Bergey's Manual (3rd edition, 1930) was the standard reference for this work.

In studies of the initial flora of Australian beef, Empey and Vickery (24) observed that 95 per cent of the bacteria capable of growth at -1° C. consisted of several types of Achromobacter while the remainder were species of Pseudomonas and Micrococcus. During storage the relative numbers of Achromobacter and Pseudomonas increased at the expense of the micrococci.

In further studies on chilled beef Empey and Scott (23) investigated the initial contamination acquired by beef surfaces during dressing and found that more than 97 per cent of the organisms viable at 20° C. were bacteria. Usually less than 1 per cent of the microorganisms growing at 20° C. were viable at -1° C., and at -1° C. yeasts and molds made up a greater share of the population. Four principal genera were found among the bacteria viable at -1° C., viz., Achromobacter, 90 per cent; Micrococcus, 7 per cent; Flavobacterium, 3 per cent; and Pseudomonas, less than 1 per cent. Empey and Scott decided that most of the organisms comprising the initial flora on beef surfaces had come from the hide and hair of slaughtered animals.

Mallman et al. (70) isolated bacteria from the surface of fresh and stored beef. They made no mention of storage conditions for this beef or

whether any of it had reached or approached the slime stage before isolations were made. The genera and number of species represented among the isolates follow: Micrococcus 22, Flavobacterium 15, Bacillus 12, Achromobacter 9, Diplococcus 1, Gaffkya 1, Staphylococcus 1, Bacterium 3, Sarcina 1; total 65.

The slime and intestinal contents of the haddock were found to contain largely species of Achromobacter by Stewart (99). In addition, organisms of the genera Micrococcus and Flavobacter and on a few occasions Pseudomonas were encountered frequently in the slime. Of 247 cultures isolated from the haddock slime, 140 were considered to be Achromobacter and 40 Micrococcus. The Achromobacter were classified into 15 types on the basis of biochemical activities and one of these contained seventy per cent of the cultures. These organisms were described as Gram-negative, non-motile coccobacilli occurring in pairs and short chains, producing no change in litmus milk and gelatin, and variable as to nitrate reduction and fermentation of glucose. None of these types could be identified with species of Achromobacter listed in Bergey's Manual; however, some types were considered to be related to the following species: Achromobacter fermentationis, A. pellucidum, A. multistriatum, A. geniculatum, A. pestifer, A. venosum, A. ravenoli, A. litoralis, A. guttatum, and A. dendriticus. Haines (35) in his study of Achromobacter from slimy beef also was unable to identify any of his types with species listed in Bergey, although he mentioned that two of them resembled Achromobacter multistriatum.

The development of a surface slime with accompanying off-odor is a very common cause of spoilage with poultry held at low temperatures above

freezing. Mallman (69) investigated slipperiness of young ducklings and found the chief slime-forming organism to be a spore-forming capsulated bacillus closely resembling B. mesentericus.

Lochhead and Landerkin (68) stored dressed poultry (presumably New York dressed) at temperatures of 30° and 32° F. They observed that the first odor was due to the development of bacteria on the skin surface, and that surface spoilage was far advanced before there was any notable increase of bacteria in the muscle. They listed the predominant bacterial types as Achromobacter (3), Micrococcus (2) and Flavobacterium (1).

Gunderson et al. (32) studied 186 cultures obtained from the surface of freshly killed chicken. The bacterial genera found and the relative numbers were: Micrococcus 50, Alcaligenes 38, Achromobacter 34, Paracolobactrum 13, Flavobacterium 9, Proteus 7, Bacillus 7, Pseudomonas 6, Staphylococcus 5, Microbacterium 4, Eberthella 3, Sarcina 2, diphtheroids 2, Gaffkya 2, Streptococcus 1, Corynebacterium 1, Neisseria 1. Also numerous Escherichia and Aerobacter were observed. From eviscerated poultry which had been stored frozen were obtained Escherichia 3, Aerobacter 1, Micrococcus 7, Alcaligenes 3, Achromobacter 10, Paracolobactrum 4, Flavobacterium 1, Proteus 1, and Staphylococcus 1.

A variety of bacteria were isolated from the skin of fresh and defrosted, frozen cut-up chicken by Ayres et al. (1). Among the genera found were Pseudomonas, Micrococcus, Achromobacter, Flavobacterium, Alcaligenes, Proteus, Bacillus, Sarcina, Streptococcus, Eberthella, Salmonella, Escherichia, and Aerobacter. However, when pieces of chicken were stored at low

temperatures to incipient spoilage it was found that the surface flora consisted almost entirely of closely related bacteria which were shown to belong to the genus Pseudomonas. Again it was not possible to make species identification using Bergey's Manual (sixth edition, 1949), but types were found which were similar in many respects to Pseudomonas ochracea, Ps. geniculata, Ps. mephitica, Ps. putrefaciens, Ps. sinuosa, Ps. segnis, Ps. fragi, Ps. multistriata, Ps. pellucida, Ps. desmolyticum, and Ps. pictorum. Aside from Pseudomonas, Gram-negative cocci were occasionally found in appreciable numbers and on rare occasions Flavobacterium on pieces of chicken which had reached or approached the sliming stage. The cocci were very inert biochemically, and their role in the spoilage of chicken was not determined; it was also impossible to identify them.

Several investigators reported that obvious deterioration first became evident at a definite level or range of bacterial numbers on the meat surface. Haines (35) reported that slime formation on beef became apparent when the number of bacteria per square centimeter of beef surface was $10^{7.5}$. The corresponding number given by Empey and Vickery (24) was 5×10^{7}. According to Scott (90) the slime point for beef with normal water content was 10^{8} or slightly greater. Schmid (89) gave a surface count of 5×10^{7} to 10^{8} as indicating the limit of saleability for beef. Lochhead and Landerkin (68) first observed odor from New York dressed poultry when the numbers of bacteria per square centimeter of skin were in the range 2.5×10^{6} to 10^{8}. Ayres et al. (1) with cut-up chicken observed off-odor at about 10^{8} and slime formation at 10^{8} to 10^{9}.

b. Molds. The early literature on mold spoilage of meat stored at low temperatures was reviewed by Brooks and Hansford (6). They found that the important mold species causing deterioration of cold-store meat coming to England from the southern hemisphere were Cladosporium herbarum (the cause of "black spot"), Thamnidium chaetocladioides, Thamnidium elegans, Mucor racemosus, Mucor mucedo, Mucor lusitanicus, Penicillium expansum, Penicillium anomalum, and Sporotrichum carnis.

The most common mold genus growing on beef stored at -1° C., according to Empey and Scott (23), was Penicillium, followed by Mucor, Cladosporium, Alternaria, Sporotrichum, and Thamnidium in that order. Empey and Vickery (24) reported that the largest part of the mold development on sides of chilled beef was caused by the species Penicillium expansum. Ayres et al. (1) reported finding Penicillium and Oospora on fresh cut-up chicken, and Gunderson et al. (32) also encountered Oospora on dressed poultry.

c. Yeasts. Descriptions in the literature of yeasts causing deterioration of chilled fresh meats were infrequent. A study of cold-store meat reaching the English market made by Brooks and Hansford (6) revealed that important yeasts were Saccharomyces sp., Torula botryoides, and Wardomyces anomala. Yeast genera found occurring in the initial contamination of Australian beef by Empey and Scott (23) and found to be viable at -1° C. were Mycotorula, Candida, Geotrichoides, Blastodendrion, and Rhodotorula.

Gunderson et al. (32) obtained Torula from the surface of commercially eviscerated poultry, and Ayres et al. (1) isolated Rhodotorula and Cryptococcus from cut-up chicken.

2. Microorganisms of cured meats

The microbiology of cured meats apparently has not been studied to the extent of that of fresh meats. It seems reasonable to expect a somewhat different flora than that encountered on fresh meats. Products which have been smoked or cooked generally should be free of all organisms but sporeformers; microbial growth developing during storage is largely due to contamination after processing. In order for microorganisms to develop on cured meats they must be able to tolerate the considerable quantities of salt usually present. One would expect only organisms capable of proliferation at low moisture contents, such as molds and some yeasts, to grow on dried sausages.

a. Bacteria. Maurel (71, 72, 73) isolated cocci repeatedly from the surface and interior of sausages. He found that these were usually diplococci and very similar in their characteristics to staphylococci. They were slightly pathogenic for rabbits whose resistance had been lowered. Maurel made no mention of the conditions under which the sausages had been stored. His incubation temperature (36° C.) probably eliminated the possibility of isolating microorganisms responsible for slime at low temperature.

Roderick and Norton (88) isolated micrococci quite consistently from slimy frankfurters and succeeded in reproducing slime on sterilized sausages with pure cultures of these organisms. A number of strains were differentiated on the basis of biochemical reactions. Most of these had no pigment although a few were yellow or orange in color. They were aerobic

and grew best on animal matter; very few were proteolytic. Gelatin rarely was liquefied and none was capable of peptonizing milk; a few formed a curd in milk. Most of the strains fermented one or more sugars, almost always without gas production. These organisms were able to tolerate salt in higher concentrations than could be used in sausages. They were killed by heating in water for 10 minutes at 60° C., and were destroyed on the sausages in the usual cooking process. Roderick and Norton concluded that slimes on sausages were caused by cocci, that sausages were infected after heat processing, and that condensation of water on the sausage surface was conducive to the spread of these bacteria.

According to Jensen (52, p. 181) almost any saprophytic bacterium was capable of producing slimes on sausage surfaces under the right conditions. However, Niven (81) thought that growth on frankfurters was largely limited to Gram-positive varieties.

Ingram (49) isolated yellow micrococci and several types of bacilli and lactobacilli from slimes on bacon. He also exposed plates of fresh pork containing various amounts of salt to the atmosphere of a cold storage room in which bacon was kept. When the concentration of salt was less than 4 per cent, he obtained Pseudomonas almost entirely. From 4 to 25 per cent salt the bacterial flora consisted largely of yellow micrococci.

Garrard and Lochhead (29) isolated Gram-negative cocci or coccoid rods from fresh pork sides on a nutrient agar containing salt. They stated that these were similar to bacteria found by Landerkin (unpublished work) to be the most numerous bacteria on 5 per cent salt agar plates from slimy

bacon. These unidentified bacteria were of interest because of their pronounced lipolytic activity. Of six types described, four reduced nitrate, five were lipolytic, two produced hydrogen sulfide, none liquefied gelatin, and none produced acid in dextrose, sucrose or lactose. They were non-motile, non-chromogenic and non-spore-forming.

b. Yeasts. Kühl (60) found a white yeast in practically pure culture on slimy sausage. The fermenting power of this yeast was unusually slight.

Mrak and Bonar (78) discovered that the pasty slime on several samples of wiener sausage was composed of a mixture of yeasts and bacteria. All of the yeast isolates were similar and were considered to be Debaryomyces Guilliermondii var. nova zealandicus Lodder. Growth was obtained on sausage from 4° to 31° C. and was heavy from 10° to 28° C.; the optimum temperature range was 22° to 28° C. The yeast tolerated high concentrations of salt, was weakly fermentative and liquefied gelatin in giant colonies after 30 days' incubation at 20° C.

c. Molds. Yesair (112) made a study of molds isolated from sausages and various cured meat products. A number of mold species were obtained from the surfaces of sausage, bacon and ham and identified as Penicillium expansum, Aspergillus glaucus, Aspergillus clavatus, Aspergillus niger, Mucor racemosus, Rhizopus nigricans, Alternaria tenuis, Monascus purpureus, and Monilia sitophila. All of the molds studied and their spores were killed within five minutes at a temperature of 140° F. Yesair gave the standard temperature for sausage dry rooms as 55° F. and standard relative

humidity as 70 per cent. These conditions did not prevent growth of molds.

3. Mode of action of CO_2 on microorganisms

From the beginning, investigators have tried to account for the inhibition of certain microorganisms by carbon dioxide. Early workers ascribed the effect either to reduction of hydrogen ion concentration or reduction of oxygen tension. Subsequently these theories were repeatedly shown to be untenable, and other possible mechanisms were suggested.

Frankel (26) emphasized the fact that CO_2 was not an inert gas, and that facultative and anaerobic as well as aerobic species were inhibited by 100 per cent levels. He concluded that the effect of CO_2 could not be explained on the basis of oxygen removal. Brown (14) found that mold growth was independent of oxygen concentration within wide limits, and Tomkins (102) observed that the influence of CO_2 on the growth of molds was also independent of oxygen concentration within wide limits. Coyne (18), Moran et al. (77) and Callow (15) proved that the substitution of nitrogen or hydrogen for carbon dioxide did not give comparable results.

Killeffer (58) and Valley and Rettger (105) thought that the effect of CO_2 on microbial growth was due to lowering of pH. Killeffer noted that the pH of meat was changed but slightly by 100 per cent CO_2; he explained that only a thin surface layer might be affected by the gas. Valley and Rettger found that pure cultures of bacteria which were most inhibited by CO_2 on an artificial medium were also most affected when

the pH was lowered by other means. By increasing the buffering capacity of the medium, they reduced the amount of inhibition caused by a given concentration of CO_2. Callow (15) objected to the findings of Valley and Rettger on the basis of their own data. He pointed out that in 18 of 27 cases, their cultures developed less in CO_2 than in air on media of the same pH.

In a study of the effect of CO_2 on meat-attacking molds, Tomkins (102) adjusted the pH of the medium to the same and lower values than those caused by the CO_2 concentrations used. The retardation in growth caused by CO_2 was much greater than that due to pH alone. Essentially the same results were obtained on artificial medium by Coyne (20) with bacteria obtained from fish slime and by Haines (37) with bacteria from slimy beef. Haines noted that 20 per cent CO_2 changed the initial pH of nutrient broth from 7.4 to 5.8. When the pH of broth was adjusted to 5.8 before inoculation, the rate of growth in air was very little different from the rate in broth of pH 7.4.

Tomkins (102) reported that CO_2 retarded both germination and growth of molds, whereas lower pH retarded growth but not germination. He considered that the effect of CO_2 on living systems was more like that of ammonia than that of other inhibiting gases and vapors, and attached significance to the fact that both produce physiologically active ions when dissolved in water.

Coyne (20) called attention to the fact that some of the cells of a

presumably pure culture were influenced less by CO_2 than others, a phenomenon which had been noted many years earlier by Frankel (26) and Frankland (27). Coyne attributed these differences to variations in cell permeability. He suggested that the permeability of cells to CO_2 might allow an internal pH which did not depend on the pH of the medium. He also suggested that CO_2 within the cell could interfere with some enzyme system. Briggs (5) discussed the influence of CO_2 on the uptake of ions by plant cells.

Jacobs (50) observed that saturated solutions of carbon dioxide were much more toxic to toad tadpoles than were solutions of hydrochloric, oxalic, salicylic, formic, acetic, or butyric acids of the same pH. Furthermore the toxicity was retained when the solution was made neutral with sodium bicarbonate. The greater toxicity of CO_2 over other acids was ascribed to its ability to penetrate living cells. An alkaline bicarbonate-carbonic acid solution was found to give a sour taste, perhaps due to the rapid penetration of CO_2 into the taste buds.

The action of CO_2 solutions on the color of flowers of Symphytum peregrinum which contain a natural indicator dye was studied by Jacobs (51). The internal pH obtained did not depend on the pH of the solution, but was apparently determined by the penetration of CO_2 molecules into the cells. After several hours, however, equilibrium apparently was attained between the cell contents and the outside environment.

Haines (37) thought that the influence of CO_2 on microbial growth might depend on its action on dehydrogenating enzymes. Preliminary experiments showed promise, but apparently this work was never finished.

It was shown by Kidd (56, 57) that CO_2 retarded germination of plant seeds by depressing aerobic and anaerobic respiration. The amount of inhibition was influenced by temperature and CO_2 concentration.

C. Effect of CO_2 on Microorganisms

A survey of the pertinent literature revealed the following facts:

(a) Microorganisms vary in their susceptibility to CO_2.

(b) The concentration of CO_2 employed determines its effectiveness within limits.

(c) Such factors as temperature, microbial load, and the amount of moisture and nutrients available affect the influence of CO_2 on the organism.

Although these factors are not independent of one another, they will be considered separately for convenience.

1. Effect of CO_2 on bacteria

Although Kolbe (59) recognized that the meat he stored under CO_2 kept better because of interference with the normal development of microorganisms, he did not conduct any studies on the organisms involved. Other investigators in the latter part of the 19th Century showed that various species of bacteria were affected by carbon dioxide. Their findings were reviewed by Frankel (26), who was the first to carry out extensive studies on the effect of CO_2 on bacteria. Pure cultures were streaked on the surface of gelatin agar in small flasks and stored under a stream of pure CO_2. The extent of growth was compared with that in control

flasks containing air. Frankel divided the many species studied into five groups on the basis of the resistance shown to CO_2. In the first group he placed those species which seemed capable of growing in pure CO_2 about as well as in air; in the second, those which developed in CO_2 but whose growth was more or less inhibited. The third group contained species which did not develop in CO_2 at room temperature but did at 37° C. In the fourth and fifth groups he placed the species which failed to grow in pure CO_2, the difference being that those in the fourth group were not killed and grew when subsequently exposed to air, whereas those in the fifth group were killed by the exposure to CO_2. The great majority of the saprophytic species was found to belong in the group which did not grow in pure CO_2 and yet was not killed by it. Frankland (27) confirmed the results of Frankel on several species of bacteria.

Carbon dioxide was found by Valley and Rettger (105) to be of small value in destroying or inhibiting bacteria in ice cream and in milk. Christyakov (17) studied the effect of CO_2 on 37 different kinds of putrefactive bacteria and found that only five were unaffected.

Coyne (18, 20) investigated the influence of CO_2 on the growth of bacteria isolated from fish slime by Stewart (99). One loopful of a broth culture was used in inoculating each plate with seven successive strokes. Plates were incubated in desiccators in which the desired concentrations of CO_2 had been obtained by evacuation and subsequent addition of the necessary quantities of CO_2 and air. For controls similar inoculated plates were

incubated in air under the same conditions. A scoring system based on the amount of growth along the various lines of inoculation was used for determining the effectiveness of CO_2. Various concentrations of CO_2 ranging from 5 to 100 per cent were employed. It was found that CO_2 generally inhibited growth, but that the amount of inhibition varied widely with the kind of organism used. In some cases complete inhibition was obtained while in others it was almost negligible. Types of Achromobacter, Flavobacterium, Micrococcus, Pseudomonas, and Bacillus were markedly inhibited at 0° and 10° C. and some effect was noticeable at 25° and 37° C. On the other hand Aerobacter, Proteus and closely related genera were only slightly affected by CO_2. Coyne concluded that the use of 20 per cent CO_2 in conjunction with storage at a temperature of 0° C. would almost completely inhibit the growth of those bacteria responsible for the spoilage of fresh fish.

The effect of CO_2 on the rate of growth of strains of Proteus, Pseudomonas and Achromobacter isolated from meat was studied by Haines (37). Bacteria were grown in flasks of nutrient broth, and the cultures shaken continuously during experiments. CO_2-air mixtures in the requisite proportions were passed through the flasks and over the surfaces of the medium. Air was supplied to the control flasks. The entire apparatus was held in a constant temperature room. At suitable intervals samples were taken aseptically for dilution and plating in order to follow the increases in numbers in the cultures. Carbon dioxide concentrations of 10 and 20 per

cent and temperatures of 0°, 4° and 20° C. were used. Haines observed that CO_2 in these concentrations had little action on Proteus, but increased the lag period and lengthened the generation time by about one-half for Pseudomonas and Achromobacter at 20° C. At 0° C. the generation times were more than doubled by 10 per cent CO_2; no benefit was obtained by increasing the CO_2 level to 20 per cent. However, there was a difference in the effects of 10 and 20 per cent CO_2 at 4° C. The maximum number of cells obtained was the same with or without CO_2, the difference being in the time required to attain this maximum.

Scott (92) inoculated thin slices of beef muscle with pure cultures of organisms known to grow well on meat surfaces at low temperature. Strips were held at -1° C. in desiccators some of which contained atmospheres having 10 per cent CO_2 and some with atmospheres of air for controls. The humidity was adjusted to 99.3 per cent so that the water content of the meat would be normal (300 per cent water on a dry weight basis). (The experimental techniques employed are discussed more extensively on page 35 of this review.) For two types of Achromobacter the rates of growth in 10 per cent CO_2 were 0.40 and 0.46 of the rates in air. For a Pseudomonas culture the rate of growth in CO_2 was 0.25 of that in air.

Mallmann et al. (70) studied the action of CO_2 concentrations of less than 10 per cent on organisms isolated from meat. Beakers containing sterile nutrient broth were inoculated in duplicate with a known number of organisms and stored at 3.5° to 5° C. One culture of each set was placed in a chamber containing CO_2; the other in a chamber with air. Covers on

the beakers were kept loose to allow free communication with the atmospheres. CO_2 concentrations employed were 10, 5 and 2.5 per cent and several species each of Flavobacterium, Achromobacter, Micrococcus, and Bacillus were tested. The data were presented as per cent increase or decrease in numbers of bacteria after 14 days. In general, 10 per cent CO_2 always gave a reduction in numbers, 5 per cent gave a small reduction or a small increase and 2.5 per cent allowed some increase, but not nearly as much as did 0 per cent. Carbon dioxide appeared to have less effect on Flavobacterium and Achromobacter then on Micrococcus and Bacillus. The authors concluded that 10 per cent CO_2 was the minimum amount that would give a reduction in the bacteria responsible for meat spoilage, and that 2.5 per cent CO_2 would make possible a longer storage period for meat and a possible increase in humidity of the storage rooms.

The finding that 10 per cent CO_2 would effect a reduction in organisms of this type is not in agreement with the results of other investigators (20, 37, 92). It is difficult to evaluate this work since neither the numbers of organisms initially present nor the numbers present after the two week storage period are given. In addition, the source of the cultures used is not clear (particularly the age and condition of the beef and the temperature at which it was stored), so it is impossible to tell whether the organisms tested were actually capable of rapid growth and slime production under the conditions of the experiments. Then, too, one wonders how accurately conclusions can be drawn for the storage of beef from work carried out with broth cultures. Haines (35) has obtained fairly good

agreement for the rate of growth of Achromobacter in broth cultures and on beef surfaces in the absence of CO_2. The generation time for the growth of a mixed flora on beef surfaces at 0° C. was about one-third longer than the generation time calculated for a pure strain of Achromobacter in broth at the same temperature. Scott (92) found the ratio of the growth rate of Achromobacter sp. in air to growth rate in 10 per cent CO_2 at -1° C. using strips of beef. His results were similar to those observed by Haines (37), who tested a like organism in broth under comparable conditions.

The effect of carbon dioxide on food poisoning organisms was investigated by Wallmann et al. (70) using the same experimental methods described above. A number of cultures of Staphylococcus and of Salmonella were held under atmospheres containing 0, 5 and 10 per cent CO_2 at a temperature of 3.5° to 5° C. (38.3-41° F.). It was found that in all cases the low temperature gave a considerable decrease in numbers in air and that the amount of reduction was not changed significantly by the presence of CO_2. The conclusion was reached that use of CO_2 as a supplement to refrigeration did not create any new health hazards.

2. Effect of CO_2 on molds

The effect of CO_2 on various fruit-rot molds including Botrytis, Fusarium and Alternaria has been studied by Brown (14). He found that germination and growth were retarded by carbon dioxide. The extent of growth was measured by determining the average length of the germ tubes,

the percentage of spores germinating on glass slides and the diameter of colonies on agar plates. The concentration of CO_2 which prevented germination of spores at ordinary temperatures was about 20 to 30 per cent when spores were sown in water and about 50 to 60 per cent when they were sown in a good nutrient medium.

Tomkins (102) observed the action of CO_2 atmospheres on molds known to cause deterioration of meat. He inoculated spores at points in agar plates which were stored at various temperatures from 0 to 20° C. and with CO_2 concentrations from 0 to 30 per cent. At suitable intervals either the extent of germination was determined microscopically or the colony diameters were recorded. In general, CO_2 was found to increase the latent period of germination, to reduce the rate of elongation of germ tubes and to decrease the rate of growth of colonies; the percentage of spores eventually germinating was not changed. It appeared that a greater reduction in growth rate was brought about by earlier increments of CO_2 than by later ones. Growth was retarded more by CO_2 than was germination. The mold species used in the study were Thamnidium elegans, Thamnidium chaetocladioides, Mucor mucedo, Penicillium expansum, Sporotrichum carnis, and Cladosporium herbarum. At temperatures up to 20° C., 30 per cent CO_2 almost completely prevented growth of all but Sporotrichum on artificial media. At 0° C., 10 per cent CO_2 reduced growth rate to about 50 per cent of the value in air.

Pieces of lean beef were sprayed with a spore suspension of Thamnidium chaetocladioides by Moran et al. (77), and stored in gas-tight jars at some unspecified low temperature with atmospheres containing 0, 20, 40,

60, 80, and 100 per cent CO_2. Although mold growth appeared in 8 days without CO_2, none of the samples stored under the various CO_2 concentrations showed mold growth on the 19th day of storage. With 20 per cent CO_2 some yeast growth appeared on the 18th day. The experiment was repeated with CO_2 levels of 0, 4, 8, and 12 per cent. The corresponding times to the first appearance of mold were 9, 11, 15 and >19 days.

In a third experiment Moran et al. inoculated plates of nutrient agar at single points with spores from pure mold cultures. The three mold species tried were Thamnidium chaetocladioides, Mucor mucedo, and Cladosporium herbarum. Plates were stored at CO_2 levels of 0, 10, 20, and 30 per cent and at temperatures at 5 degree intervals from 0 to 20° C. The time taken for each colony to reach a diameter of 0.5 cm. was recorded since this was regarded as the size of colony first clearly visible on a meat surface. At all temperatures and with all of the molds an increase in CO_2 concentration of 10 per cent caused a considerable reduction in the rate of growth.

3. Effect of CO_2 on yeasts

Frankel (26) observed that beer yeasts grew about as well in CO_2 as in air. Scott (92) investigated the influence of 10 per cent CO_2 at -1° C. upon three species of yeasts belonging to the genera Geotrichoides, Candida and Mycotorula. These species, which previously had been found capable of multiplication on beef at low temperatures, were grown on thin muscle slices in the experiment. The rates of growth in 10 per cent

CO_2 for Candida, Geotrichoides, and Mycotorula were 0.46, 0.55 and 0.83 respectively of the rates in air with muscle of normal moisture content.

B. Other Variables Concerned

As was previously mentioned the effectiveness of carbon dioxide in retarding growth of microorganisms depends not only on the concentration and the susceptibility of any particular organism to the gas, but also upon other conditions. In general it appears that any factor which tends to delay growth will enhance the growth-retarding properties of carbon dioxide. This contention has been stated in an interesting manner by Brown (14) "... the carbon dioxide retarding factor has greatest effect when the energy of growth is small". The next section of this literature review will be devoted to a detailed consideration of those elements which affect the "energy of growth".

1. Temperature

a. Effect of temperature on microorganisms. There are numerous isolated examples of the growth of particular bacteria at low temperatures in the literature, but few systematic studies of the common saprophytes over the whole range of their viability. Much of the earlier work was reviewed by Glage (31), Horowitz-Wlassowa and Grinberg (46) and Haines (39).

Haines (39) estimated the rate of growth of a number of common bacteria at the following temperatures: 37, 20, 15, 10, 5, 0, -1, -2, -3, and -5° C. Pure cultures of bacteria were streaked on the surface of nutrient agar in petri dishes and incubated at the various temperatures. The time taken

for growth to appear on the first two or four streaks was recorded. The agar remained unfrozen at all temperatures above -5° C. unless special steps were taken to bring about separation of ice. At -5° some plates froze and others remained supercooled. From the results obtained Haines divided the commonly occurring bacteria into four groups as follows: Group 1--the staphylococci, not growing below 10° C.; Group 2--most strains of B. coli, B. proteus and micrococci, ceasing to grow in the range 5 to 0° C.; Group 3--some strains of B. proteus etc. capable of growth at 0° C.; Group 4--many strains of Achromobacter, Pseudomonas, and various yeasts growing rapidly at 0° C. (sometimes in five days), and down to about -5° C. on unfrozen media. In no case was bacterial growth observed on frozen media below -3° C.

Horowitz-Wlassowa and Grinberg (46) isolated microorganisms from cold storage rooms at -2.5° C. by exposing plates of media to the air, and tested the isolated cultures for ability to grow at 0, -3 and -5° C. To prevent freezing, three per cent NaCl was incorporated with the medium for -3° C. experiments and five per cent for -5° C. A number of species were found to grow at 0 and -3° C. and some at -7° C. These included many cocci and spore-forming rods, some yeasts and a great number of molds.

Haines (34) followed the rate of growth of microorganisms on the surface of a stored carcass of lamb held frozen at -5° C. He found that there was first a decrease in numbers followed by an increase, due mainly to the growth of yeasts and molds. At -10° C. no increase in numbers

occurred. Haines observed an increase in soluble nitrogen in beef stored at 0° C. indicating that appreciable changes occurred due to growth of microorganisms. This led him to the belief that rapid cooling would extend storage life not only by slowing bacterial growth but also by decreasing the rate of production of soluble nitrogenous matter which constituted a better food supply for organisms than native protein.

Haines stated that the freezing point of muscle in full rigor was about -1° C. Meat was considered "chilled" when it was stored between 0° and -2.5° C. Of course there was slow ice crystal formation in the lower part of the temperature range for chilled meat. Frozen meat was generally stored at -5° C. and below.

The rate of growth at several temperatures of a pure strain of Achromobacter from slimy beef was studied by Haines (35). He obtained a generation time of 1.3 hr. at 20° C., 6.6 hr. at 4° C. and 9.1 hr. at 0° C. in nutrient broth. For the growth of a mixed flora on the lean surface of beef at 0° C. he calculated an "average" generation time of 12.8 hr.

Brooks and Hansford (6) found that some strains of Cladosporium herbarum would develop from spores at a temperature of -6° C. and would eventually give considerable growth. Torula botryoides, Sporotrichum carnis, Penicillium expansum and Thamnidium sp. developed slightly at this temperature and readily at 0° C. Mucor sp., Saccharomyces sp. and Wardomyces anomala did not develop at -6° C. but grew at 0° C. All of these species were known to attack cold-store meat.

The rate of growth of Sporotrichum carnis on Czapek's agar at temperatures from -10 to 30° C. was investigated by Haines (33). He obtained good growth at -5° C. and some growth at -7° C. on supercooled agar, but under no conditions was there growth on frozen agar during two months of incubation. Since growth as measured by the increase in length of the germ tubes was found to be logarithmic after the initial lag phase, the slopes of the growth curves plotted against temperature indicated the relation between temperature and the rate of growth during the logarithmic phase. The shape of the curve indicated that at -10° C. growth on supercooled agar became infinitely slow.

Temperature undoubtedly has a great effect in determining the types of microorganisms which will develop on meat surfaces. Haines (35) made plate counts at 37, 20, 4 and 0° C. from surface slime appearing on beef stored at about 0° C. The count at 37° C. was less than 0.1 per cent of the count at 20° C. and counts at 4 and 0° C. were about one-third of the count at 20° C. The organisms isolated from the 37° plates were mainly those of the groups Micrococcus and Proteus while at the other temperatures of incubation the organisms were largely or entirely Achromobacter.

Empey and Scott (24) in studying the initial contamination acquired by beef surfaces during dressing found that 99 per cent of the organisms viable at 20° C. were bacteria. Usually, less than 1 per cent of the population was viable at -1° C., and the percentages of yeasts and molds were greater. Statistical treatment of data on microbial populations obtained from beef, hides, etc. showed that the percentage incidence of

organisms viable at -1° C. decreased during summer, and also showed a decrease with decreasing geographical latitude. In other words the proportion of the contaminating organisms viable at -1° C. was higher when the environmental temperature was lower.

The effect of low temperatures on the growth of microorganisms has been summarized by Haines (38, 39) on the basis of his own experiments and those of others. The lower limit of growth of microorganisms on unfrozen media is between -5 and -10° C. and probably is close to -7° C. Bacterial growth seldom occurs on frozen tissue below -3° C., although yeasts and molds grow down to about -7° C.

Haines considered the inability of some organisms to grow on frozen substrates at temperatures at which they would otherwise be able to grow to be due to a reduction of moisture content of the medium caused by ice separation. Moran (74) studied the amounts of ice present in mammalian muscle at temperatures below freezing. At -3° C., 70 per cent of the water of muscle was present as ice, at -5°, 82 per cent, and at -10°, 94 per cent. Walter (108) by means of osmotic pressure determinations on various organisms measured their ability to extract water from their environments. He found that bacteria could exert a maximum "sucking pressure" of 52 atmospheres while for certain molds the value was 220 atmospheres. This phenomenon was cited to explain the reason that molds are better able to grow on frozen substrates than are bacteria.

Various investigations of the effect of temperature on the growth rates of organisms responsible for spoilage of meat in cold storage quite

generally have shown that they grow most rapidly at temperatures in the neighborhood of 20 to 25° C. Horowitz-Wlassowa and Grinberg (46) suggested that the term "psychrophile" be used for cold tolerant organisms and that those which grew only at low temperatures be known as "psychrobes". Evidence from their own work and the work of others convinced them that there were no true "psychrobes". They found that a species which had been held at low temperature would grow more rapidly at a given low temperature than would the same organism after holding at room temperature. Previous passage at 0° C. appeared to augment the ability of an organism to grow at -3° C., and previous passage at 0° and -3° enabled species to grow at -7° which had not grown there originally. Serial transfers at a given low temperature also improved the rate of growth.

Scott (91), on the other hand, believed that three strains of Achromobacter, a strain of Candida and a strain of Geotrichoides which he studied, were truly "cold-preferring". This concept was based on the fact that the ratio of the rate of change in growth to the rate of change of temperature became constant at less than 15° C. and attained its maximum value below 10° C., and also on the fact that the maximum cell production occurred in the region of 10 to 15° C.

Several investigators have discussed the application of the Arrhenius-Van't Hoff equation to microbial growth. Layne-Claypon (61) and Crozier (21) believed that the equation held, whereas Haines (33) and Fulmer and Buchanan (28) thought it did not. Scott (91) contended that, although the equation did not hold over the entire temperature range of growth, it

was valid for a range of sub-optimal temperatures. This question will be considered in more detail later.

Schmid (89) and Scott (91) observed that a decrease in temperature improved the inhibition resulting from a given reduction of relative humidity on the growth of microorganisms on meat surfaces.

b. Effect of temperature on CO_2 activity. Frankel (26) first observed that many species which would grow in CO_2 at their optimum temperatures failed to grow when the incubation temperature was reduced. According to Haines (37) 10 per cent CO_2 lengthened the generation times of Pseudomonas and Achromobacter by about one-half at 20° C.; at 0° C. 10 per cent more than doubled the generation times. Coyne (20) found that the inhibitory action of CO_2 on the bacteria responsible for the spoilage of fresh fish was always greater at temperatures below the optimum for growth. Wallman et al. (70) concluded that CO_2 atmospheres of 10 per cent had no significant effect upon staphylococci and paratyphoid organisms at either 3.5 - 5° C. or at 18 - 20° C.

Brown (14) reported that the retarding action of carbon dioxide on germination and growth of various molds was more marked at lower temperatures. From a study of the effect of CO_2 on meat-attacking molds Tomkins (102) found that the relative retarding effect of any given CO_2 concentration was greater at lower temperatures and that lower concentrations could inhibit growth at lower temperatures. Moran et al. (77) studied the growth rates of meat-attacking molds at various temperatures and CO_2 levels. Their data indicated that a decrease in temperature increases the effect-

iveness of CO_2. For example, the ratio of the time required for Thamnidium chaetocladioides to attain a colony diameter of 0.5 cm. in 10 per cent CO_2 to the time required in air is 1.3 at 20°, 1.7 at 15°, 1.8 at 10°, 2.5 at 5°, and 3.0 at 0° C.

2. Relative humidity

Although it long has been recognized that the humidity of the storage atmosphere influences the storage-life of meats and other foods, this subject has received much less critical attention than has the effect of temperature. Glage (31) and Richardson and Scherubel (86) recorded soon after 1900 that high humidity aided the formation of a microbial slime on meats and that desiccation served as a preventative. Another 30 years passed before fundamental studies were undertaken on this matter.

a. Effect of relative humidity on microorganisms. As was previously mentioned, Walter (108) calculated the "sucking" or "swelling" pressure of various organisms by osmotic pressure measurements. He obtained maximum values of 52 atmospheres for bacteria and 220 atmospheres for species of Penicillium and Aspergillus. Theoretical calculations indicated that the minimum relative humidities for growth were 96 per cent for bacteria and 85 per cent for molds. Microscopic examination of pure cultures of various microorganisms in equilibrium with different vapor pressures confirmed the theoretical values. Walter believed that the limiting humidities were not affected by temperature.

Tomkins (101) observed the rates of germination and growth of several

mold species at various relative humidities and temperatures. At any given temperature, the rate of growth decreased as the humidity decreased. For any one mold species there was an optimum temperature which gave the most rapid rate of growth, and the further the temperature was removed from the optimum the slower was the growth rate. As the temperature was changed from the optimum, the range of humidities allowing growth became narrower. For instance, with Alternaria citri (temperature optimum near 30° C.) Tomkins obtained growth down to 83.8 per cent relative humidity at 30° C., 85.8 per cent at 25°, 87.6 per cent at 18°, 90.8 per cent at 10° C. and 94.2 per cent at 5° C. The availability of nutrients improved the rate of growth and increased the range of humidity and temperature within which growth was possible.

Schmid (89) stored small pieces of meat under different levels of temperature and humidity with the objective of determining conditions which would give best keeping time with minimum weight loss. He emphasized that the common belief in the meat industry, that spoilage of stored meat by bacterial surface growth would occur only when the dew point was exceeded, was incorrect. Schmid interpreted his results as showing that a reduction of 1° C. in storage temperature would allow an increase in relative humidity of about 5 per cent. He suggested that higher humidities be used at a temperature of 0° C. to reduce weight losses, but thought that the highest permissible relative humidity was 90 per cent. Schmid considered that the relationship between bacterial development and relative humidity could be expressed approximately by an exponential function.

Scott (90) criticized the work of Schmid on the grounds that the vapor pressure of the muscle was not in equilibrium with that of the salt solution over which it was stored. He maintained that the relative humidity at the surface of the meat would be higher than that of the surrounding atmosphere because of diffusion of moisture from the interior to the surface. Scott avoided this difficulty by equilibrating thin slices of beef muscle with sulfuric acid solutions of various concentrations in desiccators before studies of the growth of microorganisms were undertaken. After equilibration, the moisture contents (per cent of dry weight) of sections of muscle strips were determined. Fresh beef muscle was found to be in equilibrium at 99.3 per cent relative humidity, at which humidity the mean water content was 300 per cent. (Brooks (10) obtained an equilibrium value of 99.4 per cent relative humidity for fresh frog muscle.) Some of the mean water contents obtained at other humidities were as follows: 99.0 per cent relative humidity, 245 per cent; 98.0 per cent relative humidity, 145 per cent; 97.0 per cent relative humidity, 105 per cent; 95.0 per cent relative humidity, 75 per cent; 90 per cent relative humidity 46 per cent. (These figures give some indication of the enormous weight losses that may be obtained from small pieces of meat by reduction of relative humidity.)

Scott inoculated muscle strips with pure cultures of several types of bacteria and yeasts known to grow on beef surfaces at low temperature. Growth rates under the several conditions of storage were followed by means of plate counts. The organisms used were two strains of Achromobacter, two of Pseudomonas and one each of the yeasts Geotrichoides,

Candida and Mycotorula. A bacterial slime was produced at relative humidities of 99 per cent and above, when the number of cells per square centimeter of surface was 10^8 or somewhat greater. At 98 per cent relative humidity growth became visible in the same range, but the colonies did not coalesce to form a continuous slime. At relative humidities of 96.5 and 97 per cent, the numbers of bacteria per square centimeter sometimes reached values of 10^9 without growth becoming visible to the naked eye. Yeasts produced slime at 99 per cent relative humidity when their numbers were between 2×10^6 and 10^7. When the relative humidity was 97 and 98 per cent, small transparent discrete nodules formed and at 96 per cent these became opaque white. The characteristic spoilage odor was more pronounced at high humidity.

Limiting levels of humidity, below which growth did not occur, were approximately 96 per cent for Achromobacter, 98 per cent for Pseudomonas and 90 per cent for yeasts. Moderate reductions in humidity did not change the generation time of bacteria appreciably, but did extend the lag phase; however, near the limiting humidity generation time was also extended. Scott concluded that development of bacteria on stored beef could be restricted by maintaining the water contents of surface tissues below 90 per cent (equivalent to a relative humidity of 96.5 per cent at the meat surface), and that desiccation was not a practicable method for inhibiting yeast growth on beef.

Some difficulty was experienced by Scott (92) in applying the conclusions reached above to the storage of beef quarters. He described slow growth of Achromobacter at -1° C. on muscle having a surface water

content as low as 70 per cent. It was found that the bacteria were actually growing in tissue of greater water content; about 20 per cent of the final population (3 X 10^9 per sq. cm.) was 2 mm. or more beneath the surface. Scott also suggested that the activity of water in surface tissues did not fall in accordance with the measured water contents at the surface since it had been indicated previously that crystalloids migrate from the surface to deeper layers during drying.

In a later study Scott (91) found that, at temperatures of 2 and 4° C., the critical water contents for the genus Achromobacter differed little from the values determined at -1° C., although at 4° C. a relative humidity of 96 per cent did not completely inhibit growth.

Haines (35) observed that when small pieces of beef were stored over a sulfuric acid solution giving a relative humidity of 70 per cent, the resulting keeping-time was little better than that obtained with beef stored over water at the same temperature (0° C.). Haines and Smith (40) stated that small lean pieces of meat held at 70 per cent relative humidity lost 9 per cent of their weight in three weeks and acquired a very undesirable appearance, yet the rate of growth of bacteria on the surfaces was only retarded by about one-tenth as compared with the growth rates of bacteria on pieces of meat stored with 100 per cent relative humidity.

Roderick and Norton (88) noted that low humidity and adequate air circulation were important in preventing the development of micrococcus slimes on frankfurters. Yesair (112) stated that growth of molds in sausage dry rooms could not be prevented by any practicable combination of temperature, humidity and air circulation.

The importance of air movement, in addition to relative humidity, has been recognized by several investigators. Haines and Smith (40) used the term "drying power" to denote the combined effect of these two factors. They recognized that accurate measurement of air movement in cold storage is very difficult, but suggested that drying power be evaluated by measurement of the weight lost from a block of 20 per cent gelatin of known area in a given time. Haines and Smith expressed drying power of the air in terms of grams of weight lost by a gel block per 100 sq. cm. of surface per day. These authors stated that small pieces of lean meat with cut surfaces could not be dried enough to prevent growth of microorganisms without prohibitive weight losses. In the case of sides or quarters of beef, in which the covering of fatty connective tissue hindered diffusion of moisture from the interior, effective drying of the surface was possible.

The general conclusions of Haines and Smith are supported by Scott and Vickery (93). The latter noted that, when sides of beef were being cooled, the rates of evaporation depended largely on the difference in temperature between the air and the surface of the meat, and relative humidity played a comparatively minor role. The bulk of the weight loss occurred within the first 24 hours, and the total loss during the cooling period was approximately 2.4 per cent when conditions were employed that effectively controlled microbial growth (about 88 per cent mean relative humidity at -1° C.).

Bates and Highlands (2) studied the effect of the physical conditions maintained during storage on the growth of microorganisms on meat. Some

relationships between temperature, relative humidity and air velocity, and rate of bacterial growth and weight loss were shown. Newell et al. (80) stored lots of young chickens under various types of refrigeration, half of each lot being eviscerated. Weight losses were obtained in all lots except those stored in ice. Eviscerated birds not only had the greatest weight losses but also spoiled earlier than non-eviscerated birds.

b. Effect of relative humidity on CO_2 activity. The only work found relating to this problem was that of Scott (92). He employed the procedure described on page 30, with the exception that some of the containers were given atmospheres containing 10 per cent CO_2. Presence of CO_2 extended the lag periods and this effect was markedly accentuated as the humidity was reduced. The critical relative humidity for Achromobacter was 97 per cent in the presence of CO_2 as compared to 96 per cent in air. Relative tolerance to 10 per cent CO_2 was expressed by the ratio of the rate of growth in CO_2 to the rate of growth in air. At 99.3 per cent relative humidity (normal water contents), tolerance of Achromobacter to CO_2 was about 0.43; at 98 per cent it was 0.3 and at 97.5 per cent, 0.2. Critical humidity for the growth of the yeasts Candida and Geotrichoides was 92 per cent with 10 per cent CO_2 and 90 per cent with air. Mycotorula was considerably more resistant to CO_2 and desiccation. Yeasts differed from bacteria in that tolerance to CO_2 was greatest at intermediate water contents instead of at normal water contents. Scott discussed the applications of his findings to meat storage.

3. Initial contamination

The idea that the extent of the original contamination will influence the length of time for which a meat or meat product can be successfully stored has been widely accepted by microbiologists. However, critical evaluation of the effect of amount of initial contamination on keeping-time of meats has seldom been undertaken.

a. Effect of the initial contamination on storage-life. Empey and Vickery (22) stored quarters of beef at a temperature of -1° C. and a relative humidity of about 95 per cent. In the first experiment the initial counts showed 550 bacteria and about 5 molds per sq. cm. of muscle surface capable of growth at -1° C.; in the second experiment the initial counts were only about one-tenth as great. The quarters with the higher counts kept for 16 days; those with the lower counts for 45 days.

Haines (35) followed the rate of growth of Achromobacter on pieces of lean beef at 0° C. and determined the times required for slime production with various initial counts. His values for initial counts per sq. cm. of surface and time of appearance of slime are; 40,000, 8 days; 173,00, 10 days; 2,700, 11 days; 43, 18 days. Haines and Smith (40) plotted logarithms of the initial counts against time in days for bacteria to reach the critical number producing slime and obtained a straight line.

Ayres et al. (1) determined the times for the development of slime on chicken thighs with different initial counts stored at 40 and 50° F. and 100 per cent relative humidity. The initial counts and corresponding keeping-times were as follows: at 50° F.--8 X 10^6, 1.5 days; 10^4, 2.5 days;

2×10^2, 4.5 days; at 40° F.--3×10^6, 2.5 days; 10^4, 5.5 days; 10^3, 30 days.

b. Effect of CO_2 on the influence of initial contamination. Brown (14), from a study of the effect of CO_2 on the growth and germination of molds, decided that effectiveness of CO_2 in retarding growth was somewhat greater when the density of the spores was increased, and that less effect was obtained when some growth had occurred before CO_2 atmospheres were applied.

Empey and Vickery (22) found that a 90 per cent reduction in initial contamination increased the keeping-time of quarters of beef stored in 11 per cent CO_2 at -1° C. from 23 days to 67 days. The ratio of the keeping-times with low contamination to those with high contamination were about the same with and without CO_2.

4. Composition of substrate

It is well known that chemical makeup of the microorganism's environment has a profound influence on its growth. Meat seems to be a very favorable medium for microbial development. No evidence was found in the literature that growth of spoilage organisms was better on fresh meat from one animal than on that from another.

In the case of sausage products which may be smoked and/or cured a different situation exists. Smoke is believed to impart certain inhibitory compounds to meat, and salts used in curing also affect subsequent microbial development. Urbain discussed curing methods (103, p. 675-678). Literature relative to effects of smoke on microorganisms has been summarized by Jensen

(52, p. 188-200) and Jensen (53). Investigations of the action of nitrates and nitrites on bacteria were discussed by Jensen (52, p. 11-30) and Tanner (100, p. 864-885). The same authors (52, p. 344-358; 100, p. 861-863) reported on studies carried out on the inhibitory properties of sodium chloride.

Ingram (49), in order to learn something of the flora developing on bacon, exposed plates of minced fresh pork containing various amounts of salt to the air of a bacon cold storage room. With less than 4 per cent salt, the flora developing was composed almost entirely of Pseudomonas. The molds Thamnidium chaetocladioides, Penicillium and Cladosporium, and in addition, micrococci predominated when salt concentration ranged from 4 to 10 per cent. With 10 to 25 per cent salt, the flora consisted of micrococci and an unidentified species of Penicillium. Examination of slime from bacon disclosed similar micrococci together with species of Bacillus and Lactobacillus.

Jensen (52, p. 181) states ".....practically every saprophytic mesophile and psychrophile can grow on the moisture film of the casing (of sausage) and thus form a slime." Niven (81), however, believed that the bacteria developing on frankfurters usually were limited to Gram-positive varieties.

E. Storage of Meats in CO_2

In evaluating the efficacy of meat storage in carbon dioxide atmospheres it is not sufficient to know the effect on microorganisms causing meat deterioration; the changes that take place in the meat under the conditions of storage and the influence of CO_2 on these changes must also be considered.

1. General considerations

a. Changes in lean. (1) Protein denaturation. Smith (95) observed a 30 per cent reduction in soluble protein in rabbit muscle held for 24 hours at 30° C. under sterile conditions. According to Moran (76) denaturation of proteins was very slow at 0° C. but became significant at higher temperatures. He considered this an argument for rapid cooling since denatured proteins were more rapidly attacked by bacteria. Moran also reported that freezing denatured proteins. From changes in soluble nitrogen in stored beef Haines (34) concluded that microorganisms could cause considerable changes in protein at 0° C. Gibbons and Reed (30) demonstrated that the degree of autolysis of sterile tissue made a considerable difference in the chemical changes subsequently produced by Proteus vulgaris.

No reports were found on the effect of CO_2 on changes in protein. It seems possible that the longer storage times permitted by CO_2 storage might allow increased autolysis.

(2) Tenderization. The "aging" or "ripening" of beef has been reviewed by Bate-Smith (3). He states that the rate of tenderizing was faster at higher temperatures as was the likelihood of spoilage from microorganisms. The amount of tenderizing achieved increased with time.

Carbon dioxide might be useful in tenderization of meat by allowing increased storage times or storage at higher temperature.

(3) pH. Winkler (110) showed that, to some extent, pH determined the color of meat, the meat tending to be darker at higher pH. Bate-Smith (3)

reviewed work supporting this opinion. According to Haines (37), unpublished measurements made by E.C. Smith indicated that the change caused in fresh juice by saturation with CO_2 was less than 0.1 pH unit. Killeffer (58) reported that samples of meat stored in commercial CO_2 (near 100 per cent) never dropped in pH more than 0.5 unit. Moran et al. (77) observed that beef stored in pure CO_2 for 19 days had a pH of 5.4 as compared with 5.6 to 5.9 for normal beef. According to Bate-Smith (3), beef in full rigor normally varied in pH between 5.4 and 6.0 with greater extremes occasionally encountered. Still greater variation was obtained with pork. It would appear, then, that moderate concentrations of CO_2 would have little effect on color and taste as related to pH.

(4) Flavor. Kolbe (59) found that beef stored in 100 per cent CO_2 tasted as good as fresh beef after eight days at room temperature but was slightly sour in taste after two and three weeks. Whether the sourness was due to CO_2 or microbial growth was not determined. Callow (15) believed that pieces of pork which had been stored for 70 days in commercial CO_2 at 0° C. was superior in taste to fresh pork because of increased tenderness. Moran (76) stated that CO_2 stored beef reached England from Australia and New Zealand in perfect condition. No investigators have reported off-flavors in meat caused by carbon dioxide.

(5) Pigments. Kolbe (59), Moran et al. (77), Callow (15), and others noted that storage of meats in high concentrations of CO_2 caused discoloration or loss of "bloom". It is now quite generally accepted that this phenomenon is due to the acceleration of the oxidation of the muscle pigment. Although the muscle pigment is actually myoglobin (Urbain and Jensen (104), it has commonly been referred to as hemoglobin in the earlier literature. Hereafter, when the term "hemoglobin" is used in referring to the reactions

of muscle pigment, it should be understood that the information presented is actually for myoglobin. For the study of pigment reactions in the "test-tube", blood hemoglobin is almost always used because it is so much more easily obtained than myoglobin.

Early work on the oxidation of hemoglobin was reviewed by Neill and Hastings (79). These investigators studied the effect of oxygen tension upon certain oxidations of hemoglobin. In their work oxygen was used to "activate" some easily oxidized substance which in turn oxidized the iron of the hemoglobin molecule to methemoglobin. The results obtained indicated that the substance oxidized to methemoglobin was reduced hemoglobin, and that the addition of either oxygen or carbon monoxide caused the formation of a different substance more difficult to oxidize. The optimum oxygen tension for the formation of methemoglobin was about 2 mm., which allowed most of the hemoglobin to exist in the reduced state.

Brooks (8, 11) investigated the oxidation of hemoglobin in laked ox-blood under different conditions of pH, temperature, and oxygen pressure and showed, by chemical analyses, that the decrease in hemoglobin during the reaction was accounted for by the formation of methemoglobin. A reduction in pH increased the rate of oxidation; the relation between pH and the logarithm of the velocity constant, k, appeared to be linear. Velocity constants determined with different blood samples differed, but the general relationship between pH and velocity constant seemed to be the same for all samples. The rate of oxidation increased as the temperature increased; the temperature coefficient calculated from the ratio $k_{25^\circ} \div k_{15^\circ}$ was approximately 4. The maximum rate of oxidation at 50° C. occurred with approximately 20 mm. oxygen pressure, but dropped off rapidly as pressure approached zero and changed very little above 200 mm. The

rate of oxidation at different oxygen pressures was proportional to the concentration of reduced hemoglobin. Brooks thought that the function of oxygen was to determine the concentration of the other reactant, to oxidize the unoxygenated ferri-radicals, and to inhibit the oxidation. The following possible formula for the reaction was suggested:

$$(H_3Hb)_4 = (H_2Hb)_4 + 2\ H_2O_2$$

Brooks (7) made observations on the formation of methemoglobin in pieces of fresh frog muscle between glass plates. No methemoglobin was formed in the absence of oxygen, but in the presence of oxygen it was formed slowly in the oxygenated region of the tissue and most rapidly at some distance from the surface. The depth of oxygen penetration, as determined by pigment colors, was about 2 mm. after equilibrium had been attained at 0° C.; after 100 hrs. it was about 4 mm., the increase apparently due to some decrease in oxygen consumption by the tissue. Freezing and thawing appeared to increase the rate of methemoglobin formation.

The effect of atmospheres containing carbon dioxide on the color changes of lean beef was studied by Brooks (9) who found that, with commercial CO_2 at 0° C., oxidation to methemoglobin was complete in less than 3 days as compared with 6 to 8 weeks in air. With 50 to 60 per cent CO_2 the unpleasant reddish-brown color of methemoglobin also developed rather rapidly. The rate of oxidation was not appreciably affected by 10 and 20 per cent CO_2; however, a slight increase was observed with 30 per cent. Brooks credited the effect of CO_2 on the rate of oxidation to a lowering of pH and a lowering of oxygen tension. Reduction of oxygen pressure not only increased the rate of formation of methemoglobin but also caused it to form closer to the surface where it had more effect on the external color.

Oxidation is not the only cause of loss of bloom. The importance of pH was already discussed. Brooks (9) and Winkler (111) noted that desiccation produced an undesirable darkening of color. This was attributed to an increase in opacity of tissue by Brooks and to concentration of pigments by Winkler. Changes due to drying at ordinary temperature were considered reversible by Winkler. Brooks (9) and Scott and Vickery (93) believed that modest desiccation during storage benefited bloom.

In the case of cured meats different pigments are involved. Haldane (41) concluded that the red color of cooked cured meat was due to nitric oxide hemochromogen; nitrite reacted with reduced hemoglobin to produce nitroso-hemoglobin, which was subsequently denatured to the hemochromogen by heat processing. He thought that nitrate of the cure was reduced to nitrite by bacteria and that the tissue itself might under some conditions accomplish this. Hoagland (45) confirmed the work of Haldane and made further observations. He crystallized NO-hemoglobin produced from the blood of several animals and found that the crystalline structure differed with the source, but was in all cases different than that of oxyhemoglobin crystals obtained from the same sources. The color of uncooked cured meats generally was due to NO-hemoglobin, but under some methods of processing (particularly desiccation) this compound was more or less converted to NO-hemochromogen even though cooking had not been employed.

Urbain and Jensen (104) observed that nitric oxide hemoglobin was easily oxidized to methemoglobin by atmospheric oxygen and that reduction of pH or increase in temperature accelerated the reaction. They decided

that brown discolorations of cured meat which had been commonly attributed to bacterial action were frequently due to oxidation.

Haurowitz et al. (43) showed that the autoxidation of certain unsaturated fatty acids was accompanied by destruction of heme-containing pigments. Chang and Watts (16) found that ascorbic acid protected the red color of cured meat although it caused rapid discoloration of fresh meat.

Discoloration caused by the formation of methemoglobin should be distinguished from greenish discolorations sometimes encountered in cured meats. According to Jensen and Urbain (55) the latter are formed by the action of oxidizing or hydrogen sulfide forming bacteria upon the nitroso derivatives of hemoglobin. These are considered oxidation products since they are formed under oxidizing conditions, however, spectrophotometric measurements indicated that they were different from methemoglobin. Formation of discolorations of this type are considered distinctly different from discolorations due to undercuring, wherein the pigments are not entirely "fixed" by nitrite. Jensen (52) believes that green rings in sausage products are due to the action of hydrogen peroxide, organic peroxides and hydrogen sulfide produced in the meat before heat processing, and that the location of the green area subsequently developed is determined by oxygen tension.

Niven (81) supported the opinion of Jensen on internal green discolorations and obtained evidence that surface greening of stored products was associated with the proliferation of catalase-negative lactic acid bacteria.

b. Changes in fat. Good general reviews of this subject have been made by Lea (67) and Jensen and Grettie (54).

(1) Hydrolysis. Lea (67) stated that tainted fat could contain several million bacteria per gram, although their presence was not obvious and slime was rarely produced. Lea (63) found that the amount of free fatty acids increased with the development of microorganisms, but he did not believe that these acids caused the changes in odor and flavor observed. Haines (35) showed that some of his strains of Achromobacter could hydrolyze fat. Vickery (106, 107) tested the lipolytic activity (toward beef fat) of strains of bacteria and yeasts capable of growth on beef stored at -1° C. About half of the strains of Achromobacter and Pseudomonas and all of the yeasts isolated were lipolytic. These investigators decided that measurements of free acidity of fatty tissue would not necessarily indicate the extent of microbial growth. Garrard and Lochhead (29) observed that unidentified gram-negative cocci associated with bacon were characteristically lipolytic.

It appears from the above investigations that CO_2 storage would reduce the amount of fat hydrolysis by inhibiting the growth of microorganisms. Apparently, however, the extent of fat hydrolysis does not have an appreciable effect on storage life.

(2) Oxidation. Lea (63) stored beef carcasses at temperatures in the neighborhood of 0° C. until the fat became tainted. Atmospheric oxidation in no case rendered the fat unpalatable as long as there was not an excessive exposure to strong light. Lea concluded that the storage-life

of chilled beef depended on the growth of microorganisms and not atmospheric oxidation. Lea (64) observed that storage of beef fat in moderate concentrations of CO_2 did not affect its resistance to oxidation. Although oxidation was not important in the air storage of meat, Lea (67) thought that appreciable oxidation could sometimes occur as a consequence of the longer storage periods made possible by CO_2. In the case of poultry (New York dressed) stored at 0° C. in 96 per cent CO_2, the induction period was about doubled as compared with air-stored birds; however, after long storage in CO_2 peroxide values increased considerably. Lea decided that atmospheric oxidation could be a factor in the spoilage of CO_2-stored poultry. Smith (97) observed that the fat of CO_2-stored chickens (96 per cent at -1° C.) tasted slightly rancid after 12 weeks and was definitely rancid after 18 weeks. As an index to susceptibility to oxidation Lea (67) gave the following iodine values: beef and mutton, 35-37; pork, 55-70; chickens, 70-90; rabbits, 110-180. Lea (67) stated that cured meat was much more susceptible to oxidation than was uncured.

Robinson (87) first observed that heme compounds acted as efficient catalysts for the autoxidation of an unsaturated fatty acid. Chang and Watts (16) found that ascorbic acid retarded rancidity in the presence of hemoglobin or nitrosohemoglobin and small amounts of some phenolic antioxidant.

(3) Flavor. Lea (63) and Haines (35) thought that unpleasant tastes and odors in fat of stored beef were caused by microorganisms growing either in the fatty tissue or in the adjacent muscle. Lea (63) found that the fat

of beef carcasses stored in still air at 0° C. was good after 25 days but somewhat tainted after 42 days; a tainted odor was present at 15 days. Air circulation or reduction of temperature extended the keeping time of the fat.

Lea (64) stored strips of beef fat in various CO_2 concentrations at 0° C. Strips of fat which had been stored for 50 days in 20, 50 and about 100 per cent CO_2 were excellent in flavor. Pure extracted fat was stored in approximately 100 per cent CO_2 for 66 days with no deterioration in flavor. The length of time fat strips could be stored before off-flavors developed was about doubled by the use of 10 per cent CO_2. Little advantage was gained by increasing the CO_2 concentration above 15 or 20 per cent. Reduction of relative humidity to 90 per cent caused some increase in the protection afforded by the gas.

(4) Pigments. Lea (66, 67) occasionally observed the development of bleached greyish or white patches in the external fat of beef stored with CO_2. These, he thought were due to the oxidation of hemoglobin to methemoglobin and destruction of some of the yellow carotinoid pigment. The cause of the patches was not investigated in detail, but appeared to be associated with higher peroxide values.

c. Effect of meat on CO_2 and oxygen levels. According to Moran (75), the respiration of dead muscle was between 10 and 50 per cent of that of living muscle. Beef muscle was found to take up 12 cu. mm. of oxygen per gram per hour 24 hours after death; after one month at 0° C. the rate was 4 cu. mm. per gram per hour. Muscle stored in 10 per cent

CO_2 took up 6 cu. mm. per gram per hour for the first 4 days. Moran calculated that beef quarters stored in gas-tight chambers with 105 cu. ft. of space per ton of meat would produce about 20 liters of CO_2 per week and would increase the concentration in the atmosphere by 1 per cent per week. The amount of CO_2 produced from bicarbonate by glycolysis was considered unimportant.

Brooks and Moran (13) investigated the possibility of attaining a useful CO_2 concentration in an air-tight chamber by first saturating meat with CO_2 and then allowing it to come to equilibrium with the atmosphere of the chamber. They considered that the amount of CO_2 converted to bicarbonate in the muscle would be negligible compared to the amount in physical solution. Brooks and Moran calculated that a 150 lb. quarter of beef saturated with CO_2 at 0° C. would have in solution about 70 liters (5 oz.) of the gas, and that this beef, placed in a gas-tight chamber containing air (105 cu. ft. total space per ton of meat), would produce an atmosphere of 25 to 30 per cent CO_2 when equilibrium had been attained. However, calculation based on the rate of diffusion of CO_2 through muscle indicated that quarters would have to be stored in 100 per cent CO_2 at 0° C. for 5 days to obtain 30 per cent saturation and 75 days to obtain 90 per cent saturation. Similar times would be required for the CO_2 to diffuse out of the tissue. They considered such a procedure impractical because it would have resulted in discoloration of the meat.

2. Storage of fresh meats in CO_2

a. Beef. Observations of Kolbe (59) that fumes from strong liquid acids would prevent decomposition of meat, led him to test the effect of carbon dioxide on keeping time of beef. He hung pieces of beef in a metal cylinder which was then filled with pure CO_2 and sealed. Storage was carried out at room temperature and probably varied considerably; a maximum of 32° C. was the only temperature given. After eight days' storage, Kolbe noted no change in appearance, color, odor, or taste after cooking. After 14 days the exterior had become gray in color, although the interior was normal; the flavor was very slightly acid. There was no change at 3 weeks except for an increase in tenderness. At 4 and 5 weeks, odor was still good, but broth prepared from the beef was inferior in flavor. The discoloration caused by carbon dioxide was prevented by the addition of some carbon monoxide to the CO_2 atmosphere.

Killeffer (58) stored samples of beef and other food products in large tin cans at a temperature of 40 to 45° F. Commercial CO_2 was passed through one set of cans at a rate of 3 to 4 cu. ft. per can per hour; and a duplicate set contained still air. A meat inspector pronounced the beef samples stored in air spoiled after 2 weeks; the samples stored in CO_2 did not show spoilage until the end of the third week. Subsequent experiments showed that beef could be stored for 2 to 3 times as long in CO_2 as in air. No mention was made of discoloration due to CO_2 and apparently no attempt was made to control relative humidity or to maintain equal humidities in the two sets of cans.

Empey and Vickery (22) stored quarters of beef with and without 11 per cent CO_2 at a temperature of -1° C. and a relative humidity of 95 per cent. First evidence of deterioration was the appearance of bacterial colonies, which soon coalesced to form a slime accompanied by a disagreeable odor. The end-point of storage was taken as the time at which the number of bacteria per sq. cm. of muscle surface reached a value of 5×10^7. The keeping times obtained in the first experiment were 16 days in air and 23 days in CO_2. In the second experiment when the initial contamination was one-tenth as great, the keeping times were 45 days in air and 67 days in CO_2. Weight losses in the second experiment were 2.8 per cent on the 46th day and 4.6 per cent on the 57th day; 5.5 per cent was considered the maximum loss allowable. Of the slight loss of bloom occurring, none was attributed to CO_2. The palatability of the lean was good up to the time of slime formation; the flavor of the fat was slightly off at 55 days in both the air and CO_2-stored samples.

Empey *et al.* (23) described the preparation of beef quarters for shipment from Australia to England. Strict sanitation in the slaughter house, along with rapid chilling in air with considerable drying power, were employed to hold microbial contamination to a minimum. After chilling, the counts on the exposed muscle averaged 9 and 110 bacteria per sq. cm. for the hindquarters and neck areas respectively, and the moisture content of the outer one-half mm. layer of muscle was under 55 per cent. During shipment, temperature was held at -1.4° C.; a level of 11 per cent CO_2 was maintained and the amount of air circulation was regulated accord-

ing to the relative humidity existing in the hold. This beef arrived at the wholesale market in London in excellent condition 44 days after slaughter. There was no visible microbial growth and no appreciable change in bloom.

b. Pork. Killeffer (58) found that the use of 100 per cent CO_2 in the storage of cuts of pork at 40 to 45° F. would increase the storage-life 2 to 3 times.

Callow (15) compared the storage-life of small pieces of pork in atmospheres of air, in commercial carbon dioxide and in commercial nitrogen stored at 0° C. in desiccators. After 17 days the air-stored pork was overgrown with microorganisms while the others were in good condition. After 56 days the CO_2-stored pork was in perfect condition, but the nitrogen-stored meat had a strong smell of hydrogen sulfide even though no surface growth was apparent. After 70 days, the CO_2-stored pork was as good as fresh pork only more tender. At 87 days the surface had become somewhat red in color, although the interior was unchanged. The lean meat was normal in taste, but taste of the fat was slightly abnormal; the off taste was not thought to be due to rancidity.

c. Poultry. Smith (96) studied the effect of CO_2 storage on chickens. Preliminary experiments had shown that CO_2 levels above 70 per cent were necessary to prevent microbial growth. In the first test 36 plucked and drawn chickens were hung in an air-tight tank at 0° C. Commercial CO_2 was passed through 1 per cent sodium chloride and then into the tank to give a level near 100 per cent. Birds were edible at the end of the six

months storage period, but the meat was tasteless after four months. The exposed flesh developed an unpleasant brown color. Loss of weight in six months was 2.9 per cent. In the second experiment selected birds were stored undrawn and trussed in 95 to 100 per cent CO_2 at -1° C. After one month, when the first examination was made, it was found that gas had entered the crop and gut, causing swelling. Subsequently, auto-digestion of the abdominal wall caused green discolorations and autolysis. The breast had a good flavor at the end of the four months storage period except in the green areas, which were bitter. The legs were found lacking in flavor after 89 days. Total weight loss was 3.1 per cent.

In a further experiment Smith (97) stored New York dressed chickens in an air-tight tank at about -1° C. with an average CO_2 concentration of 96 per cent, and others in air at the same temperature. At four weeks the air-stored samples were unchanged, but the CO_2 stored birds showed swelling at the crop and vent. After six weeks the air-stored birds had mold growth and the breast was dry and tasteless. At eight weeks some of the chickens stored in CO_2 had purple-brown discolorations at the neck and green areas at the vent; taste was satisfactory except in the green regions. After 10 weeks there were further cases of green discoloration and at 12 weeks this defect was quite pronounced. Because of the definite greening, 12 weeks was considered the limit of saleability; also, there was slight evidence of fat rancidity at this time. However, there was no visible evidence of microbial growth and the odor was good. A CO_2 concentration of 95.5 per cent within the alimentary canal was found to be in equilibrium

with a level of 99 per cent in the surrounding atmosphere. This was taken as evidence that the walls of the alimentary canal were permeable to CO_2 but not to air and would account for the swelling observed in the CO_2 stored birds. From his experiments Smith concluded that extended storage of poultry in CO_2 atmospheres was impractical, mainly because of a non-bacterial digestion of the gut and belly wall accompanied by green discoloration. Smith (98) stated that the greening and autolysis in CO_2-stored chickens were caused by digestive enzymes from the pancreas and duodenum.

d. Other fresh meats. Kolbe (59) obtained poor results when he stored mutton, veal and fish in 100 per cent CO_2. Killeffer (58) obtained as good results with lamb and fish as he did with beef. Coyne (19) found that at 0° C., CO_2 approximately doubled the storage life of whole fish and fillets when either gas- or ice-packed. Carbon dioxide levels of 40 to 60 per cent gave best results; higher concentrations caused softening of the flesh.

Reich (84) described a shipment of assorted packing house products by refrigerated railway car in which 500 pounds of solid CO_2 were used in addition to ice for cooling. During the four day trip the CO_2 levels at the top of the load and at the floor varied from 4.0 to 8.0 and from 5.5 to 9.5 per cent respectively. The highest levels were reached midway during the trip. No bacteriological information of any consequence was reported. According to Reich shippers believed that solid CO_2 in the car retarded bacterial growth without affecting bloom or shrinkage.

3. Storage of cured meats in CO_2

Apparently very little was done toward testing the effectiveness of CO_2 in preserving cured meats. Killeffer (58) observed that frankfurters could be stored two to three times as long in commercial CO_2 as in air at a temperature of 40 to 45° F.

Callow (15) compared the effects of atmospheres of air, of carbon dioxide and of nitrogen on the storage life of mild-cured, green (unsmoked) bacon. At 5° C., the storage life of bacon was more than doubled by an atmosphere of commercial CO_2; pure nitrogen was almost as good. The appearance of bacon stored in CO_2 and in nitrogen was described as being "brighter" than that of bacon stored in air; apparently the absence of air prevented the oxidation of the nitrosohemoglobin. Both CO_2 and nitrogen prevented the growth of microorganisms for several weeks, but bacon stored under nitrogen underwent anaerobic spoilage more rapidly.

III. EXPERIMENTAL MATERIALS AND METHODS

A. Materials

1. Meat samples

a. Chicken. Cut-up fryers ($2\frac{1}{2}$ to 4 lb.) used in experiments employing chicken were obtained from 3 sources. (1) Live birds from the College Poultry Farm were killed by bleeding; struggle was eliminated by application of an electric shock. Birds were then immersed for 30 seconds in water at 130° F. (54.5° C.), rough picked by machine and finished by hand. Following evisceration and washing, fryers were cut up and air chilled at 32° F. (0° C.) for 4 hours or overnight depending on the time of day. (2) Frozen, cut-up packaged chickens were purchased from a commercial concern and stored at -10° F. (-23.3° C.). These were thawed 24 hours at 40° F. (4.4° C.) immediately before use. (3) Fresh cut-up fryers were obtained from a local produce house immediately after slaughter and chilling and were used in experimental runs on the same day.

All birds were cut up in the same way to give, in addition to giblets, 2 legs, 2 thighs, 2 wings, ribs, back, and 2 pieces of breast.

b. Frankfurters. Skinless, uncolored, all meat frankfurters were received from a nearby packing house on the day after manufacture. They arrived with an internal temperature from 40° F. (4.4° C.) to 50° F. (10° C.) and were stored at 32° F. (0° C.) until the following morning.

2. Storage containers

In preliminary experiments several types of containers, including vacuum desiccators, Brewer and Fildes anaerobic jars, and Mason type jars were investigated for their applicability to storage experiments. One-quart and two-quart Mason type jars with rubber stoppers were found most suitable. These were considered to have the following advantages:

(1) Gas tightness compared favorably with other containers tested.

(2) Withstood complete evacuation without collapsing.

(3) Were easily sterilized by autoclaving.

(4) Initial and replacement costs were low.

(5) Were economical of space in the small refrigerators available.

(6) By simple variations in arrangement of glass and rubber tubing, could be adapted to several methods of controlling atmospheres such as evacuation and subsequent replacement with the desired gases or a constant flow of gas of predetermined composition.

In most cases jars were closed with one-hole number 12 rubber stoppers. A short piece of glass tubing having a right angle bend was inserted just through the stopper to provide a means for admitting or removing gas. A short length of rubber pressure tubing (about 5 inches) was attached to the external end of the glass tubing. Loss of gas from stoppered jars was prevented by means of a screw clamp on the rubber tubing. In some cases a sealing compound (Tackiwax) was applied at the place of contact between stopper and jar top and at tubing joints.

For runs in which a constant flow of gas was desired, a two-hole rubber stopper was used. The glass inlet tube extended to the jar bottom and the outlet tube was flush with the lower side of the stopper.

Vacuum desiccators (160 mm.) were employed for experiments in which relative humidity was the chief variable.

3. Gas control apparatus

The basic control apparatus consisted of a Fisher, Unitized, Precision Universal Model gas analyzer (Orsat type) with a built on glass manifold and mercury manometer. (Figures 1 and 2). By means of the three way stopcock at A, communication was established either to the sampling burette of the gas analyzer through capillar tubing, B, or to the manifold. A gas sample to be analyzed was drawn from the storage jar to the sampling burette by means of the leveling bulb on the sampling unit. The confining liquid in both sampling and measuring burettes consisted of mercury with a film of water on top. After transfer from the sampling to the measuring burette, the volume of sample was read. Carbon dioxide was then removed by means of a potassium hydroxide solution (500 grams per liter of solution) in the first absorption pipette and the decrease in gas volume was used to calculate per cent CO_2. After CO_2 absorption, oxygen concentration could be similarly determined by absorption in the second pipette. This pipette contained alkaline pyrogallol (one volume of a solution containing 300 grams of pyrogallic acid per liter and 5 volumes of 50 per cent KOH).

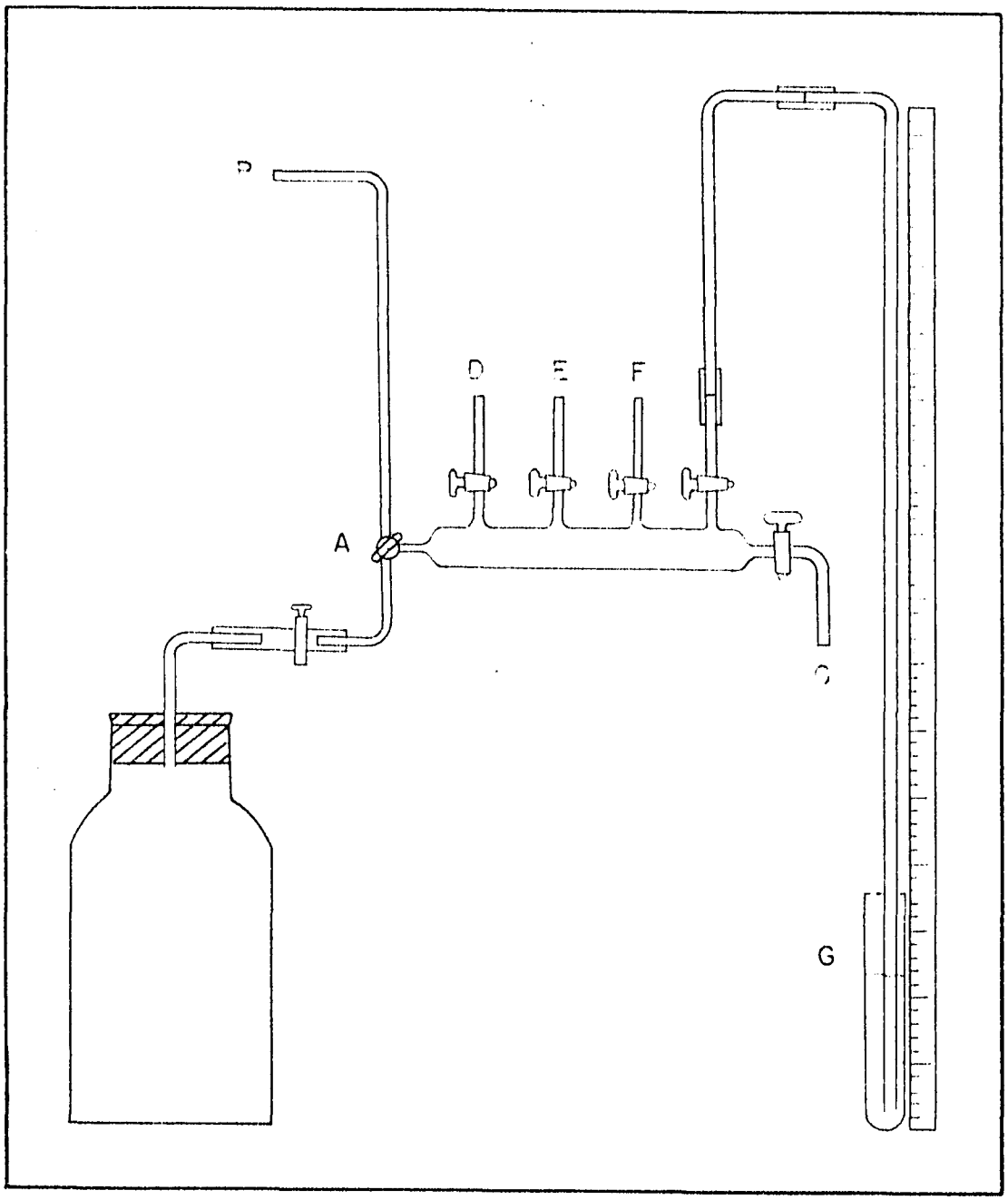

Figure 1. Apparatus for changing atmospheres in storage containers

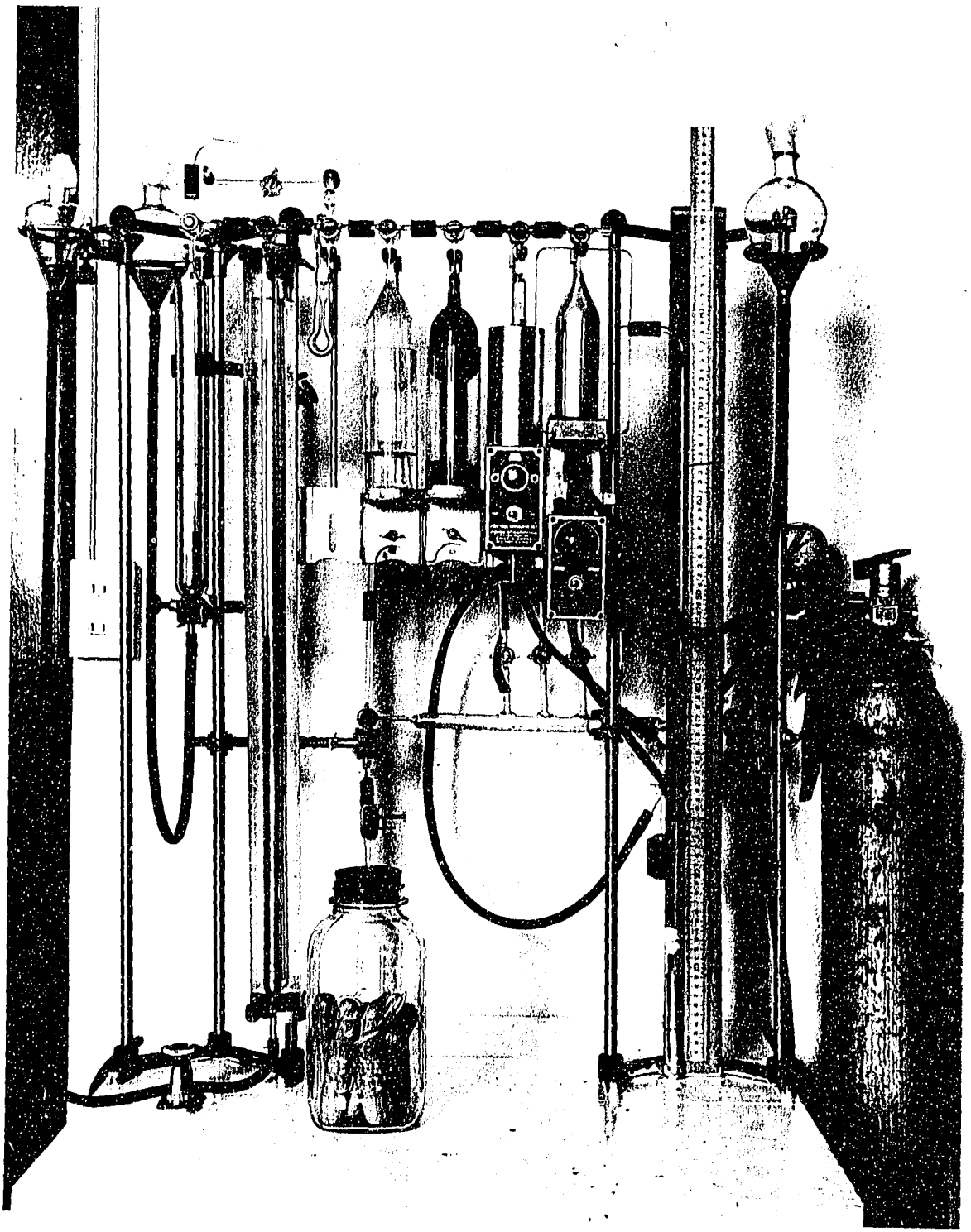

Figure 2. Gas analyzer with built-on manifold

A Conco-Hyvac vacuum pump connected to the manifold at C allowed evacuation of sample jars. Carbon dioxide and oxygen from cylinders and air from the room could be admitted to the manifold through stopcocks D, E and F. The mercury manometer, G, was employed to control the amounts of individual gases added to evacuated jars and was also used to measure vacuums in jars after storage.

Glass flow meters were used to control composition of flowing gas. These were of several designs and capacities but all operated on the basic principle of an orifice meter. A constriction within the unit caused a pressure differential which was measured by means of a U-tube manometer attached before and after the constriction. Flow meter scales were calibrated by means of a wet test gas meter. An assembly in which flow meters are employed is shown in Figure 3.

Carbon dioxide concentration of flowing gas mixtures was measured with a Cambridge Carbon Dioxide Indicator (Model M Exhaust Gas Tester, Modified). This instrument was designed to give scale readings in per cent CO_2 based on differences in thermal conductivity of a dry CO_2-air sample and a reference gas of sealed in, dry, CO_2-free air. Gas samples were passed through a calcium chloride drying tower before entering the analyzer. While in use, the scale zero was adjusted daily during passage of dry, CO_2-free air through the instrument. Weekly checks for accuracy were made by comparing analyses between the Cambridge and Fisher analyzers on the same gas sample.

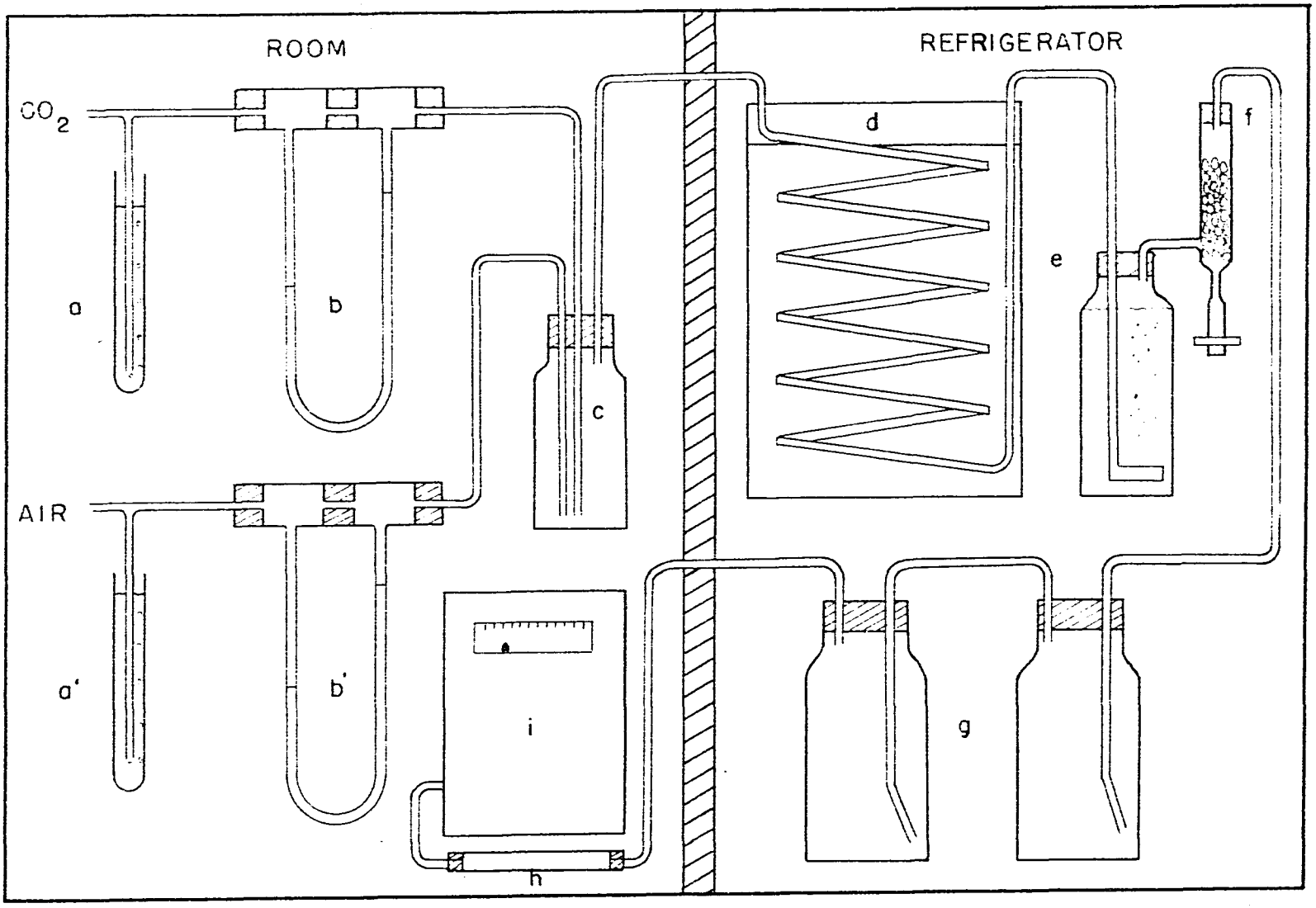

Figure 3. Apparatus for constant flow of controlled CO_2-air mixture

4. Carbon dioxide

Commercial carbon dioxide was used throughout this study. CO_2 was obtained from the Pure Carbonic Corporation in liquid form in 20 and 50 pound cylinders.

5. Refrigerators

Storage at 40° F. (4.4° C.) and 45° F. (7.2° C.) was carried out in a Super-Cold, Model 44 RS refrigerator having a capacity of approximately 40 cubic feet. Temperature was controlled to ± 1° F. by means of a Minneapolis-Honeywell temperature regulator. Temperatures of 30° F. (-1.1° C.) and 32° F. (0° C.) were obtained by a Kelvinator deep freeze unit opening at the top. This box had a capacity of about 1.8 cubic feet and a temperature regulator identical with the one described above. For 50° F. (10° C.) a Herrick 45 cubic feet box with a Par Model HA5S refrigerating unit was used. A White Rodgers Type 1609 temperature control held temperature within ± 2° F. The Super-Cold and Herrick boxes were equipped with forced air circulation.

6. Bacteriological media

These are described under the section on bacteriological methods.

B. Methods

1. Methods of obtaining and maintaining CO_2 levels

The three procedures used in controlling carbon dioxide concentration in storage containers have been designated the constant flow, the evacuation, and the displacement methods for convenience in referring to them. The apparatus used for maintaining a constant flow of gas through sample jars is shown in Figure 3. Air and CO_2 were supplied from a compressed air line and a CO_2 cylinder, both equipped with suitable reducing valves. A slight excess of gas was supplied and allowed to escape through pressure regulators a and a'. The regulators prevented fluctuations in supply pressure from causing similar variations at the flow meters, b and b', and also provided a simple means of controlling gas flow. Metered gases were mixed in chamber c and carried into the refrigerator to the cooling coil, d, by means of rubber tubing. The cooling coil consisted of a 25 ft. length of one-eighth inch i.d. copper tubing immersed in a 5 gallon can containing water. Gas was passed in tiny bubbles through distilled water in bottle e by means of a sintered glass diffusion disc in order to obtain a relative humidity near 100 per cent. Entrained water droplets were removed by a trap, f, and the gas thenvas passed through sample jars, g. The gas was taken out of the refrigerator and through a calcium chloride drying tube, h, to the Cambridge gas analyzer, i. Air alone was passed through control sample jars in the refrigerator by similar apparatus. The flow of air and of the CO_2-air mixture were held at 100 ml. per minute.

For securing atmospheres containing CO_2 by the displacement method, the apparatus was nearly identical with that shown outside of the refrigerator in Figure 3. However, larger flowmeters having a capacity of 1000 ml. of gas per minute were used. Rubber tubing carrying the gas mixture was attached to the Cambridge analyzer to make certain that flow meters were delivering correct quantities of CO_2 and air. When the desired gas composition was attained, gas was led into a sample jar through a piece of glass tubing which was inserted to the bottom of the jar. A flow of 1000 ml. per minute for 7 minutes gave complete displacement of the jar atmosphere.

In the evacuation method a jar containing a sample was attached to the manifold of the apparatus shown in Figure 2, and partially evacuated. Enough carbon dioxide was admitted to give the desired concentration as determined by pressure readings on the mercury manometer, and air was added until pressures in the jar and room were equal. A similar procedure was used when atmospheres containing additional oxygen were needed. To obtain an atmosphere approaching 100 per cent CO_2, a jar was exhausted twice to a pressure of 4 or 5 mm. of mercury and brought back to atmospheric pressure each time by addition of CO_2.

2. Storage methods

Jars in which samples were to be stored were sterilized by autoclaving 20 minutes at 250° F. (121° C.). Five ml. sterile distilled water were added to each jar to insure a relative humidity near 100 per cent

during storage. Meat samples removed temporarily from jars (for bacterial counts, weighings, etc.) were placed in sterile petri dishes.

a. Cut-up chicken. (1) Storage in containers sealed throughout holding period--CO_2 level not constant. Volumes of the one-quart jars used in this study were determined by the following procedure. A weighed jar was filled with distilled water, and the rubber stopper was pressed into place forcing water up in the glass and rubber tubing. After the screw clamp had been closed, water in the end of the tubing was removed with a towel and filter paper and the jar was again weighed. Temperature of the water was measured; volume of the jar was calculated from the weight of water and its density. Position of the screw clamp was permanently marked on the rubber tubing so that it would always be used at the same place. The specific gravity of a piece of chicken was determined from the increase in volume noted when the weighed piece was submerged in water in a 500 ml. graduate.

When a storage experiment was begun, pieces of chicken were removed from refrigeration, weighed and placed in jars. Generally, each jar contained the following pieces: 1 thigh, 1 leg, 1 wing, 1 piece of breast, 1 back or ribs, 1 piece of liver, and 1 gizzard or the heart and neck. Since samples were discarded whenever a jar was opened, it was necessary to have 4 to 6 duplicate jars for each experiment in order to get a number of observations during storage. Desired atmospheres were obtained by the evacuation method and jars were placed in a refrigerator. At intervals during storage based upon the expected keeping time, a jar was removed, the

internal pressure measured, and a gas sample withdrawn for analysis. The jar was then opened for examination of the sample.

(2) Storage in containers in which CO_2 concentration was maintained at a given level--atmosphere changed constantly or at intervals. Samples held under a constant flow of a CO_2-air mixture were stored in duplicate in one quart jars. Jars were removed for approximately 1 minute daily to permit examination of odor and appearance.

In the great majority of experiments, atmospheres of the jars were replaced periodically. Usually one, but sometimes two pieces of chicken were placed in each jar. Thighs were used in most cases. These were placed skin side down on the jar bottom. Preliminary experiments had indicated no difference in keeping time whether the piece of chicken was placed directly on the jar bottom or suspended from the stopper. In some cases pieces were weighed before and during storage. Atmospheres containing CO_2 were obtained by either the evacuation or displacement method, and jars were sealed and stored.

During the first few days of storage, jars were removed daily and the desired atmosphere resupplied in order to replace CO_2 dissolved by the samples. At intervals based upon expected keeping times, jars were removed one at a time and gas samples were withdrawn and analyzed. The chicken sample was removed briefly from each jar for bacterial counts and detailed observation. The appearance of each sample was examined through the glass on days that jars were not opened.

b. Frankfurters. Frankfurters were placed on end in two quart jars, 6 to 10 in a jar. When microbial counts were to be made, duplicate jars were prepared. A frankfurter that was removed for use in plate counts or organoleptic tests was not returned to the jar. In other respects storage procedures were identical with those used for chicken stored under constant CO_2 levels.

In the relative humidity study, weighed frankfurters were placed on the supported plates in 160 mm. desiccators. Each of 5 desiccators contained 200 ml. of sulfuric acid solution of the concentrations needed to provide relative humidities of 75, 80, 85, 90 and 95 per cent. (Data of Wilson (109) were used in preparing sulfuric acid solutions.) A sixth desiccator held 200 ml. distilled water. All desiccators were stored at 40° F. (4.4° C.) and were opened at suitable intervals for weighings and microbial counts. Densities of the solutions were determined at the termination of storage by specific gravity bulbs.

3. Bacteriological methods

a. Cut-up chicken. Bacterial counts from the lean or skin surface of chicken were made by the swab method of Ayres et al. (1). A 2 square centimeter area, as defined by a washer shaped piece of filter paper pressed to the surface, was carefully wiped with a sterile, moist, cotton swab. The swab was then transferred to a prescription bottle containing 99 ml. of sterile distilled water and given 25 vigorous shakes to separate strands of cotton and distribute bacteria. Four decimal dilutions for

each sample, selected according to the range of counts expected, were plated out with agar. In the first experiments chicken infusion agar (1) was used as the plating medium. Later, Difco nutrient agar containing an added 1 per cent salt and 0.5 per cent Difco yeast extract was substituted since no appreciable difference in counts had been obtained on the two media. Plates were incubated 4 days at 68° F. (20° C.) and then counted. Colonies selected for further study were picked from plates prepared from chicken which had reached or was approaching the slime stage and were transferred to nutrient agar slants. Cultures were screened by the following tests:

(1) Gram stains made from 24 hour subcultures on nutrient agar slants.

(2) Motility observations on hanging drops from 24 hour subcultures in nitrate broth.

(3) Reaction in Difco phenol red dextrose and maltose broth. (Tubes of media contained Durham fermentation tubes to indicate gas production.)

(4) Nitrate reduction on 48 hour cultures in nitrate broth (Difco nutrient broth plus 0.1% KNO_3). Details of the method can be found in the S.A.B. Manual of Methods for Pure Culture Study, V_{47}-9,10,11.

(5) Action on litmus milk (100 grams skim milk per liter of distilled water plus enough saturated litmus solution to give the desired color).

(6) Gelatin liquefaction (Difco nutrient gelatin in test tubes).

Tubes of media were inoculated from water suspensions of 24 hour cultures on nutrient agar slants and incubated at 68° F. (20° C.). Observations were made after 1, 2, 4, 8, 15, 30 and 60 days. All media was sterilized by autoclaving 20 minutes at 250° F. (121° C.) except litmus

milk, which was autoclaved 40 minutes at 232° F. (111° C.).

All cultures were tested for fat hydrolysis by a modification of Turner's technique. A fat emulsion containing 3.0 ml. cottonseed oil, 0.5 gram agar and 99 ml. distilled water was made up and sterilized. One ml. of a sterile 1 per cent solution of nile blue sulfate was added to each bottle (about 100 ml.) of melted nutrient agar. Ten ml. portions of agar were poured into petri dishes containing 0.5 ml. fat emulsion, and the fat globules were distributed by rotating the plates. Cultures were streaked onto the hardened agar and plates were given 4 days' incubation at 68° F. (20° C.). Fat globules adjacent to colonies were examined with a 22.5 X binocular microscope for the blue coloration indicating hydrolysis.

After screening, 3 cultures of each type were tested for hydrogen sulfide production, indol formation and motility in Difco S.I.M. agar. In addition, flagella stains were made on two cultures of each type which had evidenced motility by the method of Leifson (S.A.B. Manual of Methods for Pure Culture Study, IV46-17).

b. Frankfurters. For microbial counts on frankfurters, 10 grams of screened, washed sea sand and enough distilled water to give a total volume of 100 ml. were added to each of a number of 500 ml. wide-mouthed bottles equipped with a solid rubber stopper. Bottles and contents were sterilized by autoclaving. An entire frankfurter was placed in a bottle which was then shaken 200 times. From the resulting suspension of micro-

organisms, aliquots of suitable dilutions were plated with nutrient and tomato juice agar. Because of the diversity of microbial types a wide range of dilutions usually was necessary, a range of decimal dilutions from 10^2 to 10^8 being common. The same modified Difco nutrient agar formula used for chicken was used in obtaining counts in micrococci and total counts. Difco tomato juice agar with 0.3 per cent additional agar and 0.5 per cent Difco yeast extract was employed in counting lactobacilli, yeasts and molds. Plates were incubated 4 days at 68° F. (20° C.) before counting. The several kinds of organisms found on the agar plates were differentiated by differences in colony size and appearance. When differentiation by the unaided eye was difficult, the 22.5 X binocular microscope was employed for counting the individual groups (yeasts, micrococci and lactobacilli). To check accuracy of visual observations several colonies of each type from every plate counted were used in preparing Gram stains for microscopic examination.

Colonies were picked from plates representing different samples and storage conditions. Micrococci were subcultured on nutrient agar slants and lactobacilli were stabbed into butts of tomato juice agar slants. Tomato juice agar did not prove to be a satisfactory holding medium for lactobacilli and the majority of the cultures were eventually lost.

For the most part, procedures and media used in screening micrococci were the same as those used with bacteria from stored chicken. These included general procedure, Gram stain, motility determination (hanging drop method), fat lipolysis, and reactions in litmus milk, nitrate broth and

gelatin. Sugar cleavage was determined similarly; however, in addition to glucose, mannitol and glycerol were employed and maltose was omitted. The additional media were prepared from Difco phenol red mannitol broth and Difco phenol red broth base containing 0.5 per cent glycerol. Utilization of $(NH_4)H_2PO_4$ and urea were studied by the method of Hucker (47, 48).

For lactobacilli 0.3 per cent agar was added to the glucose broth. To determine ability of these organisms to cause greening fresh frankfurters were placed in sterile petri dishes (7 discs to a plate) and steamed 20 minutes to destroy non-sporulating surface flora. Ten ml. sterile distilled water were added to the bottom of each plate, and a loopful of each culture (from tomato juice subculture) was spread on the upper surface of a disc. The seventh piece of frankfurter in each plate was left uninoculated. Duplicate plates were incubated at 68° F. (20° C.) and 86° F. (30° C.), and inspected for greening after 1, 2, 4, and 7 days.

IV. RESULTS AND DISCUSSION

A. Evaluation of Carbon Dioxide Control Methods

In the experimental methods three means of maintaining CO_2 concentrations at the desired levels were described. These were designated the constant flow, evacuation and displacement methods. Continuous passage of a mixture of metered CO_2 and air through storage containers gave excellent control, but had a pronounced disadvantage. The considerable amount of apparatus required made the method impractical for ordinary laboratory usage when it was necessary to hold samples concurrently under several different CO_2 levels.

In an alternative procedure the gas concentration in each container was adjusted at suitable intervals during storage by one of the two methods (evacuation and displacement) previously discussed. While these methods admittedly allow some variation in gas composition, they make possible storage of samples with several CO_2 levels and at more than one temperature at the same time.

Table 1 gives the results of a series of analyses on 2-quart jars containing chicken thighs. After each analysis jars were regassed by the evacuation method. In general this procedure gave an accuracy of $\pm$ 0.5 per cent CO_2. Greater variation was obtained in the first part of the storage period due to solution of CO_2 in the meat. After the samples had dissolved enough CO_2 so that equilibrium with atmospheres having the desired

Table 1. Periodic analyses of CO_2 concentrations in jars containing thighs of commercially dressed chicken[a]

Temp. °F.	50°			40°				32°			
% CO_2 desired	0	15	25	0	5	12	25	0	5	15	25
Time stored (days)	CO_2 analyses										
1	All jars regassed but analyses not made.										
2	3.4[b]	15.9	25.1	0.3	5.1	15.0	25.1	0.1	4.5	13.8	23.5
3		16.6[b]	25.9	0.5							
4			26.7	0.9[b]	5.5						
5				3.1	5.6	15.0	25.7	0.4	5.3	15.5	24.6
6				3.8	6.5						
7					7.9[b]	15.6					
8						15.8	25.8	0.3	5.4	15.4	24.5
9						17.0					
10						16.5[b]	25.4	1.3	5.5	15.2	25.1
11							25.7				
12						19.1	26.0[b]	2.9[b]	5.7	15.4	24.9
13							26.8				
14							26.7	4.5	7.1[b]	14.8	25.1
17							35.6			15.2	25.1
19										15.7	25.3
21										16.2	24.7
23										16.8[b]	25.3
25										16.5	24.9
27											25.5
29											26.1
31											26.5[b]

[a]Conditions of storage:
Temperatures and CO_2 levels noted above
Relative humidity about 100%
Two-quart jars
Atmospheres replaced immediately after analysis by evacuation and admittance of CO_2 and air

[b]End point of storage as determined by development of off odor

compositions was approached, changes in CO_2 levels were smaller and adjustments could be made less frequently. The use of a 2-quart jar, usually containing a single piece of chicken, minimized changes in gas composition by providing a rather large ratio of gas to sample. Toward the end of storage increases in CO_2 concentration were caused by gas-producing microorganisms.

In Table 2 are listed keeping times obtained with pieces of chicken and with frankfurters stored by the two methods described above. Samples compared were from the same batch in all cases. Differences obtained by the two methods were very slight indicating that approximately the same information was given by both.

The method of changing atmospheres by evacuation was later discarded because it caused blood to come to the surface of chicken, especially around the bone, and caused wrinkled skins on frankfurters. The rapid displacement method, which controlled CO_2 concentrations equally well without changing appearance of samples, was substituted.

B. Effect of CO_2 on the Storage Life of Cut-up Chicken

Storage of cut-up chicken in carbon dioxide atmospheres can be divided into two categories on the basis of the manner of application. In the first, the container is tightly sealed after the desired atmosphere has been obtained, and the gas composition is allowed to follow its own course during the storage period. In the second, the initial gas composition is maintained during storage. To determine the effect of a given CO_2 concentration in prolonging the keeping time of meat it is, of course, important that the

Table 2. Keeping times obtained for cut-up chicken and frankfurters by two methods of maintaining gas composition[a]

Sample	Atmosphere	Keeping time under flowing gas (days)	Keeping time when gas concentration maintained by periodic adjustment (days)
Chicken thigh	Air	19	17
Chicken thigh	15% CO_2	31	31
Chicken thigh	Air	11	10
Chicken thigh	15% CO_2	21	21
Chicken back	Air	10	9
Chicken back	15% CO_2	17	16
Frankfurters	Air	20	20
Frankfurters	15% CO_2	32	31

[a]Conditions of storage:
Temperature, 40° F. (4.4° C.)
Relative humidity, approximately 100%
One-quart jars for samples stored in flowing gas
Two-quart jars for samples stored in sealed containers with atmospheres adjusted periodically
End points determined from off-odor

CO_2 level be held as constant as is feasible. However, in view of its possible practical applications, the first mentioned category deserves further attention.

1. Storage in containers sealed throughout holding period--CO_2 level not constant

Experiments of this type were undertaken primarily to study organoleptic changes in cut-up chicken stored in sealed containers; and also, to learn something about changes in pressure and concentrations of carbon dioxide and oxygen.

a. Keeping times. Average keeping times as measured by the development of off-odor, are given in Table 3 for several conditions of storage. These times were obtained from the data on the individual jars listed in Tables 1A to 7A in the appendix. At 50° F. (10° C.) carbon dioxide at any level afforded very little benefit. CO_2 gave better results at 40° F. (4.4° C.) the storage life being more than doubled for both thawed-frozen and freshly killed poultry.

Keeping times alone are not sufficient for evaluating the effect of carbon dioxide on storage life. This is amply illustrated in Table 3 which indicates a longer keeping time for freshly killed chicken (I.S.C.) stored without any CO_2 at 40° F. (4.4° C.) than for thawed-frozen chicken held under 96% CO_2 at the same temperature. Obviously, it is necessary to compare storage life of CO_2-stored samples with that of similar samples stored in air under the same conditions. This is done conveniently by computing the ratio of keeping time in CO_2 to keeping time in air. The

Table 3. Time for appearance of definite discoloration and off-odor in chicken stored in sealed containers[a]

Initial CO_2 Conc.(%)	Temp. °F.	Type of chicken	Time discoloration first observed (days)	Time off-odor observed (days)	Off odor in control (days)
96	50	Thawed-frozen	2	2-3	2
80	50	" "	4	2-4	2
80 (20% O_2)	50	" "	4	4	2
25	50	Freshly-killed	none	6	4
96	40	Thawed-frozen	6	12	5
25	40	" "	8 (slight)	5-8	4
25	40	Freshly killed	none	34	14

[a]Conditions of storage:
Relative humidity approximately 100%
Samples stored in 1-qt. jars
Atmospheres obtained by evacuation method

resulting value will henceforth be referred to by the term "storage index".

$$\text{Storage index} = \frac{\text{storage life in } CO_2}{\text{storage life in air}}$$

At 40° the storage index with 96 per cent CO_2 was 2.40 as compared with a figure of 2.43 for 25 per cent. This does not mean that 96 per cent CO_2 was not more effective in inhibiting slime forming bacteria. Indeed, the typical slime with its characteristic odor, which ordinarily develops on chicken stored in air, was never encountered to any extent with chicken stored under CO_2 in sealed containers. In the early stages the odor commonly developing could best be described as sour. Soon afterward it acquired a very offensive, putrid character. When gas samples from jars containing chicken in this condition were passed through lead acetate solution, a

black precipitate formed, suggesting that appreciable amounts of hydrogen sulfide were present. Evidently the conditions obtaining in the sealed jars favored rapid development of facultative or anaerobic bacteria at the expense of slime formers.

In Table 4 keeping times secured by the "sealed container" method are compared on the same batches of chicken with keeping times obtained by a method in which storage atmospheres were renewed at intervals in order to keep CO_2 levels constant; longer keeping times were obtained by the second method. This is not really a fair comparison since CO_2 concentrations in sealed containers are lower than initial levels through most of the storage period. However, some importance can be attached to the fact that an increase in CO_2 percentage from 15 to 25 gave a greater prolongation of storage life in the second method. This constitutes further evidence that predominant flora in the samples stored in sealed containers did not consist of the usual slime producing bacteria. The former were not influenced as much by carbon dioxide as were the latter.

b. Discoloration. Discoloration developed rapidly when high concentrations of carbon dioxide were used. As shown in Table 3 and Tables 1A to 7A, discoloration was obtained in 2 days with 100 per cent CO_2 and in 4 days with 80 per cent when storage temperature was 50° F. (10° C.). The use of 20 per cent oxygen together with 80 per cent CO_2 did not noticeably delay the appearance of off-color. At 40° F. (4.4° C.) discoloration was delayed until the sixth day when the atmosphere contained 96 per cent CO_2. Even with 25 per cent CO_2 slight darkening was observed in many cases after 8 days at 40° F. (4.4° C.).

Table 4. Keeping times for cut-up chicken stored at 40° F. by the "sealed container" and the "constant CO_2" methods[a]

Run no.	CO_2 level (%)	Sealed containers (days)	Constant CO_2 (days)
4A	15	8	10
	25	9	12
5A	15	11	12
6A	15	7	8
	25	8	12

[a]Conditions of storage:
Relative humidity approximately 100%
Sealed containers--1-qt. jars, atmospheres obtained by evacuation method
Constant CO_2--2-qt. jars, atmospheres obtained and maintained by displacement method
End points determined by off-odor

It may be concluded that high concentrations of CO_2 are not satisfactory for storing cut-up chicken because of discoloration. In the early stages the change in color due to CO_2 was evidenced as a darkening of the normal red color; in advanced stages the color became grayish brown. While this type of discoloration probably does not influence flavor, it undoubtedly decreases acceptability. A slight loss of bloom is difficult to detect because degree of pigmentation of different birds and different pieces from the same bird vary considerably. It follows that the time at which discoloration appears and the extent of discoloration are difficult to evaluate when lower concentrations of CO_2 are used in storage. One would expect that, with storage in sealed containers, loss of bloom (oxidation of myoglobin) would tend to be more rapid due to the constantly diminishing amount of oxygen than would be the case wherein the sample was held in an atmosphere of approximately constant composition.

c. Changes in pressure and gas composition. Large and rapid reduction in CO_2 concentration and pressure took place within sealed jars when a considerable proportion of the available space was occupied by chicken. Considerable changes were obtained even after jars had been opened and regassed subsequent to the initial gassing operation. Results of a series of analyses and pressure measurements on a single 1-quart jar are given in Table 5. The jar contained about 2 grams of chicken per ml. of gas. It was necessary to replace the atmosphere 4 times over a period of 5 days before CO_2 analyses on two successive days were the same.

Table 5. Change in CO_2 analysis and pressure obtained with successive gassings[a]

Time stored (days)	% CO_2 by analysis	Vacuum[b] (Cm. Hg.)
1	68.2	38.5
3	81.0	11.0
4	92.8	7.0
5	96.5	0.5
6	96.5	0

[a]Conditions of storage:
Temperature--50° F. (10° C.)
Relative humidity about 100%
Container--1-quart sealed Mason jar
Contents of jar--1 thigh, 1 leg, 1 back, 1 breast, 1 ribs, giblets
Atmosphere displaced by 100% CO_2 after each analysis

[b]Vacuum was measured immediately before gas sample was removed for analysis

In practice it was seldom possible to get CO_2 levels of over 96 to 97 per cent in the jars. This can be ascribed to a number of factors. Some CO_2 is lost from the meat whenever a jar is opened or a gas sample taken for analysis. As a result some of the CO_2 from the newly supplied atmosphere will be taken up after the jar has been sealed. A part of the atmosphere will always be water vapor, the amount depending on the temperature. At 68° F. (20° C.) the enclosed air should contain about 2.4 per cent water vapor; at 50° F. (10° C.) 1.25 per cent; and at 40° F. (4.4° C.), 0.85 per cent. Also, small amounts of oxygen and nitrogen would be expected to diffuse out of the meat and/or bones after the jar was sealed. Commercial carbon dioxide may contain up to 0.5 per cent of impurities.

Table 6 gives CO_2 and oxygen analyses and vacuums measured on jars of thawed-frozen chicken stored for various times at 50° F. (10° C.); initial CO_2 concentration in each jar was 96 per cent. Similar data for other conditions of storage can be found in Tables 1A to 8A. Vacuums of more than 30 cm. of mercury were commonly encountered during the first part of the storage experiment when initial CO_2 concentration was near 100 per cent. Even with 25 per cent CO_2, vacuums in the neighborhood of 20 cm. of mercury were obtained. Oxygen concentration tended to decrease as storage progressed while changes in CO_2 level were irregular.

The results of the vacuum measurements suggest that a large proportion of the CO_2 is lost from the atmosphere to the meat, and that the loss takes place largely during the first two days of storage. Obviously

Table 6. Changes in pressure, CO_2 and oxygen concentration, and calculated CO_2 losses during storage of thawed-frozen chicken in sealed containers[a]

Sample no.	Time stored (days)	Grams chicken per ml. CO_2 (initial)	CO_2 concen. (%)	Vacuum (cm. Hg)	O_2 concen. (%)	CO_2 loss (ml./gm.)
VII-1	1	1.93	68.2	38.5	7.7	0.35
XI-21	1	1.76	53.0	32.0	9.1	0.39
VIII-11	2	1.72	71.5	30.4	1.6	0.33
IX-15	2	1.87	72.2	39.0	2.6	0.35
X-2	2	1.61	72.4	35.7	2.0	0.39
VIII-15	3	1.82	71.7	37.3	2.4	0.35
X-3	4	1.70	90.7	28.0	0.9	0.25
VIII-6	6	1.88	70.0	14.4	1.6	0.22
IX-12	7	1.77	48.3	9.0	8.3	0.32
X-4	7	1.67	69.1	16.0	5.1	0.27
VIII-5	8	1.87	63.8	3.1	0.3	0.195
VIII-9	10	1.78	52.2	13.5	0.3	0.21
X-5	10	1.70	83.2	0.5	1.4	0.105

[a]Conditions of storage:
Temperature 50° F. (10° C.)
Initial CO_2 level, 96%
Relative humidity, approximately 100%
Storage containers, 1 qt. jars
Atmospheres obtained by evacuation method

such changes in pressure could cause serious difficulties in storing meat with CO_2 in gas tight, non-rigid containers.

A decrease in oxygen concentration would be expected due to respiration of the chicken tissue and microorganisms present. The actual amount of oxygen present in the jars was probably somewhat less than the data indicate since it was possible for some air to enter a jar from the manometer during pressure measurements. In some instances, unexpectedly high oxygen levels were found indicating that air had leaked into some jars. There were fewer irregularities in oxygen concentration in later experiments in which a sealing compound was used on the jars. The scantiness or absence of surface growth on pieces of chicken and the putrefactive odor usually encountered were evidence that conditions in most jars were anaerobic or nearly so.

Carbon dioxide analyses by themselves appear to give little information on the changes taking place in the storage containers. However, in conjunction with pressure and temperature measurements, the final concentration of CO_2 can be used to calculate the amount lost from the atmosphere (or the amount dissolved in the meat) provided that the volume is known. One-quart jars of known volume were used in all experiments. In order to determine the volume in each jar occupied by chicken it was necessary to know specific gravities of the various pieces. Table 9A gives specific gravities of pieces of thawed-frozen chicken. The average value of 1.086 was used in all cases in calculating the space occupied by the weighed pieces of chicken.

Average values for CO_2 uptake by various amounts of chicken are shown in Table 7. Individual values are given in Table 6 and in Tables 1A to 8A in the appendix. The amount of CO_2 which the chicken dissolved was determined during the first part of the storage period before microbial action became important. As storage time increased the apparent CO_2 loss to chicken became less as illustrated in Table 6. The amount of meat in a jar had a pronounced effect on the concentration of CO_2 at which equilibrium was attained. For instance with 96 per cent CO_2 (Table 7) the volume of CO_2 dissolved per gram of chicken was 0.36 ml. when chicken occupied slightly less than two-thirds of the jar; when one-fourth of the available space contained chicken the CO_2 taken up was 1.11 ml. per gram.

The changes in composition and pressure of atmospheres within the sealed jars containing meat are in accord with what would be expected from theoretical considerations. Carbon dioxide dissolves in the meat causing a reduced pressure within the container. Equilibrium is established at some pressure depending upon the temperature and the ratio of meat to gas. In order to obtain a given CO_2 concentration, equilibrium must be obtained at the desired level. For pieces of chicken equilibrium is at least approached in 1 day at 50° F. (10° C.) and within 2 days at 40° F. (4.4° C.). The apparent decrease in the amount of dissolved CO_2 in the last part of the storage time can be explained by the fact that the calculations do not take into consideration CO_2 produced by microorganisms. Reduction in calculated CO_2 uptake becomes most pronounced after the sample has developed off-odor.

Table 7. Uptake of CO_2 by chicken[a]

Initial CO_2 conc. (%)	Temp. (°F.)	Type of chicken	Average ratio of weight of chicken to volume of CO_2 (g/ml.)	CO_2 uptake[b] (ml./gm.)
96	50	Frozen-thawed	1.79	0.36
96	50	" "	0.27	1.11
96	40	" "	1.63	0.46
80	50	" "	2.13	0.30
25	40	" "	6.05	0.095
25	50	Fresh killed	6.54	0.067
25	40	" "	6.27	0.082

[a]Values obtained from measurements made on jars sampled during first phase of storage.

[b]Volume of CO_2 calculated for 740 mm. pressure and 20° C.

Solubility of CO_2 in pure water at 50° F. (10° C.) is 1.194 ml. per ml. (62; 760 mm. and 0° C.). In terms of 68° F. (20° C.) and 740 mm. pressure (the conditions used for calculating volumes of dissolved gas in this work), the solubility of CO_2 at 50° F. in water would be about 1.32 ml. per gram. Brooks and Moran (13 8a) estimated that a 150 lb. quarter of beef in equilibrium with 100 per cent CO_2 at 32° F. (0° C.) would dissolve 70 liters; the method of calculation was not given. Corrected to 60° F. the figure would be 1.13 (1.10) ml. CO_2 per gram of beef. Of course, neither the solubility of CO_2 in pure water at 50° F. or in beef at 32° F. are equivalent to the solubility of CO_2 in chicken at 50° F. It appears, however, that for storage of thawed-frozen chicken in sealed containers at 50° F. (10° C.) with 96 per cent CO_2, the gas is dissolved to the extent of roughly

one-third of the saturation value at atmospheric pressure when the ratio of meat to CO_2 is 2 and approaches saturation when the ratio is 0.25.

Carbondioxide uptake by meats conceivably could become of considerable importance in packaging in CO_2 atmospheres. In certain cases it would be expedient to allow small pieces of meat to reach equilibrium with an atmosphere containing a rather high concentration of CO_2. Subsequent packaging in gastight materials would allow a new equilibrium to be established at a lower level of CO_2. Experiments in this work were not designed primarily to study solution of CO_2 in meat, and insufficient data were obtained to show a general relationship between CO_2 uptake and the ratio of meat to gas. However, using the methods employed here, it should be possible to obtain a curve for that relationship at any given temperature.

2. Storage in containers in which CO_2 concentration was maintained at a given level--gas changed constantly or at intervals

a. Establishment of end points. It is not easy to decide upon the time at which a piece of meat is spoiled. Perhaps the best criterion would be a definite decrease in palatability as discerned in the cooked product by a taste panel. Of course, the question of how great a change in palatability is required is difficult to answer; it will vary with the individual. Also, palatability of the cooked product does not tell the whole story; raw meat products having an obvious off-odor or a disagreeable external appearance may never be cooked and tasted.

In the case of cut-up poultry stored at temperatures above freezing, development of a characteristic odor and a surface slime gave rather good

indications of the limits of storage. Detection of the first definite off-odor was the usual method of deciding upon the end point. It seemed desirable to attempt to correlate development of off-odor with other changes.

It had been noted that appreciable amounts of CO_2 were evolved by bacteria proliferating on chicken near the completion of storage experiments (see Table 1). By means of periodic analyses of gas samples, extent of CO_2 production was evaluated and used to determine storage end points. The limit of storage was considered to have been reached when an increase of CO_2 concentration of 1 per cent was obtained in 1 day at 40° F. (4.4° C.) and 50° F. (10° C.) and in 2 days at 32° F. (0° C.). In Table 8 values for storage life obtained by CO_2 measurements are compared with those from off-odor and bacterial count observations.

End points were established also by determining the times at which numbers of bacteria on chicken surfaces reached a specific value. Previous observations had indicated that off-odors became detectable on air-stored chicken when the number of bacteria per square centimeter of surface was somewhat above 10^8. A value of 2×10^8 was selected for the purpose of comparing keeping times. Keeping times obtained by bacterial counts are compared with those resulting from the other two procedures (Table 8). In most cases agreement is fairly good. This is not surprising considering that all three methods depend on manifestations of microbial growth. The fact that there is agreement for all cases suggests that the predominant flora is not markedly affected by temperature (in the range of 32° to 50° F.), or by CO_2 level (in the range 0 to 25 per cent). Keeping times as measured by bacterial counts tend to be somewhat shorter than by the other methods.

Table 8. Keeping times of fresh commercially dressed chicken as determined by development of off odor, production of CO_2 and bacterial growth curves[a]

Run no.	Temp. (°F.)	CO_2 concen. (%)	Keeping times (days)		
			Off-odor	CO_2 production[b]	Growth curves[c]
4 A	50	0	2	2	1.75
		15	3	3	3.00
		25	4	4	3.50
	40	0	4	4	4.25
		5	7	6	5.75
		15	10	9	9.25
		25	12	13	13.25
	32	0	12	10	9.50
		5	14	14	11.50
		15	23	21	18.50
		25	31	29	28.00
5 A	40	0	7	7	6.50
		5	9	10	9.00
		10	11	11	11.00
		15	12	13	11.50
		20	15	16	14.50
		25	16	18	17.00
	32	0	17	15	17.00
		5	24	24	22.50
		10	26	26	24.00
		15	39	39	35.00
		20	44	45	42.00
		25	44	48	42.00

[a]Conditions of storage:
2-qt. jars
Relative humidity about 100%
Run 4A--atmospheres changed at intervals by evacuation method
Run 5A--atmospheres changed at intervals by displacement method

[b]Time at which an increase of 1% CO_2 over the expected level was obtained

[c]Time at which no. of bacteria per sq. cm. reached 2×10^8

That is partially, at least, the result of the methods used. Keeping time can be determined to a fraction of a day from growth curves, whereas observations on odor and CO_2 increase were made at 1 day or 2 day intervals.

b. Variations in keeping time. The keeping times of different pieces of chicken from the same batch or even from the same bird were compared on several occasions and were found to vary considerably. Table 9, in which are listed keeping times of duplicate pieces from the same bird stored with air and with 15 per cent CO_2, shows the magnitude of the variation in storage indices that can be obtained. Pieces such as the back and breast were cut in two aseptically to obtain duplicates. In Table 10 keeping times and storage indices (with 15 per cent CO_2) for chicken backs from different batches are compared. Similar results were obtained for other pieces. Table 11 gives average values for keeping time and storage index for various pieces determined from a number of observations.

A single series of observations indicates a pronounced variation in keeping times and storage indices for duplicate pieces and different pieces from the same or different birds. However, when a number of comparisons are made with a given piece of chicken, the average storage index is close to that obtained with any other piece (Table 11). Consequently, it appears that results obtained with chicken thighs can be used legitimately in making predictions for other pieces, except possibly for the giblets. Giblets show a peculiar odor several days before the characteristic odor accompanying incipient slime formation can be discerned. This odor is observed both in controls and CO_2 stored giblets; its onset is delayed only slightly by CO_2. In the early stages it is not particularly objectionable,

Table 9. Effect of CO_2 in prolonging storage life of pieces from the same bird[a]

Piece	Keeping time in air (days)	Keeping time in 15% CO_2 (days)	Storage index
Thigh	11	21	1.91
Thigh	10	21	2.10
½ back	10	17	1.70
½ back	9	16	1.78
½ breast	14	18	1.29
Giblets	10	16	1.60

[a]Conditions of storage:
Temperature, 40° F. (4.4° C.)
Relative humidity, approximately 100%
Containers, 2-qt. jars
CO_2 level constant, obtained and maintained by the evacuation method
End points determined from off-odor

Table 10. Effect of CO_2 on storage life of backs from different lots of chicken[a]

Keeping time in air (days)	Keeping time in 15% CO_2 (days)	Storage index
4	9	2.25
7	12	1.72
9	16	1.77
10	17	1.70
17	27	1.59
19	27	1.42

[a]Conditions of storage:
Temperature, 40° F. (4.4° C.)
Relative humidity, approximately 100%
Containers, 2-qt. jars
CO_2 level constant, controlled by the evacuation or displacement methods
End points determined from off-odor

Table 11. Effect of CO_2 on storage life of cut-up chicken[a]

Piece	Number of trials	Average keeping time in air (days)	Average keeping time in 15% CO_2 (days)	Average storage index
Thigh	8	10.2	18.3	1.81
Leg	5	11.0	18.6	1.75
Back	6	11.0	18.0	1.74
Breast	3	8.3	13.3	1.75
Giblets	3	7.0	12.3	1.81
Wing	1	19.0	31.0	1.63

[a] Conditions of storage:
Temperature, 40° F., (4.4° C.)
Relative humidity, approximately 100%
Containers, 2-qt. jars
CO_2 level constant, controlled by the evacuation or displacement methods
End points determined from off-odor

but usually becomes so before typical spoilage odor is noted.

The keeping quality of poultry affects the relative effectiveness of a given concentration of CO_2 in prolonging storage life. This point is effectively demonstrated in Table 10 for the six pairs of chicken backs. As initial quality of chicken improves, storage index tends to decrease although keeping times become longer. The same tendency was noted with other parts of the chicken.

c. Effect of CO_2 and temperature on storage life. Figures 4 and 5 and Tables 10A through 12A in the appendix show keeping times obtained with cut-up chicken from several sources stored under different CO_2 levels and at different temperatures. Those keeping times were based on the development of the first definite off-odor. In general increased CO_2 lengthened storage life as did reduced temperature. Keeping time varied considerably

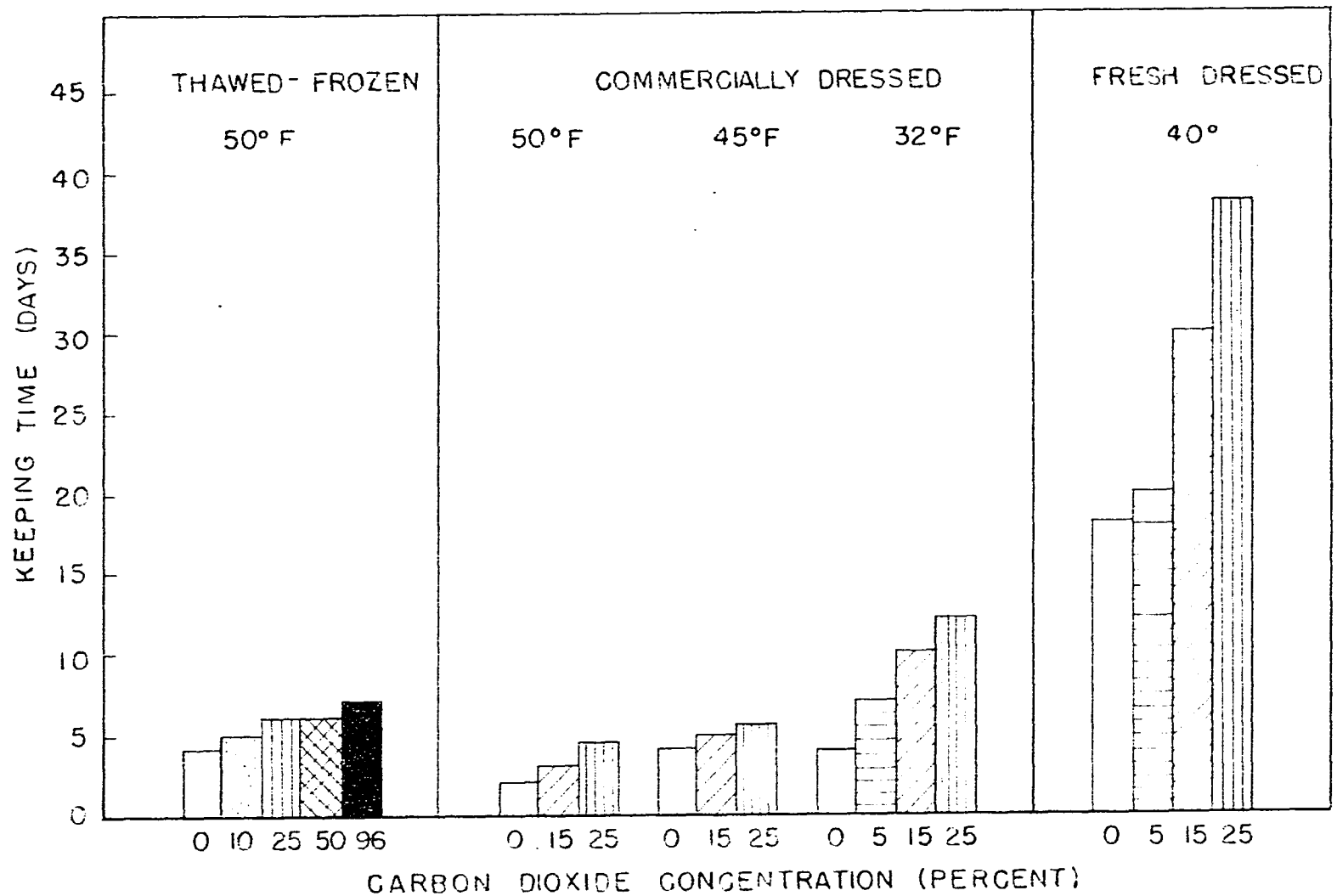

Figure 4. Effect of CO_2 concentration, temperature and source on the storage life of cut-up chicken

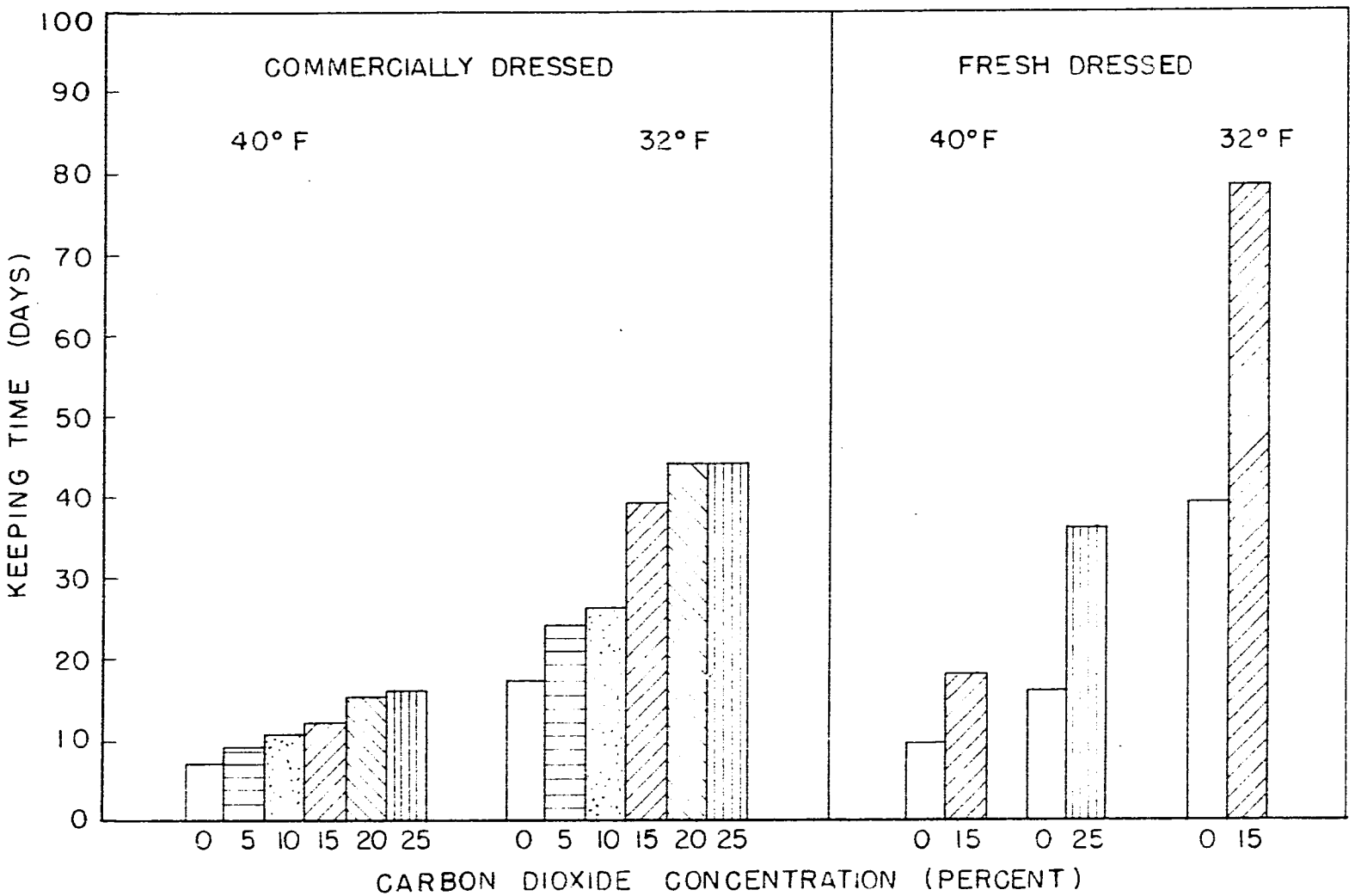

Figure 5. Effect of CO_2 concentration, temperature and source on the storage life of cut-up chicken

with the source of the chicken. Some discoloration was encountered after about 10 to 20 days at 40° F. (4.4° C.) and in 20 to 30 days at 32° F. (0° C.). In a few instances, when storage life was unusually long, considerable loss of bloom resulted.

The effect of CO_2 also is indicated in Figure 6 in which the average storage indices for each type of chicken are plotted against CO_2 concentration. Storage index likewise was increased by an increase in CO_2 level and a decrease in temperature. In addition to temperature, the source of chicken had pronounced influence on the slope of the curve. The results shown in Figure 6 suggest that the relationship between storage index and CO_2 concentration was approximately linear. To test this assumption, average values for storage index were plotted from a run in which 4 pieces from the same batch of chicken were stored at 40° F. (4.4° C.) under each of 6 CO_2 levels. In order to obtain a more accurate estimation of end points, storage indices were determined from growth curves (time to reach a surface count of 2×10^8 bacteria per square centimeter) rather than from off-odor production. Results appear in Figure 7. Apparently the relationship may be represented reasonably well by a straight line.

d. Effect of initial contamination. It was noted above that storage life depended to a large extent on the source of chicken, and that storage index was influenced also by this factor. It appeared that differences in initial contamination, in large measure, would account for the findings. Initial counts on experimentally dressed chicken were ordinarily in the neighborhood of 10^2 bacteria per square centimeter of surface; with thawed-frozen chicken counts commonly ranged from 10^3 to 10^4; initial counts on

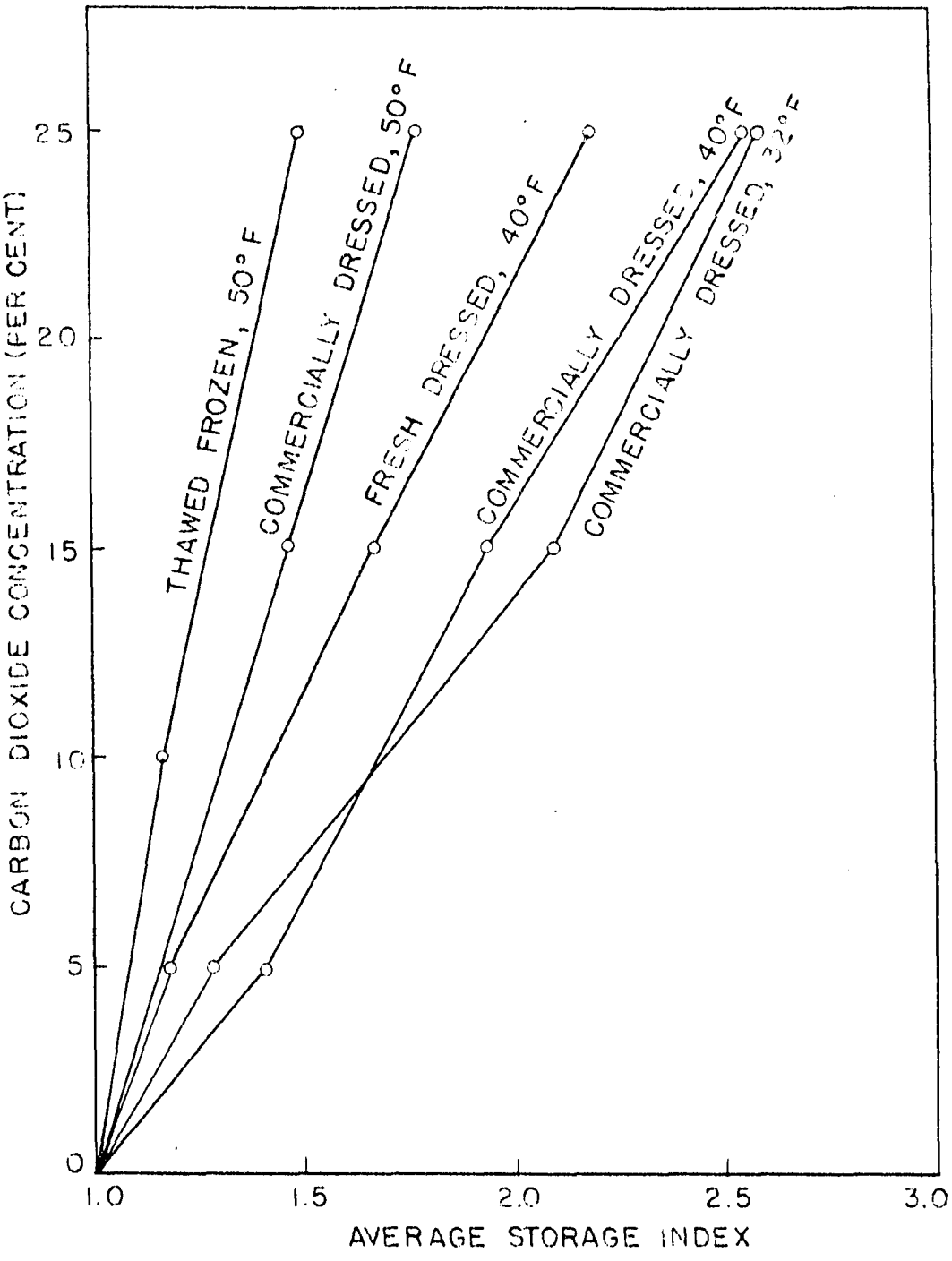

Figure 6. Relation of storage index to CO_2 concentration for different types of cut-up chicken

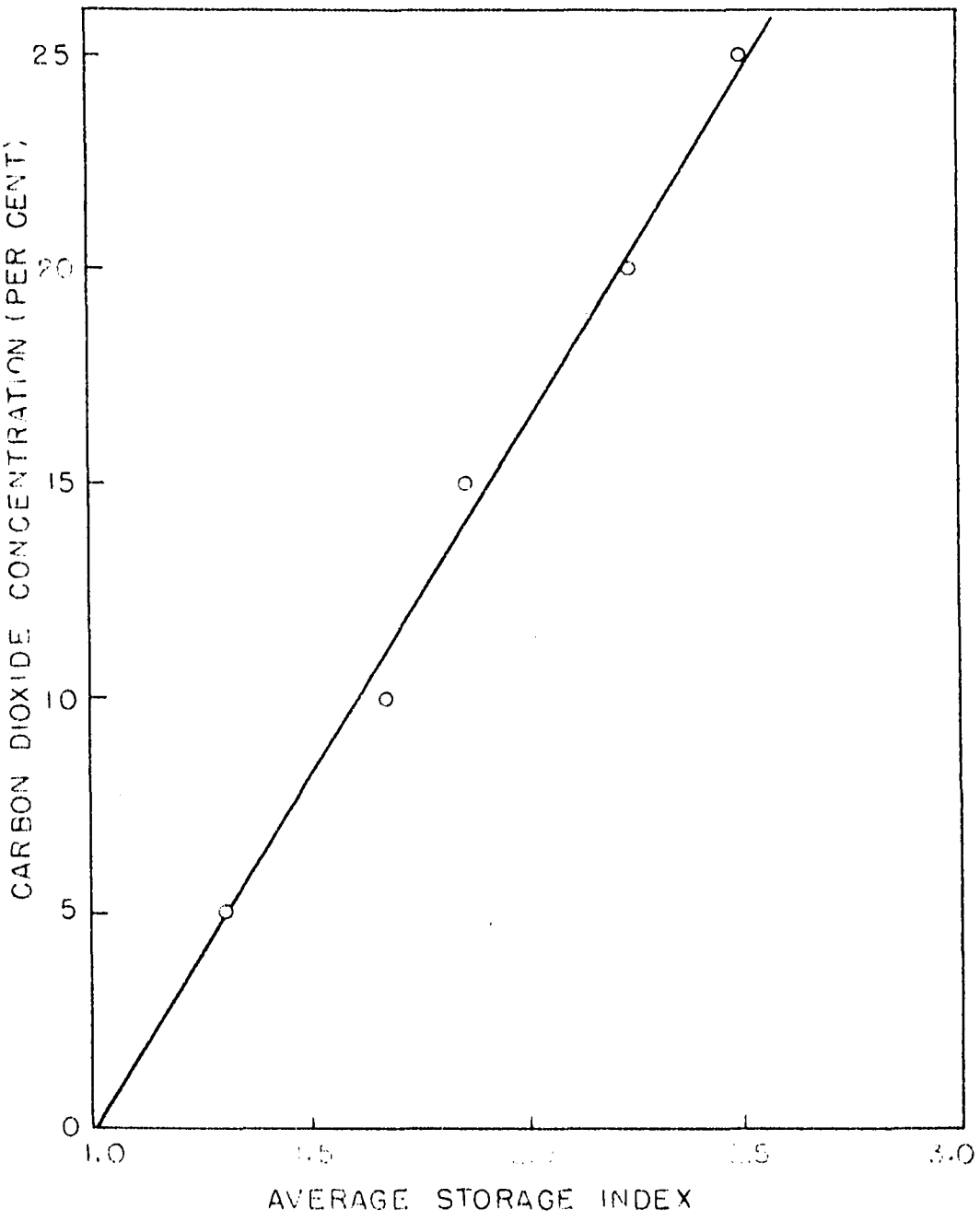

Figure 7. Relation of storage index to CO_2 concentration for fresh, commercially dressed chicken thighs[a,b]

[a]Each point represents average of quadruplicate determinations on one batch of chicken
[b]Storage temperature, 40° F. (4.4° C.)

the fresh commercial chicken employed usually ranged from 10^4 to more than 10^5.

In Figure 8 keeping times for various pieces of chicken are plotted against logarithms of initial bacterial counts. Again the relationship seems to be essentially linear. A reduction in contamination gave a marked increase in storage life. The benefits derived from a reduction of count were much greater at a lower temperature as is shown by the difference in slopes of the curves for 50° F. (10° C.) and 40° F. (4.4° C.). With any initial count, addition of 15 per cent CO_2 to the storage atmosphere resulted in a longer keeping time although the relative effectiveness was greater with higher counts.

e. Effect of relative humidity. No attempt was made to determine the effect of reduced humidities on keeping times of cut-up chicken because it appeared that no practical value would result from such a study. Previous investigators (10, 90) showed that fresh beef and frog muscle were in equilibrium with a relative humidity of more than 99 per cent. There was no reason to suppose that the corresponding figure for fresh chicken muscle would be very different. It had also been shown (40, 80) that moderate reductions in humidity caused rapid and extreme dehydration of small pieces of meat without markedly slowing the rate of microbial growth (35, 40). It seemed certain, then, that the extremely small pieces derived from cut-up poultry would have to be stored at high humidity in order to prevent excessive weight losses and loss of bloom accompanying dehydration. Very slight changes in weight were observed in the storage experiments indicating that relative humidity actually was maintained near 100 per cent.

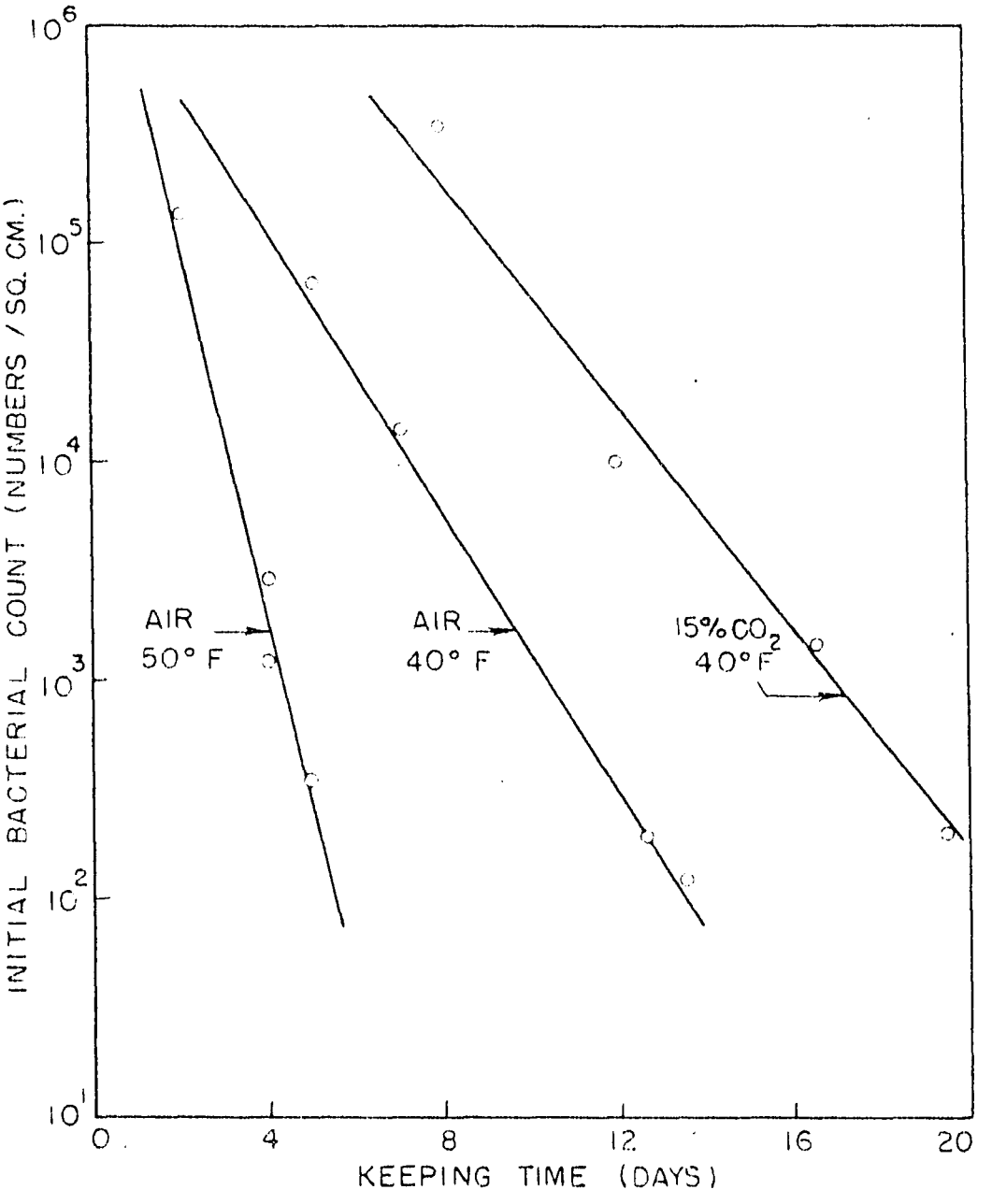

Figure 8. Relation of initial bacterial count to storage life of cut-up chicken

f. Discussion. It is evident that storage life of cut-up poultry is not merely a function of the physical conditions of storage such as temperature, composition of storage atmosphere and humidity, but depends to a great extent on the bacteriological quality of the chicken at the time storage is begun. Bacterial counts made on different pieces from the same bird, or even on different areas of the same piece may show considerable variation. The magnitude of this variation depends on the manner in which poultry has been handled previous to storage. Fresh dressed chicken was washed after plucking and evisceration and then cut up about an hour later in another location. Areas on muscle surfaces of the neck and inside of the back gave distinctly higher counts than areas on the thigh, leg or breast which were not exposed to contamination until the "cutting-up" process. Counts were more uniform on the fresh commercially dressed because cutting-up was done immediately after evisceration in the same location without an intermediate washing. All pieces were thrown into the same container of water.

Information on storage life in the presence of CO_2 is of little value in predicting storage life for chicken in general when obtained from a single sample or from a few samples of chicken. Average values on a large number of samples are also of limited value in deciding how long a particular batch of chicken will keep under specified conditions. The comparatively long keeping times of fresh dressed chicken, in spite of the fact that no special precautions were taken during processing to eliminate contamination, show that some commercial processing could be improved greatly.

When the approximate storage life in air is known, keeping time with a given CO_2 level can be predicted from the storage index. However, storage indices also are affected by the amount of contamination acquired before storage (Figure 8). Kinds as well as numbers of organisms affect storage life since only a part of the bacteria initially present can grow at refrigeration temperatures.

The extent of loss of bloom due to oxidation of heme pigments depends on CO_2 concentration, temperature and time of storage. With CO_2 levels of 25 per cent and below time assumes considerable importance. Chicken which spoils quickly will not have time to develop any discoloration while chicken with a very long storage life may discolor very badly before microbial spoilage sets in. At a temperature where some freezing occurs 30° F. (-1.1° C.) discoloration is more severe than at a temperature just above freezing (32° F., 0° C.).

g. Growth curve studies. The difficulties encountered in attempts to express the effect of CO_2 atmospheres and temperature in terms of storage life and storage index led to the search for a more fundamental method which would be independent of the numbers of bacteria originally present. Counts obtained from various samples were used in plotting bacterial growth curves on semi-logarithmic paper. Growth rates were determined from the slopes of logarithmic portions of the curves. Fresh, commercially dressed poultry from a local source was used in all of this work because of its comparatively uniform (although high) initial bacterial load.

(1) Effect of CO_2 on bacterial growth. Figure 9 gives growth curves obtained from a series of bacterial counts made on chicken thighs stored at 50° F. (10° C.) with atmospheres of 0, 15 and 25 per cent CO_2 (Run 4A). Figures 10 and 11 show similar curves obtained from thighs of the same batch of chicken at 40° F. (4.4° C.) and 32° F. (0° C.) with CO_2 atmospheres of 0, 5, 15 and 25 per cent CO_2. The horizontal distance from the initial count to the point of intersection with the logarithmic portion of the curve was considered to represent the lag time. At any of the temperatures studied an increase in CO_2 level definitely decreased the slope of the linear part of the growth curve. There was also a tendency for lag to increase with CO_2 concentration although results were not consistent in this respect.

The velocity coefficient, k, was computed from the logarithmic part of each curve by the formula:

$$k = \frac{2.3}{t} (\log b - \log B) \quad \text{in which:}$$

B = number of bacteria at the beginning of a given time ($t = 0$).

b = number of bacteria at the end of time t.

The average generation time, g, was obtained from the formula:

$$g = \frac{\ln 2}{k} = \frac{0.694}{k}$$

Values obtained for b, B, t, k and g are listed in Table 13A in the appendix. Logarithms of average generation times at each temperature were found to be approximately proportional to carbon dioxide concentration in the range 0 to 25 per cent as is shown in Figure 12.

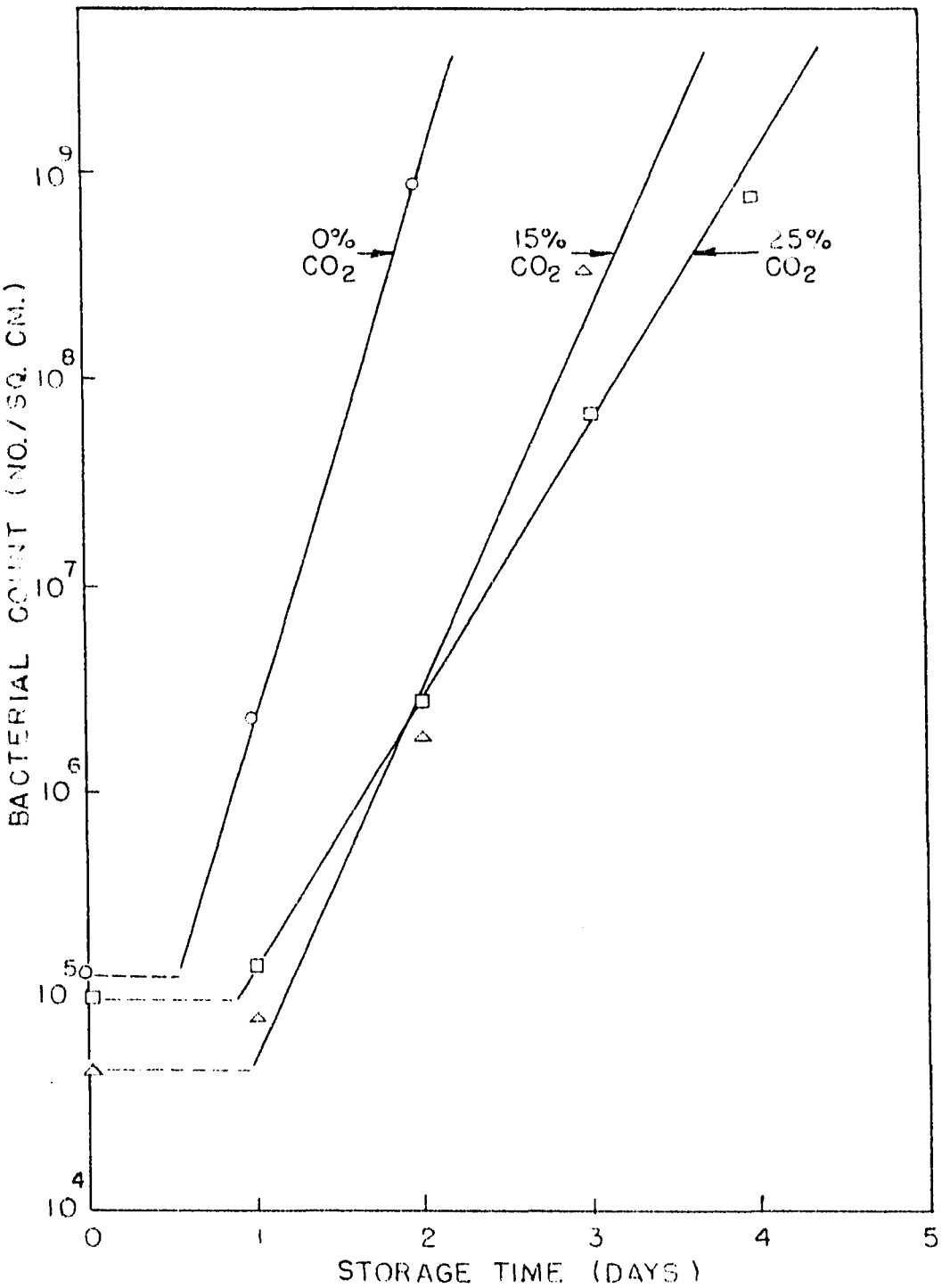

Figure 9. Effect of CO_2 on growth curves of bacteria on chicken thighs at 50° F.

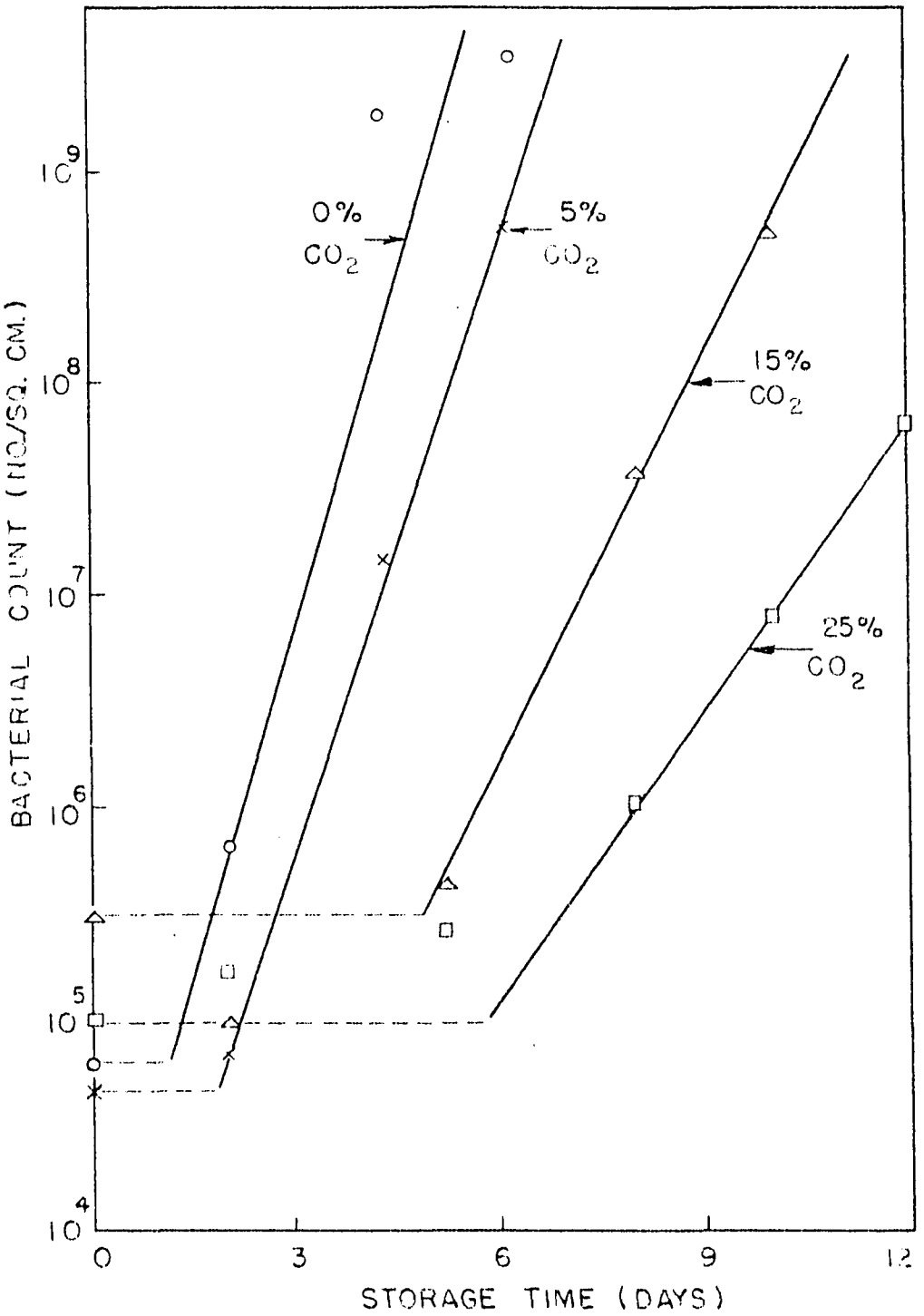

Figure 10. Effect of CO_2 on growth curves of bacteria on chicken thighs at 40° F.

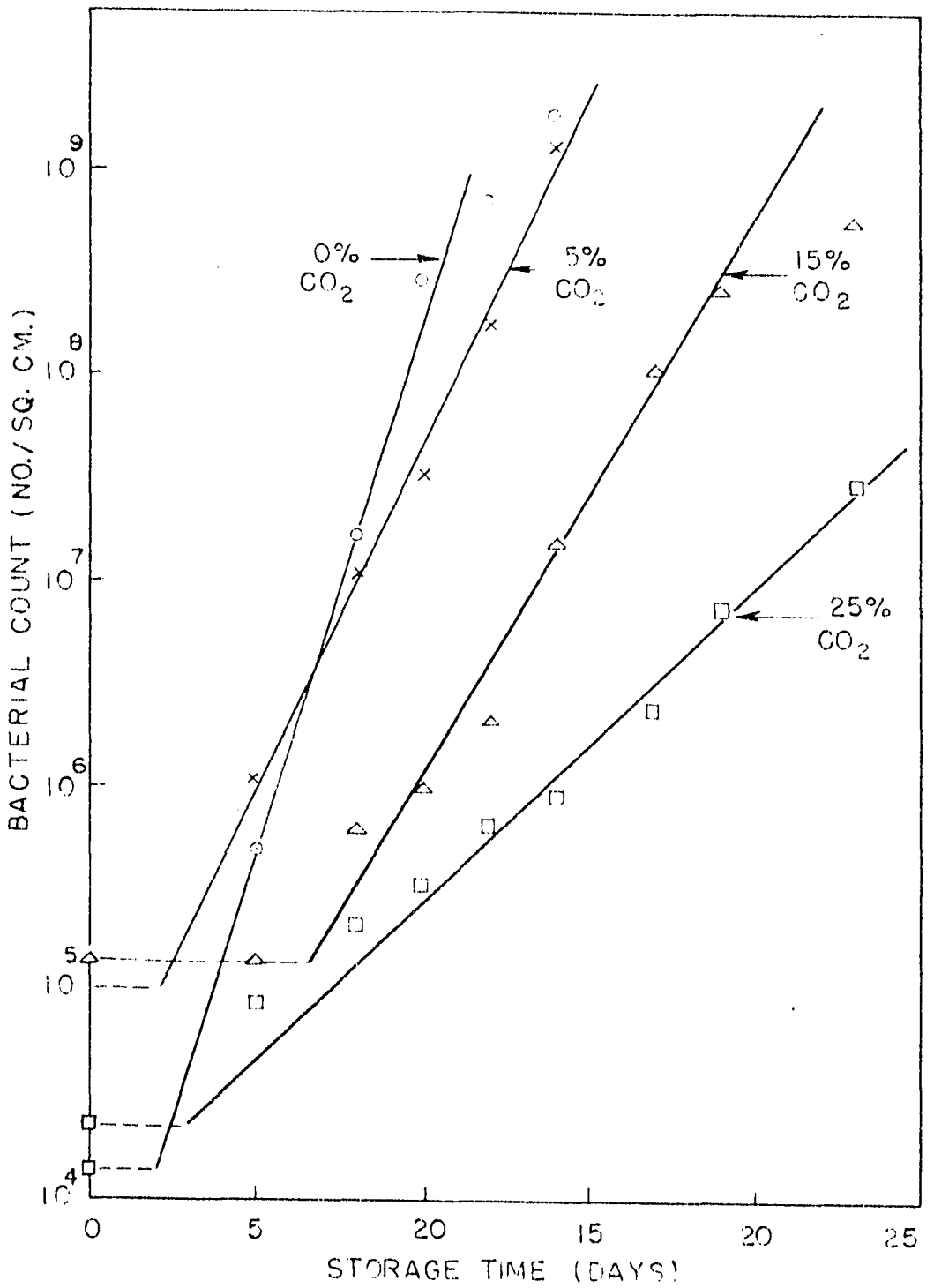

Figure 11. Effect of CO_2 on growth curves of bacteria on chicken thighs at 32° F.

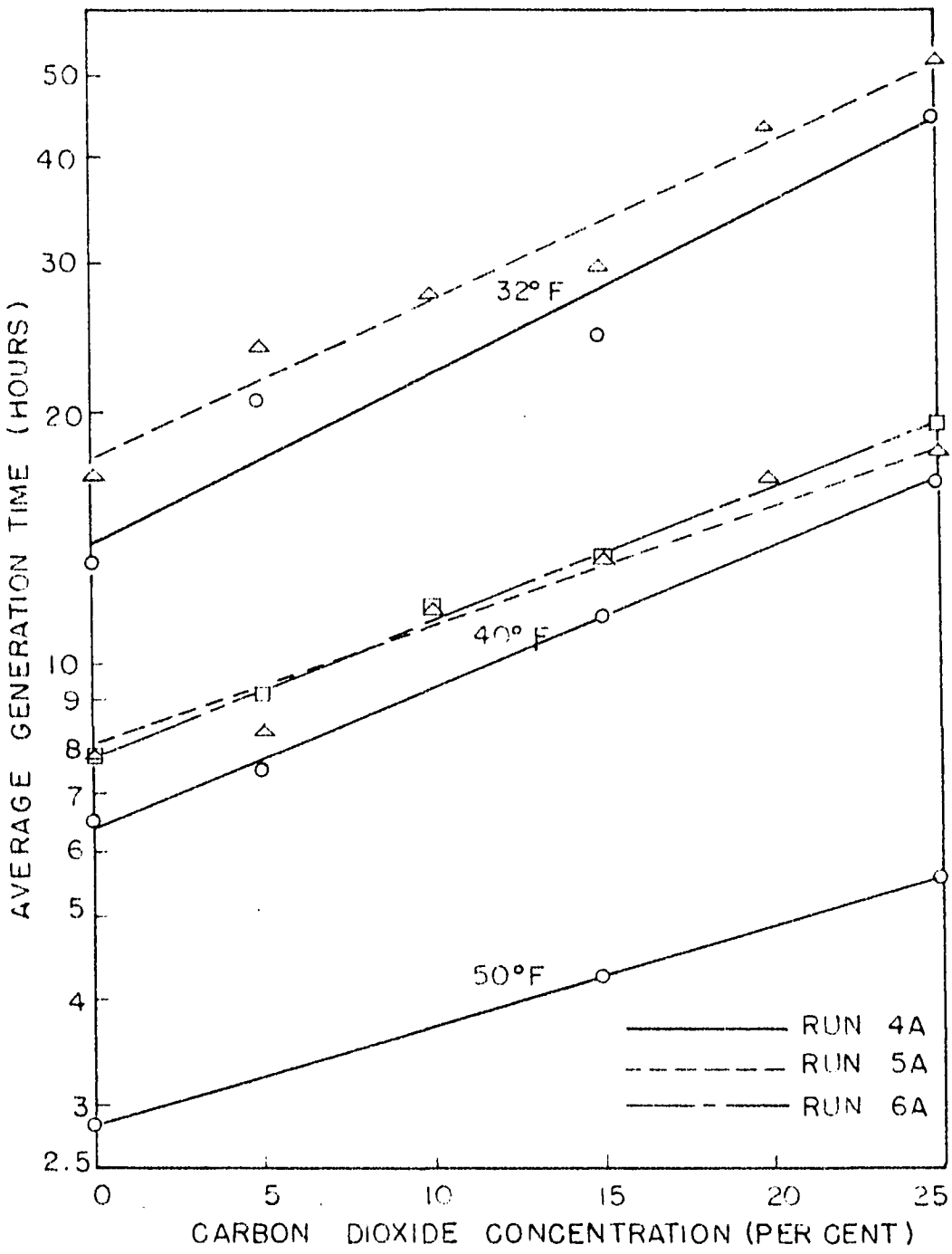

Figure 12. Relation of CO_2 concentration to average generation time at temperatures of 50°, 40° and 32° F.

To test the reproducibility of the above results, an additional storage experiment (Run 5A) was run at 40° F. (4.4° C.) and 32° F. (0° C.) on thighs from another batch of chicken using CO_2 levels of 0, 5, 10, 15, 20 and 25 per cent. Bacterial counts are given in Table 14A in the appendix. Again CO_2 was found to increase lag time and retard rate of reproduction. Values of k and g were again computed (Appendix Table 15A), and logarithms of average generation time were plotted against CO_2 concentration as before. In Figure 12 the resulting curves are compared with those obtained from Run 4A. The new curves were approximately parallel to the previous ones but average generation times at equivalent temperatures were somewhat greater than previous values.

As a final check one more experiment (6A) was undertaken at 40° F. (4.4° C.) in which 3 thighs and 1 leg were stored with CO_2 levels of 0, 5, 10, 15, 20 and 25 per cent. (All pieces were from the same batch of chicken). The individual and average counts obtained at each CO_2 concentration are presented in the appendix, Table 16A. Average counts were used to plot the growth curves shown in Figure 13. Counts from legs were made on the skin surfaces. Growth curves from skin surfaces varied no more from the average curves than did individual curves from muscle. It was concluded that growth rates on skin and muscle were approximately equal. Logarithms of figures for average generation time (Appendix Table 17A) when plotted against CO_2 level (Figure 12) gave a curve which practically coincided with the one obtained for 40° F. in Run 5A. Apparently the logarithm of generation time is a linear function of CO_2 level, and similar results can be obtained with chicken from various batches.

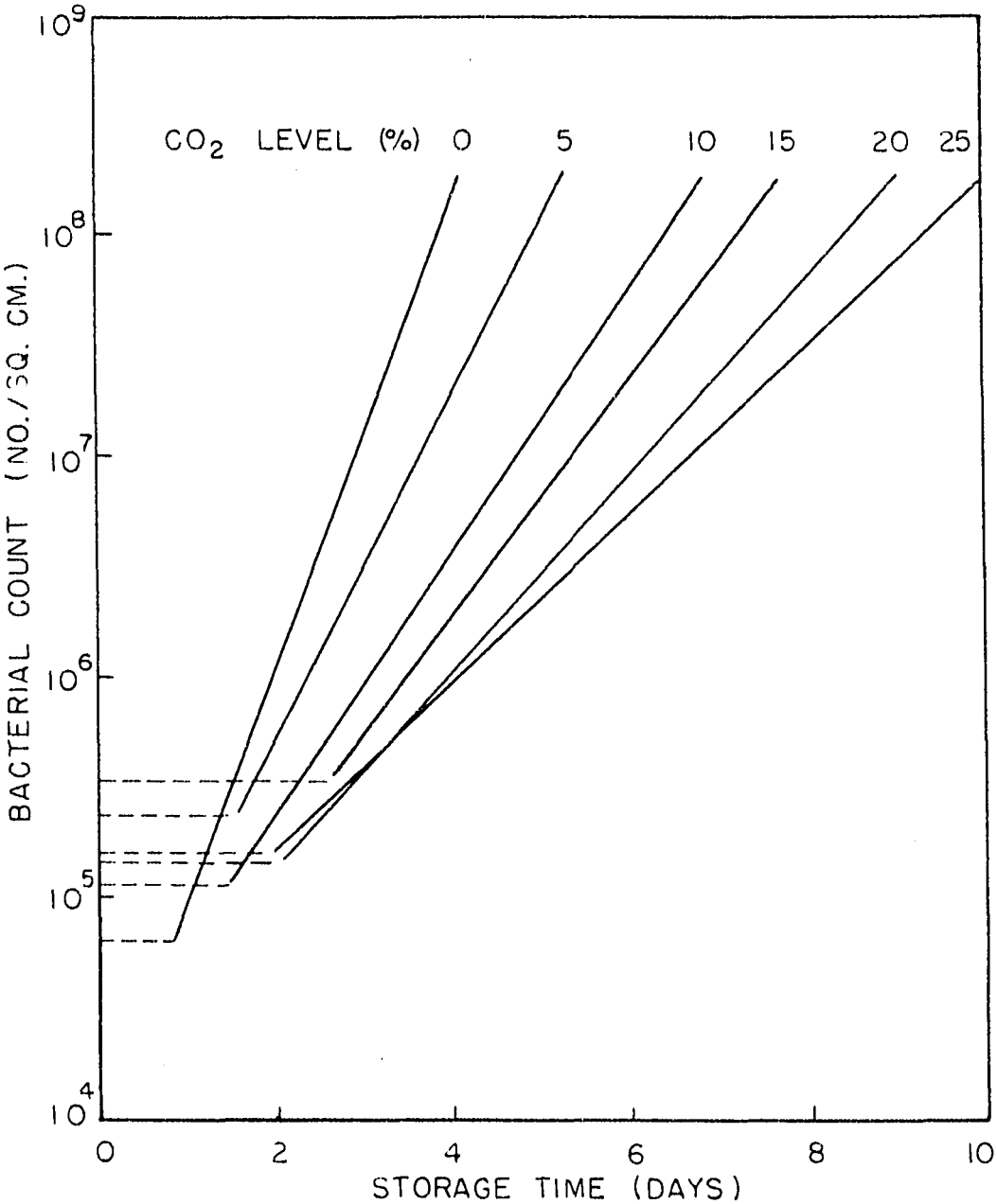

Figure 13. Effect of CO_2 on growth curves of bacteria on chicken thighs and legs at 40° F.[a]

[a]Each curve represents average counts from three thighs and one leg.

(2) Effect of temperature on bacterial growth. The influence of temperature on growth rate can be seen by comparing growth curves at different temperatures in Figures 9, 10 and 11. Figure 14 gives growth curves at 50° F. (10° C.), 40° F. (4.4° C.) and 32° F. (0° C.) in the absence of CO_2. A reduction in temperature increased log time and slowed rate of bacterial multiplication in the logarithmic phase. This was found to be true both with air storage and CO_2 storage. Using the data from Run 4A, logarithms of average generation times were plotted against temperature (° F.) for CO_2 concentrations of 0, 15 and 25 per cent (Figure 15). Straight lines were obtained. According to the Arrhenius-van't Hoff equation, log k plotted against the reciprocal of absolute temperature gives a straight line. In this equation k is the velocity constant at any absolute temperature T or in the case of bacteria it is the velocity coefficient of growth. Since generation time is proportional to k, the straight line relationship also would result when the logarithm of average generation time was plotted against temperature. The results shown in Figure 15 indicated that the Arrhenius-van't Hoff equation applied to the relation of rate of growth to temperature in the range 0 - 10° C. This was true both in the absence and presence of CO_2.

Values for the constant μ, the temperature characteristic, were computed from the following form of the Arrhenius-van't Hoff equation:

$$\mu = 4.6 \frac{(\log k_2 - \log k_1)\ T_2\ T_1}{T_2 - T_1}$$

For 0 per cent CO_2 μ was 23,600; for 15 per cent, 27,200; for 25 per cent, 32,000. Q_{10} values were also calculated. These were 4.62, 5.83 and 7.94

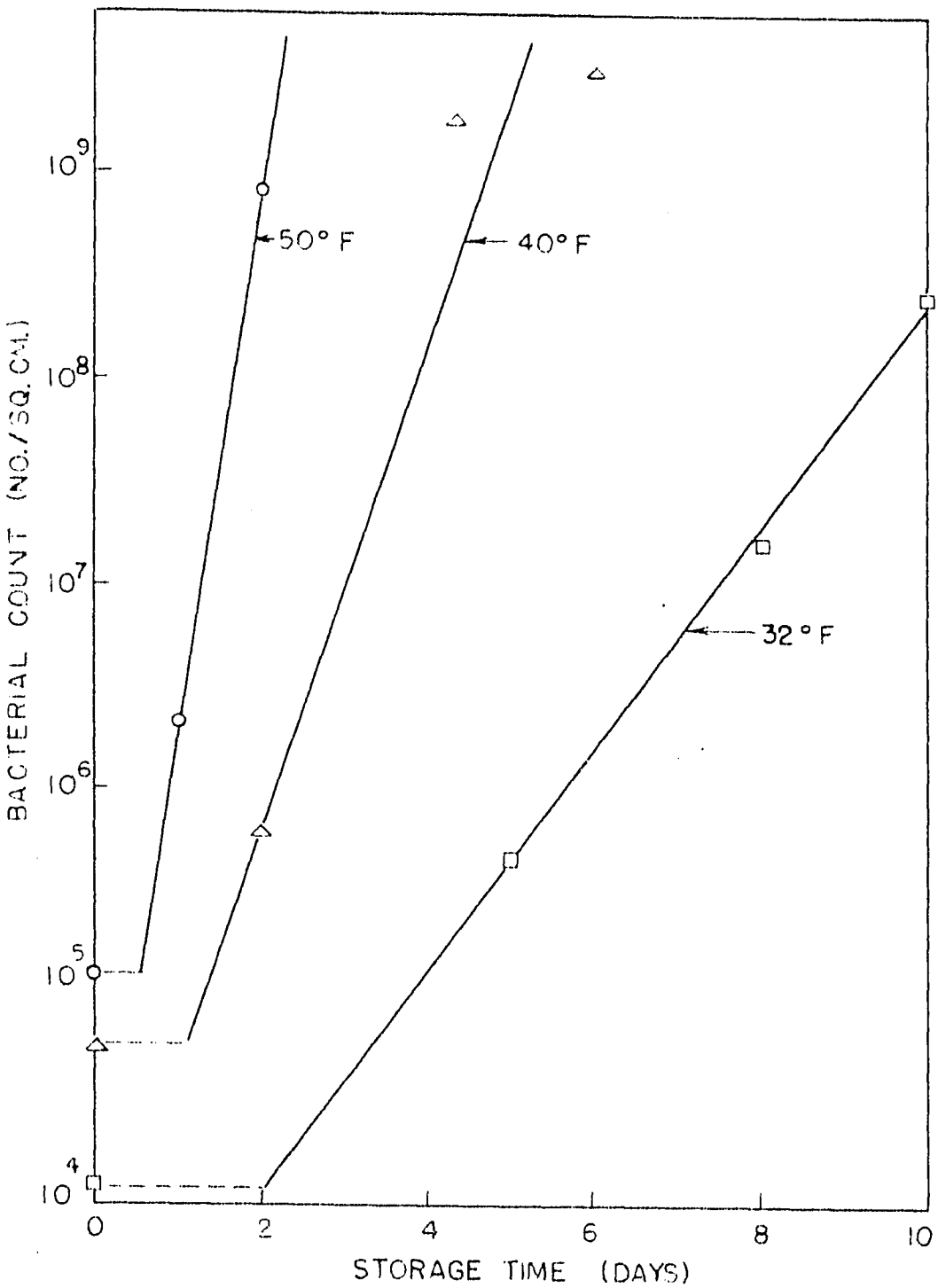

Figure 14. Effect of temperature on growth curves of bacteria on chicken thighs stored in air

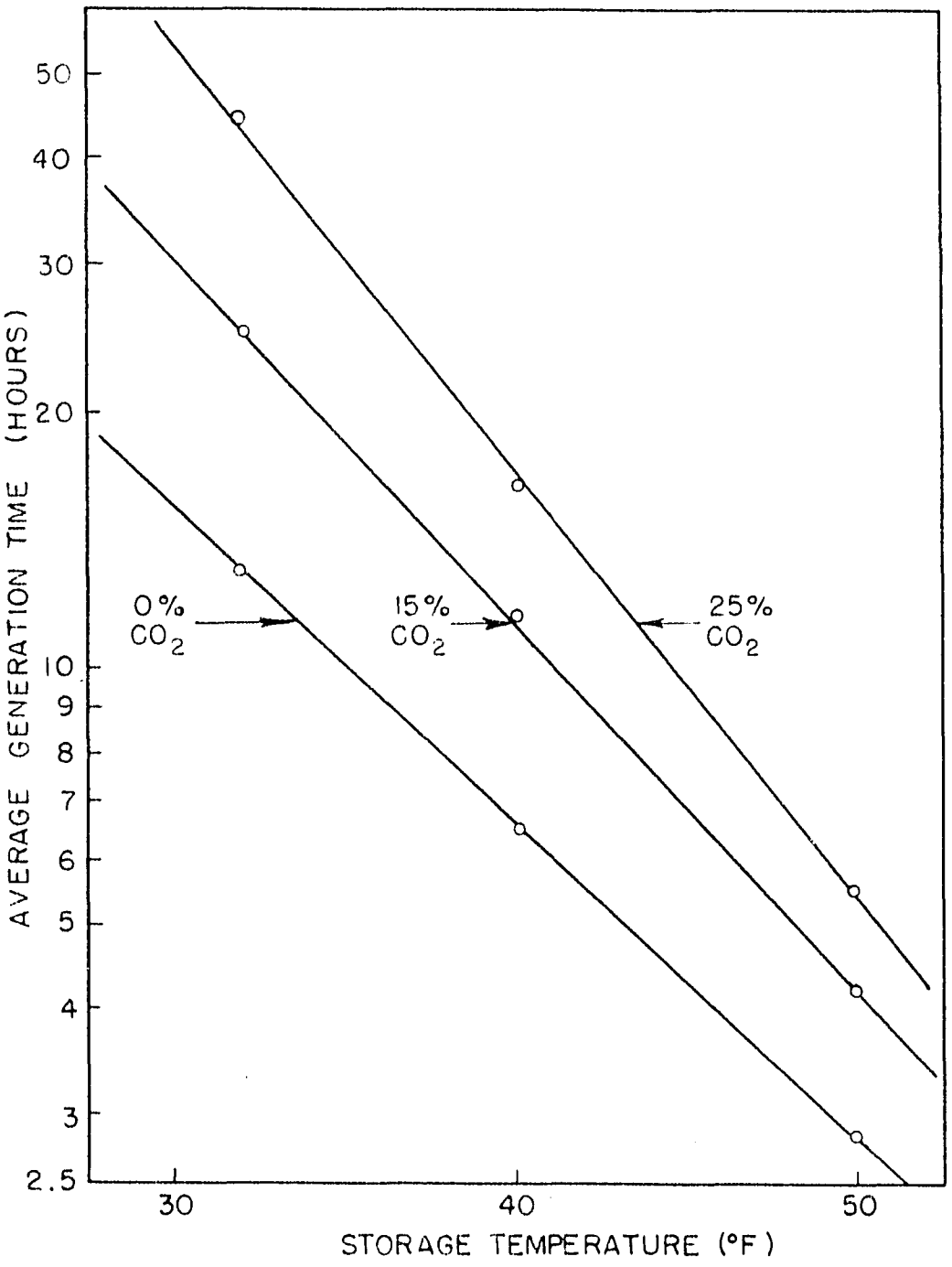

Figure 15. Relation of temperature to average generation time at CO_2 concentrations of 0, 15 and 25 per cent

for 0, 15 and 25 per cent CO_2 respectively.

(3) Discussion. Particular attention should be called to the differences in slope of the curves plotted in Figures 12 and 15. Considering Figure 15, it can be seen that the curves diverge as temperature is reduced and become greater in slope as CO_2 concentration is increased. The combined effects of temperature and CO_2 are greater than would be indicated by the sum of individual effects of these factors. For example, reducing temperature from 40° F. (4.4° C.) to 32° F. (0° C.) with 0 per cent CO_2 increases generation time by 6.6 hours; increasing CO_2 level from 0 to 15 per cent at 40° F. prolongs generation time by 4.9 hours. However, the combined effect is to increase generation time by 18.0 hours instead of 11.5 hours (6.6 + 4.9). At 50° F. (10° C.) an increase in CO_2 concentration from 0 to 25 per cent gives a two-fold increase in average generation time; at 40° F. the corresponding increase is approximately $2\frac{1}{3}$ times, and the use of CO_2 with low temperatures gives very long keeping times.

Figure 15 also brings out the value of applying CO_2 atmospheres in situations in which further reduction of storage temperature is impractical or undesirable. For instance, the use of 15 per cent CO_2 at 40° F. (4.4° C.) has the same effect on average generation time as a reduction in temperature to 34° F. (1.1° C.), and 25 per cent CO_2 gives a greater retardation than any temperature short of one which would freeze the meat. Application of CO_2 at 50° F. (10° C.) is of much less practical value.

Expression of the influence of CO_2 or temperature in terms of generation time of the microbial flora concerned has the obvious disadvantage that the effects on lag time are not considered. Since a reduction of

temperature and an increase in CO_2 level tend to extend the lag period, interpretations from Figures 12 or 15 give underestimates of the effectiveness of these agents. Unfortunately there seems to be no precise method of expressing relationships between CO_2 level or temperature and lag phase. Other investigators have experienced similar difficulty. Lag to a great extent appears to be a function of the past history of the individual microorganisms concerned (44). In this work a regular increase in log time with CO_2 concentration generally was observed up to 15 per cent CO_2. At higher concentrations results were more variable.

Some doubt exists as to whether the relationship between log generation time and CO_2 concentration actually is linear as indicated in Figure 12. Data obtained over a narrow range may often appear to fit a straight line although the true relationship may be given by a curved line. No previous work of a suitable nature is available for comparison. It seems unlikely that a linear relationship would hold at higher concentrations of CO_2 since application of high levels does not markedly improve storage life. For practical purposes the use of straight lines in the range 0-25 per cent CO_2 is probably satisfactory.

Application of the Arrhenius-van't Hoff equation to the influence of temperature on microbial growth has been studied by a number of investigators. Crozier (21) decided that the thermal increment, μ, was constant over certain ranges of temperature with sharp breaks at so-called critical temperatures. Fulmer and Buchanan (28) and Haines (33) observed that μ varied continuously with temperature and failed to find critical temperatures. According to Fulmer and Buchanan the graphical method of deter-

mining μ with small scale ordinates (as used by Crozier) does not accurately interpret the data. Scott (91) obtained constant values of μ for a range of sub-optimal temperatures, but found that μ varied at higher temperatures. Data obtained in this work are not voluminous or exact enough to clarify this problem, but indicate that the equation at least gives a close approximation in the temperature range 0° to 10° C. (32° - 50° F.).

Figure 12 shows that generation times obtained in Run 4A were less than those obtained in Runs 5A and 6A at comparable temperatures, although the general relation between generation time and CO_2 concentration was about the same in all cases. Two possible reasons for this discrepancy can be suggested. In Run 4A CO_2 levels were attained by the evacuation method, whereas the displacement method was used in Runs 5A and 6A. The results do not support the contention of Scott (62) that vacuums increase toxicity of CO_2 toward slime forming bacteria. There is a possibility that blood brought to the surface in the evacuation method might cause improved microbial growth. Variations in microbial flora from one run to the next might also account for differences in average generation time under identical conditions of storage. In the next section it will be shown that there were differences in the bacterial types predominating between Runs 4A and 5A, although genera represented were apparently the same in both.

h. Short term CO_2 storage. The value of holding chicken thighs in CO_2 for a part of the total storage period followed by storage in air was

studied for one batch of fresh commercial chicken. Table 12 gives the results of holding samples in CO_2 for 3 days and 6 days as compared with the usual air storage or CO_2 storage. As would be expected, keeping times for combinations of air and CO_2 storage fell between those for straight CO_2 storage and air storage. These results are in accord with what would be predicted from growth curve studies of the spoilage bacteria involved. Initial holding in CO_2 prolongs lag time and reduces rate of multiplication when the logarithmic growth phase or "steady state" has been reached. When CO_2 is removed, slope of the growth curve will increase and storage life will be correspondingly reduced. A short term of holding under CO_2 at the beginning should be more helpful than an equal period later since the advantage due to extended lag time is not obtained in the latter case.

Table 12. Comparison of short term with regular CO_2 storage of fresh commercially dressed chicken thighs at 40° F.

Storage atmosphere	Keeping time (days)
Air	5
15% CO_2 for 3 days, then air	7
15% CO_2 for 6 days, then air	8
15% CO_2	8
25% CO_2 for 3 days, then air	8
25% CO_2 for 6 days, then air	9
25% CO_2	12

3. Microbiology of stored chicken

a. Organisms. The only organisms studied in detail were bacteria causing slime formation on cut-up chicken stored under constant CO_2 levels and aerobic conditions. The principal objective in studying these bacteria was to determine whether the presence of CO_2 in the storage atmosphere influenced the type of flora predominating. A total of 163 cultures from Runs 4A and 5A were isolated and separated into two groups on the basis of morphology and physiological reactions. These groups could not be distinguished on the basis of colony characteristics. A general description of the two groups follows:

Group I (144 cultures) Gram-negative, small to medium sized, non-sporulating rods occurring singly, in pairs or chains; motile with polar flagella. Usually produce acid from glucose, but seldom from maltose. Gelatin liquefaction and nitrate reduction and action on litmus milk variable.

Group II (19 cultures) Gram-negative medium to large coccoid rods usually occurring in pairs; non-motile, non-sporulating. No acid from sugars; sugars and litmus milk usually become alkaline. Do not liquefy gelatin; seldom reduce nitrates. Strongly lipolytic.

Group I was divided into 13 different types on the basis of reactions in gelatin, dextrose, maltose, litmus milk and nitrate broth. These types can be separated from each other and from Group II on the basis of the following scheme:

I. Gelatin liquefied

A. Acid from glucose

1. Acid from maltose

a. Litmus milk peptonized ..... Type 1

b. Litmus milk becomes alkaline ..... Type 2

2. No acid from maltose

a. Litmus milk peptonized

(1) Nitrates not reduced ..... Type 3

(2) Nitrates reduced to nitrites ..... Type 4

(e) Nitrates reduced beyond nitrites ..... Type 5

b. Litmus milk becomes alkaline

(1) Nitrates not reduced ..... Type 6

(2) Nitrates reduced to nitrites ..... Type 7

c. Litmus milk becomes acid or reduced without peptonization ..... Type 8

B. No acid from glucose (becomes alkaline)

1. Nitrates not reduced ..... Type 9

2. Nitrates reduced beyond nitrites ..... Type 10

II. Gelatin not liquefied

A. Acid from glucose

1. Litmus milk becomes alkaline

a. Nitrates not reduced ..... Type 11

b. Nitrates reduced to nitrites ..... Type 12

2. Litmus milk becomes acid ..... Type 13

B. No acid from glucose ..... Group II

Physiological reactions of the 13 types in Group I are listed in Table 13. In addition all cultures were tested for indol and hydrogen sulfide production and found to be negative. All inoculated tubes of glucose and maltose broth in which no acid was formed became alkaline by the end of two weeks. Tubes that showed an acid reaction usually did so within two days and became alkaline within a month.

Cultural characteristics varied considerably within each type. Agar surface colonies ordinarily fitted into one of the following two classifications:

(a) Circular, entire, convex, glistening, white, opaque.

(b) Circular, entire, flat, translucent, greyish white.

Organisms in Types 1, 6, 8, 11 and 13 were usually better described by (a). Most cultures in Types 2, 3, 4, 5, 7, 9, 10 and 12 agreed more closely with (b). Almost any combination of properties intermediate between (a) and (b) could be found in at least one culture. Nearly all were to a greater or lesser degree viscid. Many of the cultures produced a ropiness in sugar broths and litmus milk, although this ability seemed to be well distributed among the various types. Several members of Type 10 imparted a soluble green pigment to the agar when first isolated.

Table 14 shows the percentages of cultures of each type isolated from pieces of chicken stored under various concentrations of carbon dioxide. Types with less than 5 cultures were not included. It should be emphasized that all of the cultures were picked from plates made when chicken had reached or was approaching the slime stage. Table 14 indicates that CO_2 had little influence on the flora comprising slime. A possible exception

Table 13. Reactions and number of cultures of Group I bacteria

Type	Gelatin liquefied	Acid from glucose	Acid from maltose	Litmus milk	Nitrate reduced	Fat hydrolyzed
1	+ (incomplete)	+ (slight)	+ (slight)	Peptonized Some reduction	-	+
2	+	+	+	Alkaline	+	-
3	+ (usually incomplete)	+	-	Rapid alkaline peptonization More or less reduced	-	++
4	+	+	-	Rapid alkaline peptonization More or less reduced	+	++
5	+ (usually incomplete)	+	-	Rapid alkaline peptonization More or less reduced	+[a]	++
6	+ (slight)	+	-	Alkaline	-	-
7	+ (incomplete)	+	-	Alkaline	+	+++
8	+ (usually slight)	+	-	More or less reduced, sl. A, sl. alk. or no change Occasionally some coagulation	-	+++ to -
9	+ (complete)	-	-	Rapid alk. peptonization More or less reduced	-	++
10	+ (usually complete)	-	-	Rapid alk. peptonization More or less reduced	-[a]	+++
11	-	+	-	Alkaline or no change	-	-
12	-	+	-	Alkaline	+	-
13	-	-	-	More or less reduced; sl. A, sl. alk. or no change Occasionally some coagulation	-	-

[a]Reduced past nitrite

Reactions and number of cultures of Group I bacteria

	Acid from glucose	Acid from maltose	Litmus milk	Nitrate reduced	Fat hydrolyzed	No. of cultures studied
	+ (slight)	+ (slight)	Peptonized Some reduction	-	+	1
	+	+	Alkaline	+	-	1
	+	-	Rapid alkaline peptonization More or less reduced	-	++	27
	+	-	Rapid alkaline peptonization More or less reduced	+	++	15
	+	-	Rapid alkaline peptonization More or less reduced	+[a]	++	3
	+	-	Alkaline	-	-	2
	+	-	Alkaline	+	+++	1
	+	-	More or less reduced, sl. A, sl. alk. or no change Occasionally some coagulation	-	+++ to -	38
	-	-	Rapid alk. peptonization More or less reduced	-	++	2
	-	-	Rapid alk. peptonization More or less reduced	-[a]	+++	15
	+	-	Alkaline or no change	-	-	8
	+	-	Alkaline	+	-	22
	-	-	More or less reduced; sl. A, sl. alk. or no change Occasionally some coagulation	-	-	9

t nitrite

Table 14. Effect of CO_2 concentration upon types of bacteria isolated from stored cut-up chicken

CO_2 level %	Group I (% of cultures)							Group II (% of cultures)
	Type 3	Type 4	Type 8	Type 10	Type 11	Type 12	Type 13	
0	26	40	18	27	25	23	11	5
5	22	27	18	13	25	23	11	11
10	11	20	8	-	12.5	-	11	5
15	15	7	35	33	12.5	23	45	37
20	0	6	5	-	12.5	-	11	16
25	26	0	16	27	12.5	31	11	26

was Type 4 whose incidence decreased regularly as CO_2 level was increased. There was some increase in incidence of Group II bacteria with CO_2 level; however, in other experiments, these organisms have been found to comprise a majority of the types found in the slime of cut-up chicken stored without CO_2.

As indicated in Table 15, the types of bacteria found on stored chicken were not affected by temperature. Cultures obtained from samples held at 50° F. were not included in this table since that temperature was employed in only one of the two runs.

The effect of source of chicken on the kinds of bacteria obtained is given in Table 16. All types which consisted of 2 or more cultures have been included. Type 5 was found on chicken used in Run 5A but not Run 4A while Type 13 also was confined largely to 5A. On the other hand, Types 6, 10 and 12 obtained from samples used in 4A were not found in 5A. Lots of chicken used in Runs 4A and 5A were obtained from the same produce house within the same month. Differences in types secured in the two cases suggest that spoilage bacteria, in some measure at least, are brought into

Table 15. Effect of temperature upon types of bacteria isolated from stored cut-up chicken

Temperature (° F.)	Group I (% of cultures)							Group II (% of cultures)
	Type 3	Type 4	Type 8	Type 10	Type 11	Type 12	Type 13	
40	48	40	55	42	75	21	67	44
32	52	60	45	58	25	79	33	56

Table 16. Effect of source of chicken upon types of bacteria isolated from stored cut-up chicken

Run no.	Group I, Type: (% of cultures)										Group II (% of cultures)
	3	4	5	6	8	9	10	11	12	13	
4A	33	33	0	100	66	50	100	25	100	13	26
5A	67	67	100	0	34	50	0	75	0	87	74

the processing establishment on the birds and do not come solely from the resident flora of the establishment.

Although the presence of CO_2 in the storage atmosphere had little effect on the types of slime forming bacteria predominating, there were indications that another organism benefited from the CO_2. Pin-point, subsurface colonies were occasionally observed in nutrient agar plates made from surface swabs of samples stored under CO_2 levels of 15 per cent and higher. These were found to consist of Gram-positive, chain-forming bacteria similar culturally and morphologically to members of the genus Lactobacillus. These organisms were non-motile and non-sporulating; produced acid from glucose; failed to reduce nitrates or liquefy gelatin; litmus milk was unchanged. Their importance in storage of chicken is not known. Apparently CO_2 depresses the growth of slime-forming bacteria sufficiently to allow the "lactobacilli" to be detected.

An entirely different flora was found when chicken was stored in sealed jars with high CO_2 levels until the onset of spoilage. In this case CO_2 and low oxygen pressure suppressed growth of slime-forming types. Spoilage was accompanied by a putrefactive odor. Only 8 spore-forming obligate anaerobes per gram were obtained from a sample of spoiled chicken. An aerobic count of 8×10^9 organisms per gram was found with the same sample. Cultural characteristics and microscopic examination indicated that two general types were present in about equal numbers. The first group had characteristics of lactobacilli. Organisms of the second group were Gram-negative rods, but were decidedly different than the aerobic slime-formers.

An offensive odor was associated with members of the second group. Descriptions of both groups follow.

First group. (3 cultures studied)

Morphology: Gram-positive rods with square ends, in chains.

Nutrient agar colonies: Sub-surface, pin-point.

Tomato agar colonies: Sub-surface, angular, white.

Non-motile.

Litmus milk: Partially reduced, acid, no coagulation.

Acid from glucose.

Nitrates not reduced.

Gelatin not liquefied.

Second group. (5 cultures studied)

Morphology: Gram-negative rods, occurring singly.

Nutrient agar colonies: Surface colonies large, flat, round, entire, butyrous, grey, opaque. Sub-surface colonies lens shaped.

Tomato agar colonies: Similar to those in nutrient agar.

Motile--3 cultures, non-motile--2 cultures.

Litmus milk: Alkaline in 2 weeks (20° C.)

Acid and gas from glucose and glycerol, indicator (phenol red) decolorized.

Nitrates reduced.

Gelatin not liquefied.

Storage of cut-up chicken with carbon dioxide atmospheres in containers sealed throughout the storage period resulted in spoilage from certain

microaerophilic and facultative bacteria.

b. Discussion. The slime-forming bacteria described in this work appear to be, in many cases, identical with those reported by Ayres et al. (1). Similarly, the Group I bacteria unquestionably fit into the genus Pseudomonas (Bergey, Manual of Determinative Bacteriology, 6th ed., 1948), although species identification is not possible. A number of types previously reported as Achromobacter, isolated from slimy beef by Haines (35) and from slimy fish by Stewart (99), probably belong in the genus Pseudomonas since they were reported to have monotrichous flagella. This apparent anomaly is due to changes in classification made in the more recent editions of Bergey's Manual.

Group II bacteria cause more difficulty in classification. It is difficult to tell from microscopic examination whether they should be considered cocci or rods. In physiological properties these organisms are in no way similar to any of the described species of micrococci. If they are considered rods, there is no trouble in placing them in the family Achromobacteriaceae; indeed, both morphological and physiological characteristics are very much like those given for Alcaligenes viscosus in Bergey's manual. Although only 19 of 163 cultures isolated from stored chicken in Runs 4A and 5A belonged in Group II, these organisms have occasionally been found in almost pure culture in chicken slime. Comparatively inert biochemically, they show a pronounced ability to hydrolyze fat. A few cultures reduce nitrates. Judging from the information available, Group II bacteria may be closely related to "coccobacilli" found by Stewart (99) to be the most numerous types in haddock

slime, to "coccoid rods" isolated from slimy pork and mild cured bacon by Garrard and Lochhead (29), and to Gram-negative "micrococci" observed on the skin of stored, dressed poultry by Lochhead and Landerkin (68).

Information obtained from this work and from the published works of other investigators leads to the belief that slimes on any kind of fresh meat stored at refrigeration temperatures are almost always due to closely related types belonging to the genus Pseudomonas and the family Achromobacteriaceae. Since it has been shown repeatedly that the rate of proliferation of these organisms is reduced in the presence of carbon dioxide, it appears that CO_2-storage of fresh meats has universal applicability within definite temperature limits.

C. Storage of Frankfurters in CO_2 Atmospheres

Considerable differences in results would be expected from the storage in carbon dioxide atmospheres of the frankfurter, a cured, comminuted meat product, than from the similar storage of chicken, a fresh meat. The frankfurter contains a different pigment than that occurring in fresh meat; the salt concentration is much higher and the moisture content is lower; the predominant flora developing during storage is quite different. The influence of these dissimilarities and others will be brought out by the following results and discussions.

1. Determination of storage end points

More difficulty in deciding when the limit of storage had been reached was encountered with frankfurters than with poultry. Owing to

the strong natural odor of the samples and to the many different types of spoilage determination of off-odor was not a sensitive method of measuring storage life. As a result definition of end points generally was based on undesirable changes in external appearance due to microbial activity. A sample was considered spoiled when surface slimes or greenish discolorations were first noted or when discrete colonies attained an approximate diameter of 1 mm. Under some conditions of storage neither odor nor appearance could be used satisfactorily for judging the conditions of stored samples. In such cases flavor may constitute the only satisfactory criterion.

2. Effect of CO_2 on frankfurters

Storage of frankfurters in CO_2 caused no deleterious effects on flavor or color. Concentrations as high as 96 per cent produced no noticeable discoloration. High CO_2 levels tended to reduce color fading which sometimes occurred when air or low CO_2 levels were used in storage.

Frankfurters stored in jars containing high concentrations of CO_2 often developed a wrinkled, shrunken appearance. The appearance was very similar to that of frankfurters which had been subjected to a vacuum and subsequent release of pressure and was attributed to vacuums arising in jars due to solution of CO_2 in the samples.

3. Effect of CO_2 and temperature on storage life

a. Keeping times at various temperatures and CO_2 levels. Table 17 shows keeping times obtained with frankfurters stored with different CO_2

Table 17. Keeping time of frankfurters stored at various temperatures and CO_2 levels[a]

CO_2 level (%)	Temperature (°F.)				
	30	35	40	45	50
			(days)		
0	38	26	18	10	6
5	65	-	28	15	-
15	90	-	35	22	10
25	-	60	50	32	-
50	138	-	ca 60	ca 40	14
75	-	-	ca 60	ca 40	-
96	-	ca65	ca 60	ca 40	ca 19

[a]Conditions of storage:
Relative humidity, approximately 100%
Containers, 2-qt. jars
Constant CO_2 levels obtained and maintained by the displacement method
Keeping times based on changes in appearance due to micro-organisms

levels at several temperatures. These times were determined from visual manifestations of microbiological growth (greening, colony formation, sliming) and were not necessarily the same as keeping times based on palatability tests. An increase in carbon dioxide concentration gave an improvement in storage life up to levels of 50 per cent. Additions of CO_2 above 50 per cent made little difference in the time at which microbial growth could be discerned. This fact is brought out by Figure 16. The slopes of the curves decrease rapidly near the 25 per cent level and curves are practically horizontal at the 50 per cent level. End points with high CO_2 concentrations could not be defined with exactness. Microbial growth was evidenced visually as a thin, watery slime. This developed gradually and was difficult to detect in the initial stages.

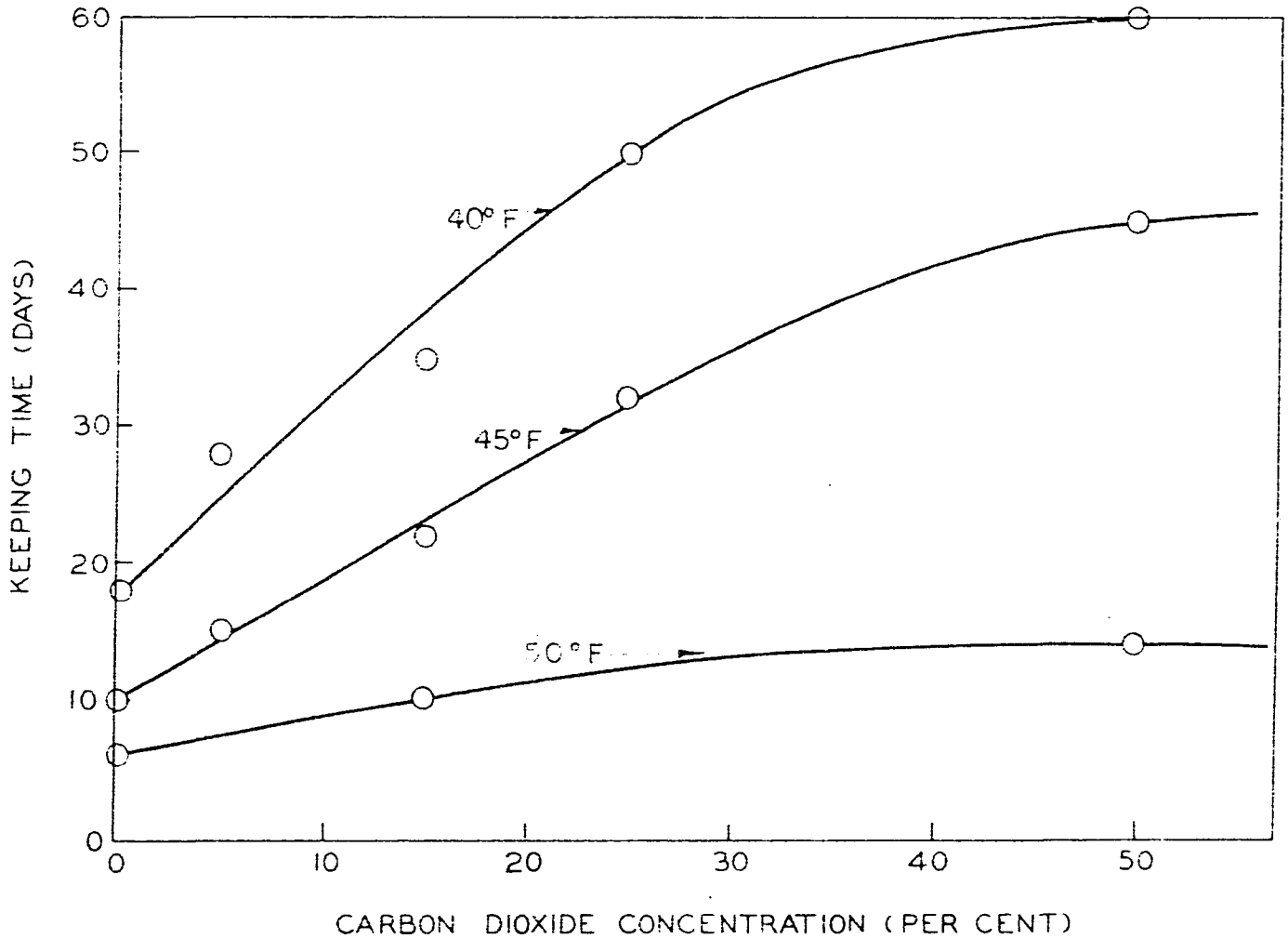

Figure 16. Effect of CO_2 concentration on storage life of frankfurters at 40°, at 45° and at 50° F.

The tremendous influence of temperature on keeping time also is indicated by Table 17. A 5 degree reduction in temperature near the bottom of the temperature range was much more effective than a 5 degree reduction at a higher temperature. In addition, the action of CO_2 was increased by a decrease in temperature. These effects are readily seen from the shapes and positions of the curves in Figure 17. Contrary to the experience with poultry, there was no freezing in frankfurters stored at 30° F. (-1.1° C.).

(1) Flavor tests. In Table 18 keeping times as determined from visual evidence of microbial growth are compared with those deduced from flavor changes on a batch of frankfurters stored at 40° F. (4.4° C.). These taste tests were made on unheated samples by the author and also by others in the laboratory. The tests were intended to detect gross changes in flavor; no attempt was made to judge small differences or to give samples a numerical score. Keeping times based on taste were somewhat longer, indicating that small isolated colonies, moderate greening and the first evidence of a watery slime (with high CO_2 levels) all precede by several days a pronounced deterioration in flavor.

A different situation was encountered with samples stored at 30° F. (-1.1° C.). Frankfurters stored with 15 per cent CO_2 were found to have a slightly rancid flavor after 67 days' storage although microbial growth was not observed until 90 days. With 50 per cent CO_2 a rancid taste was noted at 95 days, whereas there was no visible growth when the experiment was terminated after 138 days. It was concluded that carbon dioxide

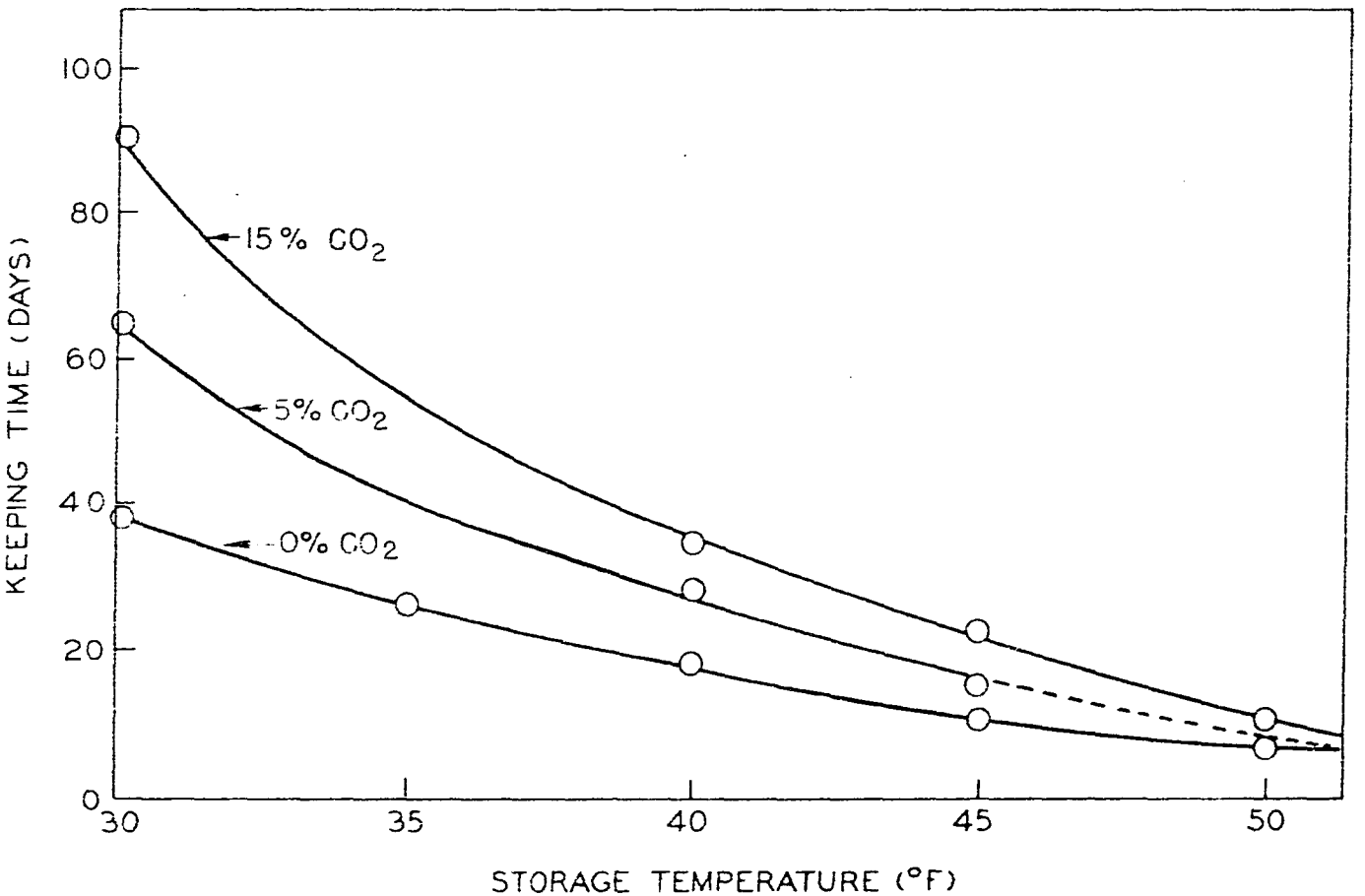

Figure 17. Effect of temperature on storage life of frankfurters with 0, with 5 and with 15 per cent CO_2

Table 18. Keeping time for frankfurters stored under various CO_2 levels as determined from microbiological and flavor end points[a]

CO_2 conc. (%)	Keeping time from gross flavor changes (days)	Keeping time from visual observation of microbial growth (days)
0	26	18
5	33	28
15	41	35
25	>52, <72	50
50	ca 72	ca 60
75	>72	ca 60
96	>72	ca 60

[a]Conditions of storage:
Temperature, 40° F. (4.4° C.)
Relative humidity, approximately 100%
Containers, 2-qt. jars
Constant CO_2 levels obtained and maintained by the displacement method

in conjunction with low temperature so restricted the development of microorganisms that oxidative rancidity became the limiting factor in storage.

Samples from one storage experiment were scored on palatability by a panel of judges. Unfortunately, these samples were all more or less rancid in spite of having been held frozen from the time of removal from the experimental jars to the time of judging. Rancidity, and to a lesser extent, inexperience on the part of judges, caused results to be of little value. Judges almost invariably preferred CO_2 stored samples to controls which had been frozen from the time that fresh samples were received. This leads one to speculate as to whether treatment with CO_2 may in some manner reduce susceptibility to fat oxidation. Because of the condition of frozen-stored controls, fresh samples of the same type were used as

controls in most of the judging. All of the samples were scored rather low, and there was no means of separating the effect of rancidity on taste from that of microbial activities. Flavor scores and keeping times determined from visual examination are shown together in Table 19. Although the scores were rather irregular, they tended to hold up past the microbiological end point. The results obtained for samples stored at 45° F. (7.2° C.) under the higher CO_2 levels showed that a very definite change in flavor did occur eventually. In these cases samples usually were described as being acid in taste.

(2) pH determinations. The fact that samples stored under high CO_2 percentages acquired an acid flavor suggested that pH determinations might constitute a reliable physical method for determining storage life. pH's measured at intervals during storage for CO_2 concentrations of 0, 25 and 96 per cent at several temperatures are listed in Table 1B in the appendix. For fresh frankfurters, pH normally varied from 6.0 to 6.2. With CO_2 levels under 25 per cent, little change in hydrogen ion concentration was observed during storage, and at 25 per cent results were variable and of little value. However, at 96 per cent CO_2, considerable reductions in pH were noted near the times that samples were considered spoiled by other methods. Similar results were obtained at 50 and 75 per cent CO_2. Use of pH measurements for finding limits of storage with high CO_2 concentrations appears promising.

(3) Effect of greening on keeping time. The keeping times given in Table 17 were obtained with samples which were not particularly prone to greening. When frankfurters were more heavily contaminated with bacteria

Table 19. Flavor scores and estimated keeping times for frankfurters stored under various CO_2 concentrations at 50°, 45° and 35° F.[a]

Storage temp. (° F.)	CO_2 conc. (%)	Time stored (days)	Flavor scores (0-10; 10 high)	Keeping time from visual observation of microbial growth (days)
50	0	7	8.8	7
	25	7	7.8	12
		11	6.3	
		16	6.8	
	96	7	7.0	ca 19
		11	6.5	
		16	6.8	
45	5	8	7.5	15
	15	24	6.4	22
	25	15	6.5	32
		22	7.3	
		45	5.4	
	50	23	7.1	ca 40
		45	6.0	
		66	3.4	
	75	17	6.0	ca 40
		23	7.5	
		45	7.0	
		66	4.2	
	96	22	7.0	ca 40
		45	6.7	
		66	4.8	
35	0	19	8.5	26
		29	7.7	
	25	19	6.5	60
		29	7.8	
		39	8.1	
		66	6.3	
	96	19	6.5	65
		29	6.6	
		39	5.5	
		66	7.4	

[a]Flavor score on fresh controls was 9.0.

capable of producing green discolorations, the effect on storage life was very noticeable. In Table 20 are compared keeping times of two batches of frankfurters at 40° F. (4.4° C.). One batch of frankfurters was obtained at a time when outbreaks of greening were prevalent in the meat industry; the other was typical of normal production. Without CO_2, keeping times were the same and greening was not the primary cause of spoilage in either case. With CO_2, the frankfurters susceptible to greening spoiled more rapidly than the others although an increase in CO_2 level still improved storage life. No greening occurred at 30° F. (-1.1° C.) or with 96 per cent carbon dioxide.

b. Effect of CO_2 and temperature on microbial growth. (1) Initial contamination. The initial bacterial flora on frankfurters ordinarily consisted of Gram-positive types. Micrococci, bacilli and sarcinae were most numerous. Lactobacilli and Gram-negative bacteria were present in smaller numbers. Appreciable numbers of yeasts often were encountered; molds, while probably present in all cases, were comparatively few in number.

Initial counts on frankfurters showed a rather remarkable consistency. Out of 20 counts (nutrient agar, incubation temperature 20° C.) made on the surfaces of fresh frankfurters from 5 different batches over a period of 2 years, the numbers obtained ranged from 800 to 9,600 per frankfurter. A range from 100 per cent above to 50 per cent below the average count of 4,120 included 70 per cent of the individual counts. Counts made on meat from the interior of frankfurters averaged 5,800 bacteria per gram.

Table 20. Effect of "greening" on the storage life of frankfurters at 40° F. (4.4° C.)

CO_2 level %	Average keeping time of normal samples (days)	Average keeping time of samples susceptible to greening (days)
0	18	18
15	35	25
25	50	40
50	ca 60	50

All of these seemed to be sporeformers. The uniformity of external contamination helps to explain the excellent agreement in keeping times obtained with samples from different batches.

(2) Total counts during storage. In order to get a better understanding of the growth of microorganisms on frankfurters stored in carbon dioxide atmospheres, plate counts were made at intervals on samples stored at 40° F. (4.4° C.). The resulting growth curves are shown in Figure 18, and individual counts can be found in Table 2B in the appendix. These are total counts exclusive of molds from nutrient agar plates incubated at 20° C. Counts made on samples stored with 25 per cent CO_2 were too erratic to permit construction of a curve. Keeping times determined for these samples are listed in Table 17. No consistent relationship could be demonstrated between keeping time and total count.

(3) Influence of CO_2 on types of microorganisms causing spoilage. The order in which various kinds of microbiological deterioration usually became visible is listed for a number of CO_2 levels ranging from 0 to 96 per cent in Table 21. The pattern was much the same at temperatures from 35° F. (1.7° C.) to 50° F. (10° C.). At 30° F. (-1.1° C.), no greening

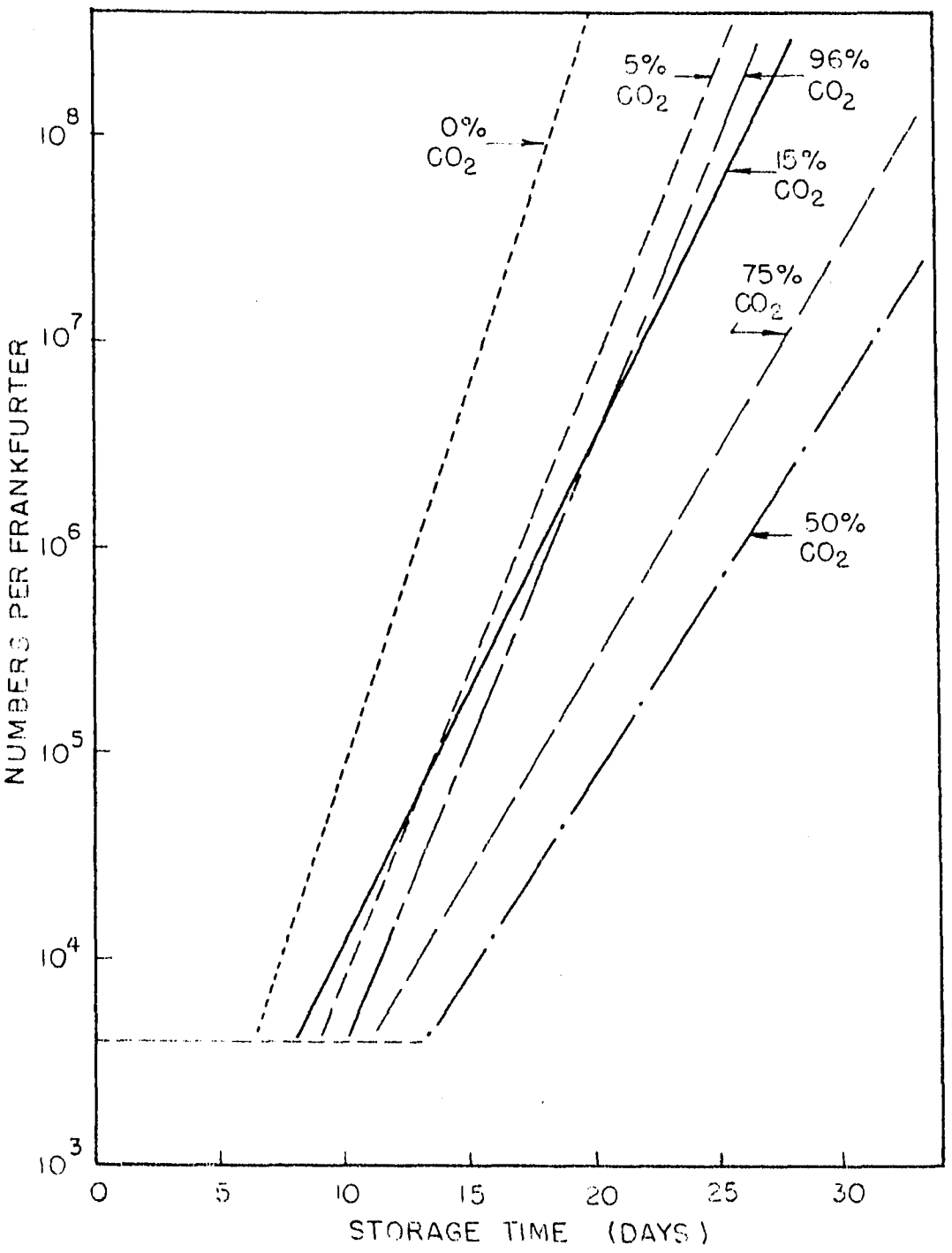

Figure 18. Effect of CO_2 on growth curves of microorganisms on frankfurters at 40° F.

Table 21. Effect of CO_2 on type of microbial spoilage of frankfurters stored at temperatures from 35° to 50° F.

CO_2 level %	Type of spoilage (listed in usual order of appearance)
0	Mold colonies, micrococcus and yeast colonies or slimes, greening.
5	Mold colonies, micrococcus and yeast colonies or slimes, greening.
15	Micrococcus and yeast colonies or slimes, mold colonies, greening.
25	Micrococcus and yeast colonies or slimes, greening, mold colonies.
50	Thin colonies or watery slime of lactobacilli, greening, micrococcus colonies, yeast colonies, mold colonies.
75	Lactobacillus colonies and slimes, micrococcus colonies.
96	Lactobacillus colonies and slimes.

was observed and molds played a more prominent role when CO_2 concentrations were 0 and 25 per cent. With batches of frankfurters which were particularly inclined to development of green discoloration, greening was often the first indication of spoilage at CO_2 percentages from 5 to 50. The pronounced effect of carbon dioxide concentration on the kind of spoilage obtained prompted a more detailed study of its action on the development of microorganisms.

(4) Growth curve studies on spoilage types. The individual development of micrococci, yeasts, molds, and lactobacilli was followed by

means of plate counts made at suitable intervals from frankfurters stored at 45° F. (7.2° C.) at CO_2 levels of 0, 5, 15, 25, 50, 75 and 96 per cent.

The micrococcus counts obtained are given in Table 3B (appendix), and the corresponding growth curves are shown in Figure 19. Carbon dioxide had a very decided inhibitory influence on the rate of growth of micrococci, its effectiveness increasing with concentration. There was apparently little or no lag with CO_2 levels of 15 per cent and below; a regular increase was observed with higher levels. With 96 per cent CO_2, numbers of micrococci declined with time; none were found at 45 days. In this case lack of oxygen may have been a more important factor than the presence of CO_2.

Yeast counts are shown in Table 4B (appendix) and Figure 20. Yeast colonies appeared infrequently and irregularly in the initial plates. Counts made during storage tended to be erratic, especially at intermediate CO_2 concentrations. Apparently some samples had so few yeasts that colonies were completely hidden by the more numerous microorganisms. For Figure 20 an initial count of 10 yeasts per frankfurther was arbitrarily selected as the starting point for the various curves. Of course, the straight lines in the figure cannot be regarded as true growth curves; they merely serve to indicate the relative effectiveness of different CO_2 levels in inhibiting yeast growth. Yeasts multiplied very rapidly in air; however, growth became progressively slower as the CO_2 level was increased. Growth occurred very slowly at the 25 and 50 per cent levels; no yeasts were found during 45 days' storage with 75 and 96 per cent CO_2.

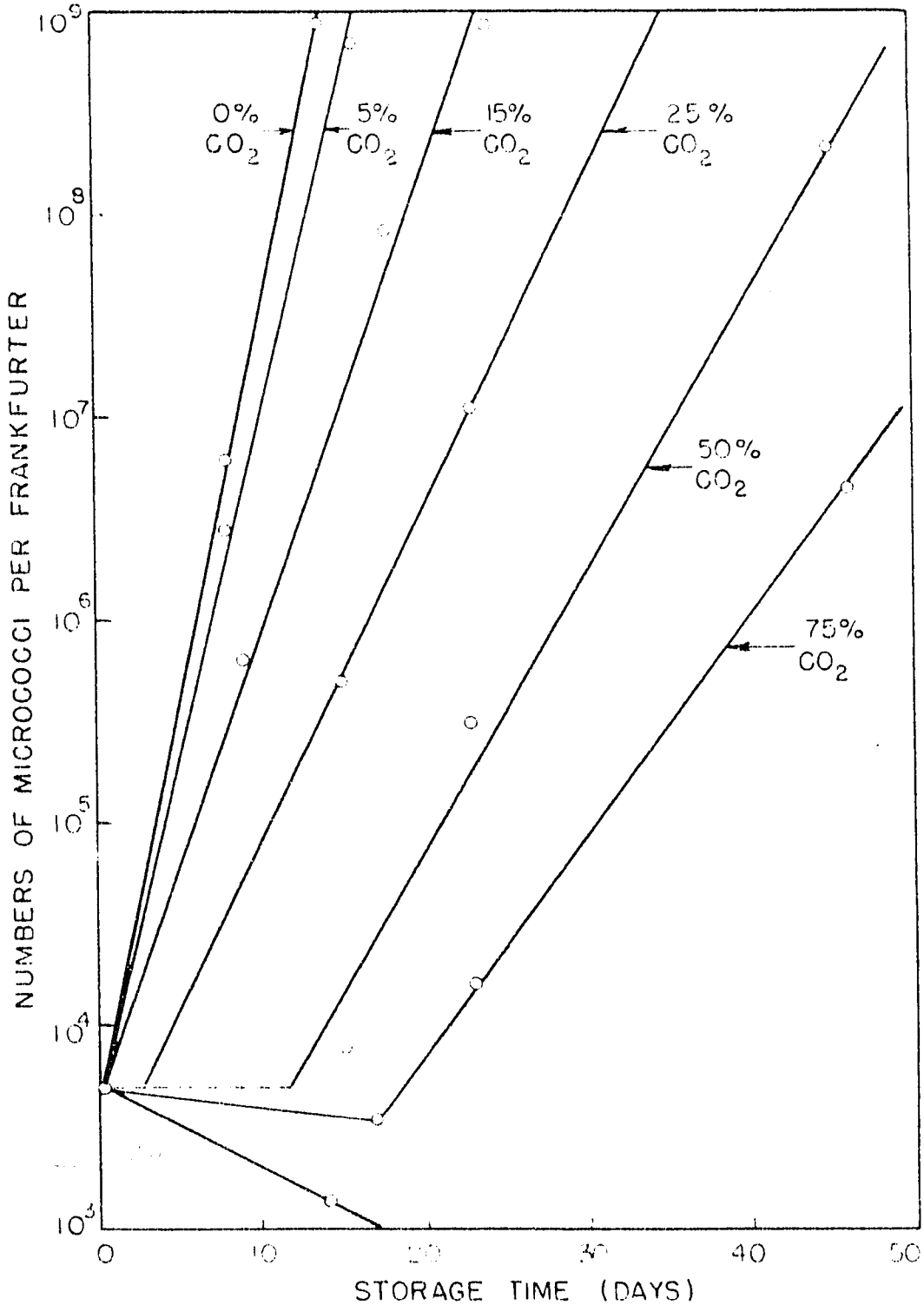

Figure 19. Effect of CO_2 on growth curves of micrococci on frankfurters at 45° F.

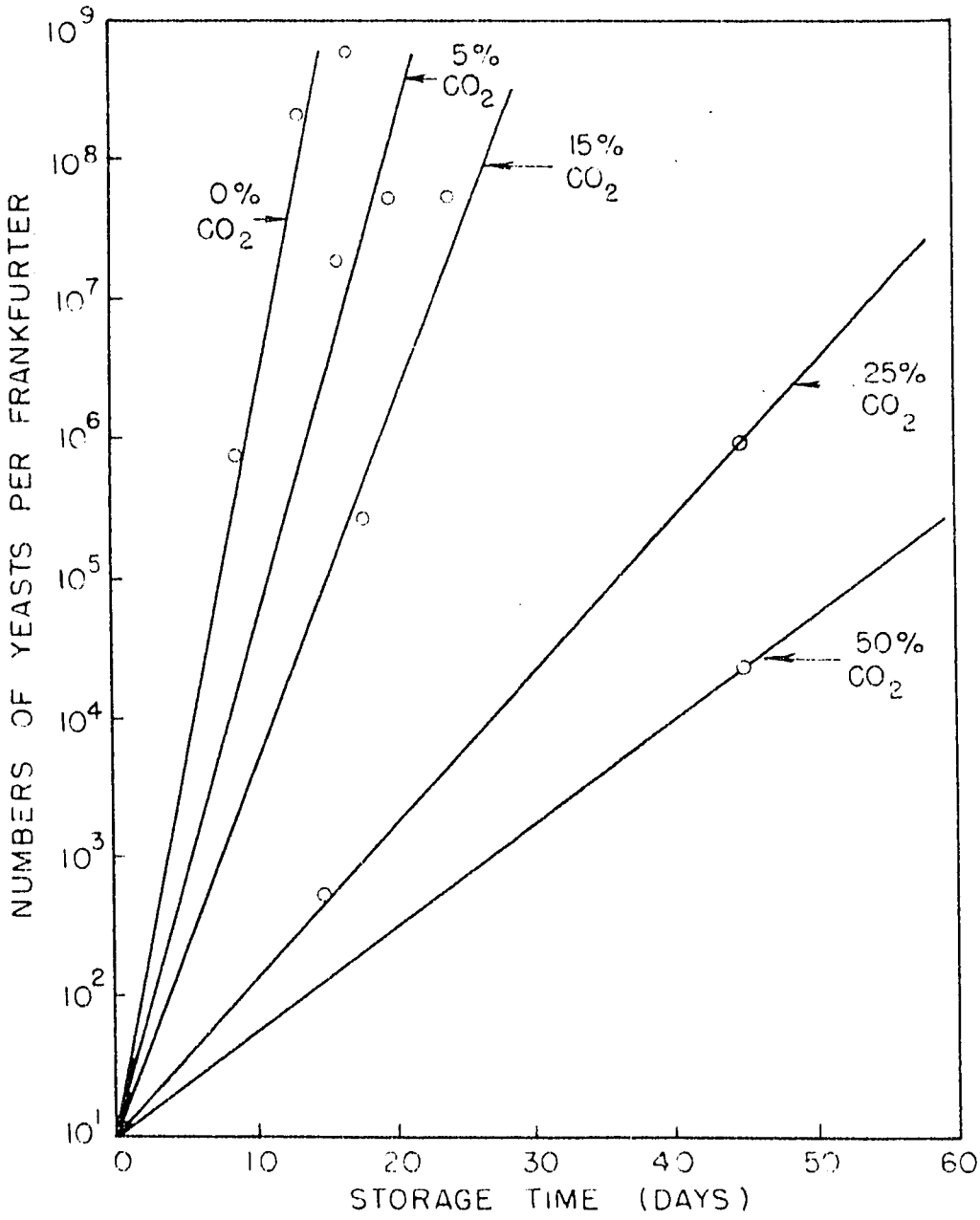

Figure 20. Effect of CO_2 on the growth of yeasts on frankfurters at 45° F.

It was not considered practicable to represent mold increase by a regular curve based on plate counts because of their peculiar manner of growth (slow development of a vegetative mycelium followed by the comparatively abrupt production of numerous spores). Plate counts indicated heavy mold growth at 14 days on samples stored without CO_2 and at 16 days on samples stored with 5 per cent CO_2. With 15 per cent CO_2 no molds were found at the 18 day counts, but there were numerous colonies in plates made at 24 days. At 25 per cent, molds were obtained at 45 days, but not at 23 days. Above 25 per cent, plate counts demonstrated no molds even at 45 days. These results along with visual observations showed that molds could be controlled at CO_2 levels of 15 per cent and higher and probably could be entirely disregarded at levels of 50 per cent and up.

Table 5B (appendix) shows counts of lactobacilli which were used in preparing the growth curves of Figure 21. Counts could not be obtained from samples stored in air because other faster growing organisms obscured lactobacillus colonies in the plates. Curves for 15 and 25 per cent CO_2 were so nearly identical in the logarithmic portions that they were combined in order to avoid confusion. The same was true for the 50, 75 and 96 per cent levels. Contrary to the results secured with micrococci, yeasts and molds, lactobacilli were not influenced to any great extent by the presence of CO_2. As measured by the time required to attain a given number of organisms, 15 and 25 per cent CO_2 were somewhat better than 5 per cent. Carbon dioxide at the 50 per cent level

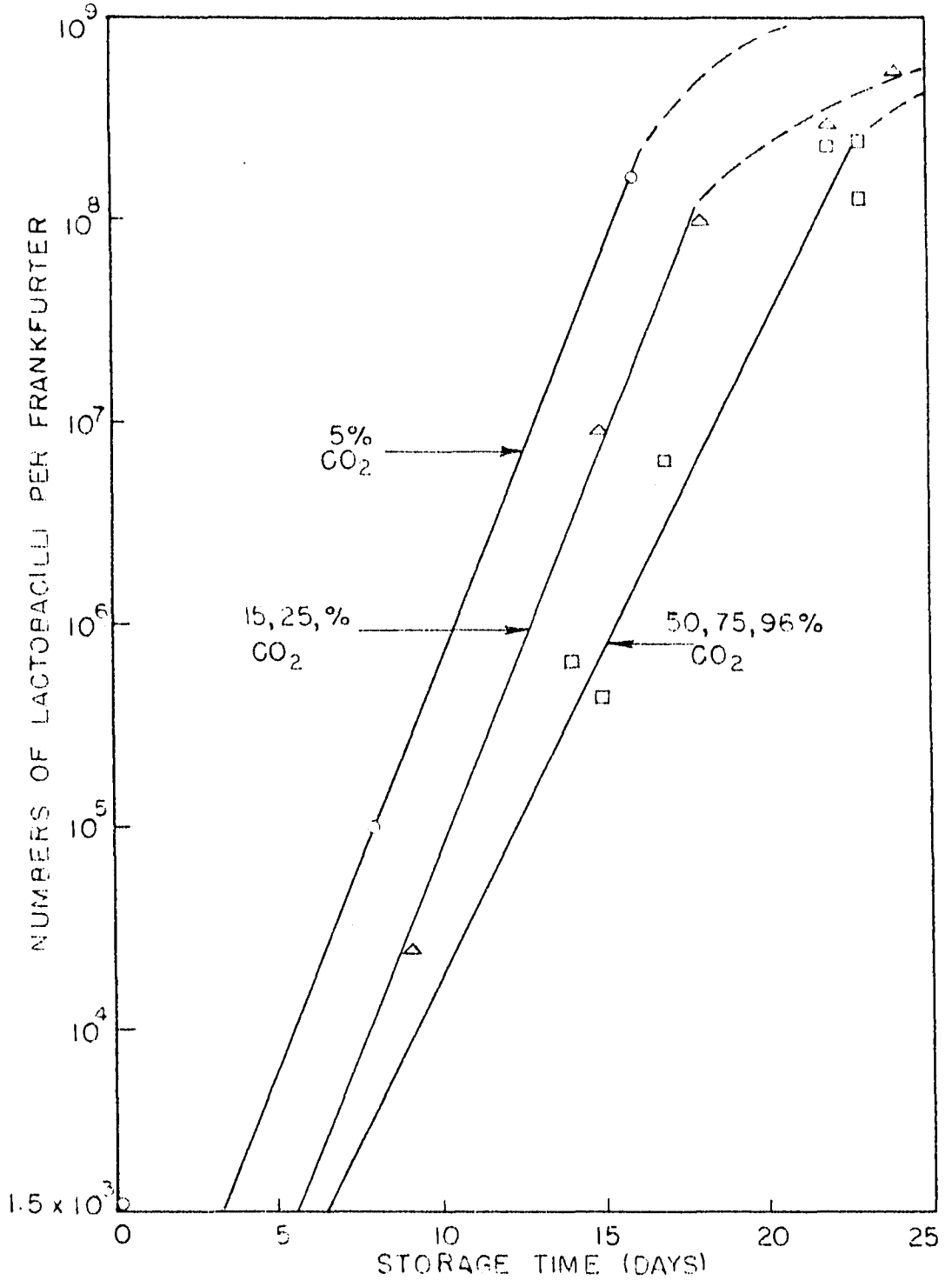

Figure 21. Effect of CO_2 on growth curves of lactobacilli on frankfurters at 45° F.

gave some improvement, but no appreciable advantage resulted from higher concentrations. Such differences as were attained appeared to depend as much or more on extension of lag times as on an increase in generation time during logarithmic reproduction.

(5) Discussion. On the basis of the preceding results a reasonably clear picture of the microbiological spoilage of frankfurters can be drawn. With storage in air, spoilage is due to molds, micrococci and yeasts. Which of these is the primary agent will be determined by such factors as initial contamination, relative humidity and, to some extent, temperature. This study and information obtained from workers in the meat industry indicate that molds normally are the greatest trouble makers. However, there is ample evidence that, under some conditions, micrococci (88), yeast (78) and members of the family Lactobacteriaceae (82) can cause spoilage outbreaks of great economic importance.

Application of carbon dioxide to storage atmospheres changes the picture markedly. In general, mold growth is considerably restricted by moderate concentrations (15-25 per cent) and is substantially reduced by 5 per cent. High CO_2 levels probably prevent any important development of most frankfurter-attacking molds. Growth of micrococci and yeasts is progressively reduced as carbon dioxide concentration is increased becoming very slow at higher percentages. Lactobacilli, on the other hand, are influenced much less by CO_2. Use of levels of more than 50 per cent seems to be of no value in retarding proliferation of these bacteria. In fact, there is evidence (Figure 19) that very high

concentrations may sometimes permit more rapid growth than lower levels. The influence of CO_2 in retarding development is probably opposed by the tendency of low oxygen tensions obtaining with high CO_2 levels to promote growth of the microaerophillic lactobacilli. At any rate, the surface flora found with high CO_2 percentages (50-96) is composed almost entirely of those organisms. The failure of changes in CO_2 concentration in the range 50-96 per cent to affect substantially the growth rate of lactobacilli explains the constancy of the keeping times found in this range.

The relationship between lactobacilli and keeping time of stored frankfurters is not yet clearly understood. Certain types of lactobacilli have been reported to cause green discolorations (82). Still, it is possible to have enormous numbers without any sign of greening. In the high CO_2 range, counts of lactobacilli attain very high values some time before deterioration is evidenced by marked changes in appearance, flavor or pH. In addition to numbers of organisms, time may be an important factor here. Even after maximum numbers have been reached, an additional period of metabolic activity may be required for the production of definite changes in the characteristics of the frankfurter.

Assuming that certain types of lactobacilli cause green discolorations, one wonders why samples stored in 75 ot 96 per cent CO_2 showed no greening while replicates held under lower percentages did. Lack of oxygen could prevent completion of the reaction in the storage container. In that event, greening should develop upon subsequent exposure to air. This was not the case. Perhaps CO_2 inhibits green-producing lactobacilli

more than it does others although no explanation for such a supposition is apparent.

A study of the effect of carbon dioxide on a mixed flora such as is found on the frankfurter is complicated by the difficulty in evaluating the influence of one type of microorganism upon another. For instance, is the inhibition of micrococci, yeasts and molds with high CO_2 levels due entirely to carbon dioxide, or does the acid produced by the lactobacilli play a part? Such questions probably can be answered satisfactorily only by investigation of microorganisms in pure culture.

c. Short term storage. The effect on storage life of a limited holding period in CO_2 followed by storage in air also was studied in the case of frankfurters. In Table 22 keeping times resulting from 5 and 10 day periods in atmospheres containing 5, 25 and 96 per cent CO_2 are compared with direct storage in air, 5 per cent, 25 per cent and 96 per cent CO_2. A short term in 5 per cent CO_2 was not helpful, but similar treatment with 25 and 96 per cent gave a marked improvement in keeping time.

4. Effect of relative humidity on storage life

It was thought that reduced relative humidities might be employed successfully for prolonging storage life in the case of frankfurters. Moisture determinations on frankfurters, as received, gave an average water content of 52 per cent or 118 per cent on a dry weight basis. It seemed possible that the low moisture content and high salt content as compared to fresh meat might allow storage in humidities sufficiently low

to retard microbial growth without resulting in excessive weight losses. Weighed frankfurters were stored under relative humidities ranging from 75 to 100 per cent at 5 per cent intervals at a temperature of 40° F. (4.4° C.). Plate counts were made at intervals and weight changes were

Table 22. Comparison of short term with uninterrupted storage of frankfurters at 45° F.

Storage atmosphere	Keeping time (days)
Air	10
5% CO_2 for 5 days, then air	10
5% CO_2 for 10 days, then air	11
5% CO_2	15
25% CO_2 for 5 days, then air	14
25% CO_2 for 10 days, then air	20
25% CO_2	32
96% CO_2 for 5 days, then air	16
96% CO_2 for 10 days, then air	23
96% CO_2	ca 40

determined on the 15th day. Results were affected by the fact that the relative humidities increased during storage. The volumes of the sulfuric acid solutions used to control humidity were too small relative to sample size. This error was accentuated by the use of larger samples at lower humidities. For this reason the weight losses given in Table 23 were undoubtedly too small for the lower humidities. Individual weight changes can be found in Table 6B in the appendix.

Combined bacterial and yeast counts taken during storage are listed in Table 24. In addition to initial humidities, this table shows final humidities as determined from specific gravity measurements on the acid

Table 23. Effect of relative humidity on changes of weight of frankfurters stored at 40° F. (4.4° C.) for 15 days

Relative humidity (%)	No. of frankfurters per desiccator	Average weight change (%)
100	4	+ 0.7
95	4	- 1.3
90	5	- 4.0
85	5	- 6.1
80	5	- 7.2
75	6	- 7.3

Table 24. Counts of yeasts and bacteria on frankfurthers stored with various humidities at 40° F. (4.4° C.)

Initial relative humidity (%)	Relative humidity at 31 days (%)	Storage time (days)			
		9	15	20	31
100	100	3.0×10^3	1.3×10^4	9.5×10^6	2.3×10^8
95	97	1.1×10^4	3.4×10^4	2.3×10^8	2.3×10^9
90	94	1.7×10^3	2.7×10^3	5.0×10^5	1.5×10^7
85	92	2.6×10^3	5.3×10^4	2.2×10^7	6.5×10^7
80	90	3.5×10^3	2.2×10^5	2.7×10^7	1.4×10^7
75	90	1.5×10^3	8.3×10^4	6.4×10^6	4.2×10^5

solutions at the conclusion of the experiment (31 days). At the end of 15 days, molds were beginning to appear at all humidities except at 90 per cent. By 20 days rather large mold colonies were present on all samples. Colonies of lactobacilli were found only on the plates made from samples stored at 100 per cent humidity. Samples stored at relative humidities initially under 90 per cent acquired a very undesirable appearance due to dehydration, and this effect was noted to some extent at 90 per cent.

In spite of the obvious shortcomings of the relative humidity study some valuable observations can be derived from it. Judging from weight losses, continued use of humidities below 95 per cent probably would be economically impractical. A humidity of 95 per cent does not retard the rate of microbial growth. Frankfurters stored at 40° F. (4.4° C.) are in equilibrium with a relative humidity of more than 95 per cent. The actual value is estimated from the data on weight changes to be near 98 or 99 per cent. There does not, then, appear to be much hope in restricting microbial growth by humidity control. The failure to find lactobacilli on samples stored with humidities of 95 per cent and lower suggests that there may be merit in combining controlled humidity with carbon dioxide storage. If a relative humidity of about 95 per cent can delay growth of lactobacilli without causing prohibitive dehydration, carbon dioxide should be capable of reducing or preventing growth of organisms which tolerate such humidity.

5. Effect of CO_2 on packaged frankfurters

The first problem encountered in studying the effect of CO_2 on keeping time of packaged frankfurters was that of getting sufficient CO_2 into the package. Since the usual one pound package contains very little gas space relative to the quantity of meat, addition of gaseous or solid CO_2 to the package was not considered a satisfactory method. In such a procedure nearly all of the added CO_2 would be dissolved by the meat after the package was sealed. A method in which frankfurters were given a preliminary "soaking" in CO_2 before packaging gave good results. A one day holding period in 96 per cent at 45° F. (7.2° C.) resulted in the liberation of enough CO_2 in 24 hours after transfer to a smaller gastight jar to give a concentration of 37.5 per cent in the enclosed atmosphere. (Volumes of meat and gas in the second container were approximately equal.) In a second experiment, identical except that the preliminary "soaking" period was 2 days, a concentration of 44.0 per cent was obtained in 24 hours. It was estimated that somewhat less than one-fourth of the total volume in a one pound package of frankfurters was occupied by gas. Similar soaking before packaging should give even higher CO_2 levels provided that the gas did not escape.

In experiments in which frankfurters were held 2 days in 96 per cent CO_2 before packaging, the CO_2-treated samples showed evidence of spoilage at 40° F. (4.4° C.) and 45° F. (7.2° C.) at exactly the same time as the controls. It was concluded that CO_2 was lost through the wrap as rapidly as it diffused out of the frankfurters.

The L.S.A.T. (semi-moisture vapor proof) cellophane wraps used in this study were identical with materials commonly used in commercial practice for packaging frankfurters. It appears that packaging with carbon dioxide will not be practical unless a more nearly impermeable wrap is employed.

6. Microbiology of frankfurters

a. Yeasts and molds. No study was made of yeasts from frankfurters. Molds were identified as to genus only. Nearly all of the molds isolated from stored frankfurters were found to belong to the genus Penicillium. Other genera found occasionally were Sporotrichum, Zygorhinchus, Monilia, Mucor, Aspergillus and Alternaria. An increase in the relative numbers of Monilia was noted at the lower storage temperatures (30° and 35° F.).

b. Spore forming bacteria. Members of the genus Bacillus were found frequently on the surface and always in the interior of fresh frankfurters. However, these organisms played little part in spoilage of samples at refrigerator temperatures below 50° F. (10° C.). At 50° F. large numbers of aerobic spore formers were obtained on several occasions from the interior of frankfurters stored without carbon dioxide.

None of the samples stored at temperatures of 50° F. and below showed any evidence of putrefaction. Apparently sporulating anaerobes cause no difficulties at such temperatures.

c. Micrococci. A detailed study was made of 57 cultures of Gram-

positive micrococci isolated from stored frankfurters which had reached or were approaching the slimy stage. Cultures were divided into 14 types by means of the following scheme:

I. Yellow pigment

A. Nitrates not reduced, litmus milk alkaline ..... Type 1

B. Nitrates reduced, litmus milk reduced ..... Type 2

II. Pink pigment ..... Type 3

III. White or buff pigment

A. Nitrates not reduced

1. Utilizes $(NH_4)H_2PO_4$ as sole source of nitrogen

a. Gelatin not liquefied, litmus milk alkaline ... Type 4

b. Gelatin liquefied, litmus milk acid or peptonized ..... Type 5

2. Does not utilize $(NH_4)H_2PO_4$ as sole source of nitrogen

a. Litmus milk peptonized, gelatin usually not liquefied ..... Type 6

b. Litmus not changed or becomes slightly acid or alkaline ..... Type 7

B. Nitrates reduced

1. Utilizes $(NH_4)H_2PO_4$ as sole source of nitrogen (white pigment)

a. Gelatin liquefied

(1) Litmus milk peptonized ..... Type 8

(2) Litmus milk usually reduced, slightly acid or alkaline ..... Type 9

b. Gelatin not liquefied

(1) No acid from glucose ..... Type 10

(2) Acid from glucose ..... Type 11

2. Does not utilize $(NH_4)H_2PO_4$ as sole source of nitrogen

a. Gelatin liquefied (white pigment) ..... Type 12

b. Gelatin not liquefied

(1) No acid from glucose (white pigment) ..... Type 13

(2) Acid from glucose (buff pigment) ..... Type 14

A description of each type and the number of cultures in each are shown in Table 7B (appendix). None of the types could be identified definitely with any of the species of Micrococcus described in Bergey's Manual (6th ed.). Types 8 and 9, which contained 30 per cent of the cultures, resembled the description given for Micrococcus caseolyticus in many respects.

The results differ from those of Roderick and Norton (88) in that most cultures isolated in this work liquefied gelatin and quite a few peptonized milk. Results are similar in that most cultures produced white colonies on agar and none produced gas from sugars.

Another group whose classification was not clear was included with the Gram-positive cocci. This group was composed of Gram-negative cocci or coccobacilli usually occurring in pairs. Microscopic examination also revealed sticky conglomerations of cells in most cases and occasionally tetrads. These organisms were frequently found in large numbers together with Gram-positive micrococci on nutrient agar plates made from stored

frankfurters and were similarly affected by carbon dioxide. Surface colonies on agar were a greyish-white, convex and butyrous in consistency. No cultures liquefied gelatin or produced acid from glycerol; ability to hydrolyze fat varied widely. Some other physiological characteristics of the 15 cultures studied are listed in Table 25.

Table 25. Physiological characteristics of Gram-negative bacteria isolated from stored frankfurters

Number of cultures	Nitrates reduced	Gelatin liquefied	Reactions in litmus milk	Acid from glucose[a]
2	-	-	Sl. alkaline	-
3	-	-	Peptized	+
9	+	-	Acid, little or no coag.	+
1	+	-	Sl. alkaline	-

[a]No cultures produced gas from glucose

d. Lactobacilli. Of 120 cultures of lactobacilli isolated, all produced acid from glucose but none gave gas in the Durham fermentation tubes employed. Only two cultures produced visible greening on the cut surfaces of steamed pieces of frankfurter, and it was impossible to repeat those results at a later trial. Individual strains were not studied in detail. Morphologically, organisms varied from long thin rods with rounded ends to small, short rods with square ends. All were Gram-positive in young cultures; chains were usually present. In nutrient agar plates colonies were subsurface and very small. In tomato juice agar subsurface

colonies were larger, usually 1 to 2 mm. in diameter, white, and lens shaped or angular. In addition, tiny feathery colonies, barely visible to the naked eye, were frequently encountered. In several cases lactobacilli were found growing in small (1 to 3 mm.), raised surface colonies on tomato juice agar.

The term "lactobacillus" has been used rather loosely in this work to refer to a group of bacteria which may include several members of the family Lactobacteriaceae. Although none of the organisms isolated was thought to belong to the genus Leuconostoc, there remains some doubt that Leuconostoc can always be separated from certain types of Lactobacillus by morphological and cultural characteristics. The fact that some cultures grew fairly well aerobically suggests that some of the cultures could more properly be placed in the genus Microbacterium.

The failure of the vast majority of cultures to cause greening when inoculated on steamed frankfurters is not necessarily in disagreement with results reported by Niven et al. (82). Greening, although present, was not a primary cause of spoilage for the frankfurters from which cultures were obtained, and no effort was made to select organisms from green areas specifically. The failure to produce gas in dextrose indicates that cultures were different than those found by Niven and co-workers to be responsible for green discolorations, although the use of Eldredge tubes (82) might have given different results.

V. CONCLUSIONS

The following conclusions were reached in regard to storage of cut-up chicken in atmospheres containing carbon dioxide:

1. Increase of CO_2 concentration within the range 0 to 25 per cent progressively prolonged storage life.

2. Storage index (ratio of keeping time in CO_2 to keeping time in air) gave a useful estimate of the effectiveness of carbon dioxide. A linear relationship was indicated between CO_2 concentration and storage index.

3. Use of CO_2 levels above 25 per cent was not feasible because of the resulting discoloration. Color changes were sometimes caused by 15 and 25 per cent CO_2 when keeping times were long.

4. Carbon dioxide increased generation times and lag times of the slime forming bacteria. Average generation time appeared to be a logarithmic function of CO_2 concentration.

5. Uptake of carbon dioxide and oxygen by chicken stored in sealed containers caused large reductions in pressure and, eventually, led to anaerobic conditions.

6. Reduction in temperature resulted in better storage life and enhanced inhibition of bacteria by carbon dioxide. The relationship of average generation time to temperature conformed to the Arrhenius-van't Hoff equation.

7. Length and regularity of keeping times with given storage conditions depended on the amount and uniformity of the initial bacterial load.

8. Types of bacteria causing spoilage were not changed appreciably by differences in temperature (32° to 50° F.) or in CO_2 concentration (0 to 25%). Source of chicken made some difference in the types predominating. Under low oxygen tension an entirely different flora developed.

The following conclusions were reached in regard to storage of frankfurters in atmospheres containing carbon dioxide:

1. Increase of CO_2 concentration within the range 0 to 50 per cent progressively prolonged storage life.

2. Use of CO_2 levels above 50 per cent gave no apparent increase in keeping time.

3. Carbon dioxide had no adverse effects on color or flavor.

4. The type of microbial spoilage encountered varied widely with CO_2 concentration. The tolerance of microorganisms to carbon dioxide increased in the following order: molds < yeasts < micrococci < lactobacilli.

5. Carbon dioxide effectively inhibited growth of molds, yeasts and micrococci but was much less effective against lactobacilli.

6. Reduction in storage temperature gave longer keeping times and increased effectiveness of carbon dioxide.

VI. SUMMARY

A study was made of the effect of atmospheres containing carbon dioxide on storage life of cut-up chicken. Evaluation of this effect was made difficult by tremendous differences in keeping time encountered with pieces of chicken stored under the same conditions. The differences were ascribed to variations in initial bacterial load. Ratio of keeping time in CO_2 to keeping time in air (storage index) gave a useful indication of the action of carbon dioxide; however, storage index also was dependent on original bacteriological quality. Storage indices obtained for chicken backs stored at 40° F. (4.4° C.) with 15 per cent CO_2 varied from 1.42 to 2.25. Storage index appeared to be directly proportional to carbon dioxide concentration.

Multiplication of slime forming bacteria on surfaces of stored chicken was followed by means of plate counts. Average generation times computed from growth curves gave a good measure of the influence of carbon dioxide on bacterial growth. Average generation time was a logarithmic function of CO_2 concentration within the range 0 to 25 per cent. Increasing CO_2 level also tended to prolong lag time.

Reduction of storage temperature markedly improved keeping time of cut-up poultry. Temperatures of less than 50° F. (10° C.) were considered essential for satisfactory storage life with or without carbon dioxide storage. Effectiveness of CO_2 in retarding bacterial proliferation was enhanced by decrease in temperature. In the range 32° to

50° F. (0-10° C.), relation between rate of bacterial growth and temperature conformed rather well with the Arrhenius-van't Hoff equation. This was true both for storage in air and for storage in atmospheres containing 15 and 25 per cent carbon dioxide.

Keeping times for cut-up chicken were determined from the time required for: (1) a definite off-odor to be detected; (2) a specified increase in CO_2 to be observed in storage containers; (3) numbers of bacteria to reach a value of 2×10^8 per sq. cm. of chicken surface. Good agreement was obtained by the three methods.

A straight line gave a reasonably good expression of the relationship of storage life of cut-up poultry to the logarithms of initial bacterial count. The original bacteriological quality had tremendous effect on keeping time, whether or not CO_2 storage was employed.

Discoloration limited use of high CO_2 concentrations for storing chicken. Some loss of "bloom" was noted even with 15 and 25 per cent CO_2 when keeping times were long.

Slime producing bacteria were predominantly members of the genus Pseudomonas. Another important group was similar in many respects to Alcaligenes viscosus. Temperature (32° to 50° F.) and CO_2 level (0 to 25%) did not affect the types of bacteria isolated from 2 lots of stored chicken. Some differences in type were noted between lots. When jars containing chicken and CO_2 atmospheres remained sealed throughout storage, a different type of bacterial flora developed. This phenomenon was ascribed to reduced oxygen tension.

Carbon dioxide and oxygen concentrations changed markedly when cut-up poultry was stored for some time in a confined atmosphere. These changes were accompanied by large pressure fluctuations. At low oxygen tension CO_2 was of less value in prolonging keeping time and spoilage was putrefactive in nature. The quantities of carbon dioxide dissolved in the chicken were calculated in some experiments.

Effect of atmospheres containing carbon dioxide on storage life of frankfurters also was investigated. Keeping time becaue progressively longer as CO_2 concentration was increased to 50 per cent. CO_2 levels above 50 per cent appeared to be of no additional value. Visible evidences of microbial deterioation were noted before definite changes in flavor or pH were detected.

Reduction of temperature improved storage life and increased the effectiveness of carbon dioxide. Rancidity developed before microbial spoilage was observed when a storage temperature of 30° F. (-1.1° C.) and atmospheres containing 15 and 50 per cent CO_2 were used.

Relative growth rates of the various kinds of microorganisms varied with the CO_2 level employed. Organisms primarily responsible for microbial deterioration varied from molds through yeasts and micrococci to lactobacilli with increasing carbon dioxide concentration. Rates of growth of molds, yeasts and micrococci were found to be more and more retarded as CO_2 level was elevated. Growth rates of lactobacilli were affected much less.

Measurement of pH showed promise as a means of determining storage end points when high CO_2 percentages were employed.

Color of frankfurters was not affected adversely by carbon dioxide.

In one experiment use of relative humidities below 95 per cent caused excessive dehydration of frankfurters without appreciably prolonging keeping time.

L.S.A.T. cellophane did not maintain a carbon dioxide atmosphere around packaged frankfurters.

A holding period in atmospheres containing CO_2 followed by storage in air resulted in longer keeping times for chicken and frankfurter samples than did storage in air only.

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VIII. ACKNOWLEDGMENT

The author wishes to express his appreciation to Dr. John C. Ayres for his continuous guidance and encouragement; to Dr. George F. Stewart for his interest and advice; to Dr. Frances Carlin and her co-workers for organoleptic evaluation of stored frankfurters; and to Mr. K.H. Steinkraus for his helpful suggestions. Appreciation is expressed to Mr. C.W. King, Mr. A.J. Granata and the Pure Carbonic Co. (a division of Air Reduction Co., Inc., New York, N.Y.) for financial support of this undertaking; and to Mr. E. Frank Penne and the Rath Packing Co., Waterloo, Iowa for furnishing frankfurter samples used in this work.

IX. APPENDIX

Table 1A. Storage of defrosted-frozen chicken at 50° F. with an initial CO_2 concentration of 96 per cent

Sample no.	Time stored (days)	Grams chicken per ml. gas space	Grams chicken per ml. CO_2 (initial)	CO_2 conc. (%)	Vacuum (cm. Hg)	CO^2 loss (ml./gm.)	O_2 conc. (%)	Extent of discoloration	Extent of off-odor[a]
VII-1	1	1.93	1.93	68.2	38.5	0.35	7.7	0	0
XI-21	1	1.76	1.76	53.0	32.0	0.39	9.1	0	0
VIII-11	2	1.72	1.72	71.5	30.4	0.33	1.6	++	+
IX-15	2	1.87	1.87	72.2	39.0	0.35	2.6	++	0
X-2	2	1.61	1.61	72.4	35.7	0.39	2.0	0	0
VIII-15	3	1.82	1.82	71.7	37.3	0.35	2.4	++	+
X-3	4	1.70	1.70	90.7	28.0	0.25	0.9	+	+
VIII-6	6	1.88	1.88	70.0	14.4	0.22	1.6	+++	++
IX-12	7	1.77	1.77	48.3	9.0	0.32	8.3	+++	++
X-4	7	1.67	1.67	69.1	16.0	0.27	5.1	++	++
VIII-5	8	1.87	1.87	63.8	3.1	0.195	0.3	+	+++
VIII-9	10	1.78	1.78	52.2	13.5	0.21	0.3	+	+++
X-5	10	1.70	1.70	83.2	0.5	0.105	1.4	++	+
VII-3	1	0.28	0.28	86.3	16.0	1.06	6.4	0	0
VII-4	3	0.26	0.26	83.7	14.0	1.15	11.4	0	0

[a]Off-odor in controls in 2-3 days.

Table 2A. Storage of defrosted-frozen chicken at 50° F. with an initial CO_2 concentration of 80 per cent

Sample no.	Time stored (days)	Grams chicken per ml. gas space	Grams chicken per ml. CO_2 (initial)	CO_2 conc. (%)	Vacuum (cm. Hg)	CO^2 loss (ml./gm.)	O_2 conc. (%)	Extent of discoloration	Extent of off-odor[a]
IX-8	1	1.65	2.05	32.8	15.5	0.32	11.3	0	0
IX-3	2	1.82	2.27	27.6	-1.0	0.28	8.6	0	0
XI-16	2	1.65	2.06	45.7	25.5	0.295	6.2	0	+
IX-6	4	1.64	2.05	56.0	24.0	0.25	2.2	++	+
XI-17	4	1.65	2.07	50.1	25.5	0.27	5.5	+	+
IX-9	6	2.04	2.55	44.8	-5.0	0.15	0.4	+	+++
XI-18	6	1.66	2.08	52.1	5.0	0.18	5.6	++	+
IX-11	7	1.71	2.13	47.3	2.0	0.18	2.6	++	+++
XI-19	7	1.68	2.10	54.9	5.5	0.17	4.7	+	+++
XI-20	10	1.69	2.12	57.1	2.5	0.135	2.4	+	+++

[a]Off-odor in controls in 2-4 days

Table 3A. Storage of defrosted-frozen chicken at 50° F. with an initial atmosphere of 80% CO_2 and 20% O_2

Sample no.	Time stored (days)	Grams chicken per ml. gas space	Grams chicken per ml. CO_2 (initial)	CO_2 conc. (%)	Vacuum (cm. Hg)	CO^2 loss (ml./gm.)	O_2 conc. (%)	Extent of discoloration	Extent of off-odor[a]
XIII-16	2	1.66	2.07	65.9	27.5	0.22	14.9	+	+
XIII-17	4	1.62	2.02	-	23.5	-	-	+I	+I
XIII-18	4	1.71	2.13	68.6	33.5	0.24	5.0	+	+
XIX-19	6	1.68	2.10	59.6	19.5	0.21	6.4	+	++
XX-20	10	2.02	2.53	41.7	6.5	0.20	8.7	+I	+++

[a]Off-odor in controls in 2 days

Table 4A. Storage of frozen-defrosted chicken at 40° F. with an initial CO_2 concentration of 96 per cent

Sample no.	Time stored (days)	Grams chicken per ml. gas space	Grams chicken per ml. CO_2 (initial)	CO_2 conc. (%)	Vacuum (cm. Hg)	CO_2 loss (ml./gm.)	O_2 conc. (%)	Extent of discoloration	Extent of off-odor[a]
XII-1	2	1.62	1.62	35.0	19.0	0.45	10.8	0	0
XII-2	4	1.63	1.63	43.2	34.0	0.47	10.1	±	0
XII-3	6	1.69	1.69	57.3	37.0	0.415	7.5	+	0
XII-4	12	1.71	1.71	79.8	34.0	0.33	2.9	++	+
XII-5	17	1.76	1.76	43.1	0	0.32	10.4	++	+++

[a]Off-odor in control at 5 days

Table 5A. Storage of frozen-defrosted chicken at 40° F. with initial CO_2 concentration of 25 per cent

Sample no.	Time stored (days)	Grams chicken per ml. gas space	Grams chicken per ml. CO_2 (initial)	CO_2 conc. (%)	Vacuum (cm. Hg)	CO_2 loss (ml./gm.)	O_2 conc. (%)	Extent of discoloration	Extent of off-odor[a]
XIV-1	1	1.74	6.97	9.9	22.5	0.102	16.7	0	0
XIV-2	5	1.13	4.53	15.4	20.8	0.118	8.3	0	++
XIV-3	6	1.66	6.65	18.1	18.0	0.065	0.1	0	+
XIV-4	8	1.16	4.63	18.8	0.5	0.046	0.7	+	+
XIV-5	14	1.17	4.68	27.7	-5.0	-0.062	2.0	±	++

[a]Off-odor in control at 4 days

Table 6A. Storage of fresh chicken at 50° F. with initial CO_2 concentration of 25 per cent

Sample no.	Time stored (days)	Grams chicken per ml. gas space	Grams chicken per ml. CO_2 (initial)	CO_2 conc. (%)	Vacuum (cm. Hg)	CO_2 loss (ml./gm.)	O_2 conc. (%)	Extent of discoloration	Extent of off-odor[a]
XVI-1	2	1.63	6.54	18.2	20.0	0.067	5.2	0	0
XVI-2	6	1.59	6.38	19.4	5.0	0.038	0.8	0	++
XVI-3	6	1.72	6.90	18.2	2.0	0.038	1.8	0	+
XVI-4	8	1.70	6.78	20.1	2.0	0.027	0.9	0	++

[a]Off-odor in control at 5 days

Table 7A. Storage of fresh chicken at 40° F. with initial CO_2 concentration of 25 per cent

Sample no.	Time stored (days)	Grams chicken per ml. gas space	Grams chicken per ml. CO_2 (initial)	CO_2 conc. (%)	Vacuum (cm. Hg)	CO_2 loss (ml./gm.)	O_2 conc. (%)	Extent of discoloration	Extent of off-odor[a]
XV-16	1	1.57	6.27	15.9	21.0	0.062	9.5	0	0
XV-17	14	1.73	6.93	19.3	19.5	0.056	0.8	0	0
XV-18	24	1.49	5.94	24.4	20.5	0.042	0	0	0
XV-16	34	1.57	6.27	26.8	18.0	0.040	0.1	0	+
XV-19	41	1.75	6.95	20.5	5.0	0.026	4.5	0	+++

[a]Off-odor in control at 14 days

Table 8A. Weight-volume relationships and CO_2 uptake obtained with cut-up chicken stored in sealed containers

Run no.	Jar no.	Original CO_2 conc. (%)	Storage temp. (° F.)	Weight chicken (gm.)	Days stored	Final CO_2 conc. (%)	Vacuum (cm. Hg)	CO_2 loss (ml./gm.)	Grams chicken per ml. gas	Grams chicken per ml. CO_2	Type of chicken
VII	1	96	50	626.4	1	68.2	38.5	0.35		1.93	Frozen
	2	96	50	634.5	1	68.2	-	-		1.94	Frozen
	3	96	50	204.3	1	86.3	16.0	1.06		0.28	Frozen
	4	96	50	187.9	3	83.7	14.0	1.15		0.26	Frozen
VIII	9	96	50	594.2	0	94.8	0	-		1.78	Frozen
	11	96	50	609.3	2	71.5	30.4	0.33		1.72	Frozen
	15	96	50	619.7	3	71.7	37.3	0.35		1.82	Frozen
	6	96	50	635.4	6	70.0	14.4	0.22		1.88	Frozen
	5	96	50	622.5	8	63.8	3.1	0.195		1.87	Frozen
	9	96	50	594.2	10	52.2	-13.5	0.21		1.78	Frozen
IX	15	96	50	626.0	2	72.2	39.0	0.35		1.87	Frozen
	12	96	50	609.4	7	48.3	9.0	0.32		1.77	Frozen
X	1	96	50	589.1	1	54.5	18+	-		1.61	Frozen
	2	96	50	594.7	2	72.4	35.7	0.39		1.61	Frozen
	3	96	50	598.7	4	90.7	28.0	0.25		1.70	Frozen
	4	96	50	593.1	7	69.1	16.0	0.27		1.67	Frozen
	5	96	50	592.5	10	83.2	0.5	0.105		1.70	Frozen
XI	21	96	50	605.9	1	53.0	32.0	0.39		1.76	Frozen
XII	1	96	40	590.5	2	35.0	19.0	0.45		1.62	Frozen
	2	96	40	596.6	4	43.2	34.0	0.47		1.63	Frozen
	3	96	40	596.7	6	57.3	37.0	0.415		1.69	Frozen
	4	96	40	598.7	12	79.8	34.0	0.33		1.71	Frozen
	5	96	40	600.8	17	43.1	0	0.32		1.76	Frozen

Table 8A. (continued)

Run no.	Jar no.	Original CO_2 conc. (%)	Storage temp. (° F.)	Weight chicken (gm.)	Days stored	Final CO_2 conc. (%)	Vacuum (cm. Hg)	CO_2 loss (ml./gm.)	Grams chicken per ml. gas	Grams chicken per ml. CO_2	Type of chicken
IX	8	80	50	580.3	1	32.8	15.5	0.32	1.65	2.05	Frozen
	3	80	50	616.6	2	27.6	-1.0	0.28	1.82	2.27	Frozen
	6	80	50	602.3	4	56.0	24.0	0.25	1.64	2.05	Frozen
	9	80	50	623.1	6	44.8	-5.0	0.15	2.04	2.55	Frozen
	11	80	50	606.6	7	47.3	2.0	0.18	1.71	2.13	Frozen
XI	16	80	50	596.9	2	45.7	25.5	0.295	1.65	2.06	Frozen
	17	80	50	634.4	4	50.1	25.5	0.27	1.65	2.07	Frozen
	18	80	50	574.4	6	52.1	5.0	0.18	1.66	2.08	Frozen
	19	80	50	620.1	7	54.9	5.5	0.17	1.68	2.10	Frozen
	20	80	50	576.3	10	57.1	2.5	0.135	1.69	2.12	Frozen
XIII	16	80 (02)	50	598.1	2	65.9	27.5	0.22	1.66	2.07	Frozen
	17	80	50	629.2	4	-	23.5	-	1.62	2.02	Frozen
	18	80	50	579.7	4	66.6	33.5	0.24	1.71	2.13	Frozen
	19	80	50	619.4	6	59.6	19.5	0.21	1.68	2.10	Frozen
	20	80	50	615.8	10	41.7	6.5	0.20	2.02	2.53	Frozen
XIV	1	25	40	608.0	1	9.9	22.5	0.102	1.74	6.97	Frozen
	3	25	40	500.0	5	15.4	20.8	0.118	1.13	4.53	Frozen
	2	25	40	602.0	6	18.1	18.0	0.065	1.66	6.65	Frozen
	4	25	40	505.0	8	18.8	0.5	0.046	1.16	4.63	Frozen
	5	25	40	504.0	14	27.7	-5.0	-0.062	1.17	4.68	Frozen
XV	16	25	40	585.0	1	15.9	21.0	0.082	1.57	6.27	Fresh
	17	25	40	646.0	14	19.3	19.5	0.056	1.73	6.93	Fresh
	18	25	40	548.0	24	24.4	20.5	0.042	1.49	5.94	Fresh
	19	25	40	628.0	41	20.5	5.0	0.026	1.74	6.95	Fresh
XVI	1	25	50	593.0	2	18.2	20.0	0.067	1.63	6.54	Fresh
	2	25	50	592.0	6	19.4	5.0	0.038	1.59	6.38	Fresh
	3	25	50	601.0	6	18.2	2.0	0.038	1.72	6.90	Fresh
	4	25	50	597.0	8	20.1	2.0	0.027	1.70	6.78	Fresh

Table 9A. Specific gravities of pieces of thawed-frozen chicken[a]

Piece	Density
Leg	1.111
Wing	1.084
Thigh	1.066
Wishbone	1.047
Ribs	1.075
Breast	1.143
Back	1.102
Giblets	1.064
Average	1.086
Mean	1.095

[a]Specific gravities determined from volume of water displaced.

Table 10A. Effect of CO_2 on the storage life of commercially dressed, thawed-frozen chicken[a]

CO_2 conc. (%)	Keeping time (days)				Average storage index
	Run III	Run IV	Run VI	Average	
0	4	4	4	4.0	-
10	5	5	4	4.6	1.17
25	7	6	5	6.0	1.50
50	7	6	6	6.3	1.67
96	8	7	7	7.3	1.92

[a]Conditions of storage:
Temperature, 50° F. (10° C.)
Relative humidity, approximately 100%
Containers, 2-qt. jars
Constant CO_2 levels, obtained and maintained by the displacement method
End points determined from off-odor

Table 11A. Effect of CO_2 on the storage life of fresh dressed chicken[a]

Run no.	Temp. (° F.)	CO_2 level (%)	Average keeping time control (days)	Average keeping time in CO_2 (days)	Storage index
1A	40	25	16	36.5	2.28
2A	40	5	17	20.0	1.18
		15	18.3	30.0	1.64
		25	18.3	38.5	2.10
3A	40	15	10.7	18.2	1.70
	32	15	39.5	78.5	1.99

[a]Conditions of storage:
Temperature, indicated in table
Relative humidity, approximately 100%
Containers, 2-qt. jars
Constant CO_2 levels, obtained and maintained by the evacuation method
End points determined from off-odor

Table 12A. Effect of CO_2 on the storage life of fresh, commercially dressed chicken[a]

Temp. (° F.)	CO_2 conc. (%)	Keeping time (days)				Storage index				Average storage index
		4A	5A	6A	7A	4A	5A	6A	7A	
50	0	2			1.75	-			-	
	15	3			2.50	1.50			1.43	1.47
	25	4			2.75	2.00			1.57	1.78
	50	-			3.25	-			1.86	
45	0				4				-	
	15				5				1.25	
	25				5.5				1.38	
	50				6				1.50	
40	0	4	7	5		-	-	-		
	5	7	9	6		1.75	1.28	1.20		1.41
	10	-	11	7		-	1.57	1.40		
	15	10	12	8		2.50	1.72	1.60		1.94
	20	-	15	10		-	2.14	2.0		
	25	12	16	12		3.00	2.29	2.40		2.56
32	0	12	17			-	-			
	5	14	24			1.17	1.41			1.29
	10	-	26			-	1.53			
	15	23	39			1.92	2.29			2.10
	20	-	44			-	2.59			
	25	31	44			2.58	2.59			2.59

[a]Conditions of storage:
Temperature, indicated in table
Relative humidity, approximately 100%
Containers, 2-qt. jars
Constant CO_2 level, obtained and maintained by the evacuation method (4A) and the displacement method (5A, 6A, 7A)
End points determined from off-odor

Table 13A. Calculation of growth rates of bacteria on chicken in terms of velocity coefficient, $\underline{k}$, and average generation time, $\underline{g}$[a,b] (Run 4A)

Temp. (° F.)	CO_2 level (%)	b (no./cm^2)	B (no./cm^2)	t (hours)	k	g (hours)
50	0	8.4×10^8	7.0×10^3	48	0.243	2.86
	15	1.75×10^8	1.2×10^3	72	0.165	4.21
	25	2.4×10^8	7.8×10^3	84	0.123	5.64
40	0	1.35×10^9	6.5×10^5	72	0.106	6.54
	5	4.3×10^9	7.0×10^4	120	0.0918	7.56
	15	2.6×10^9	1.2×10^3	240	0.0606	11.54
	25	6.5×10^7	2.5×10^3	240	0.0423	16.40
32	0	2.8×10^8	5.0×10^5	120	0.0527	13.17
	5	2.3×10^8	7.0×10^4	240	0.0337	20.6
	15	1.9×10^8	2.1×10^5	240	0.0283	24.5
	25	4.6×10^7	1.1×10^6	240	0.0155	44.7

[a] $\underline{k} = \frac{2.3}{t} \quad (\log \underline{b} - \log \underline{B})$

[b] $\underline{g} = \frac{\ln 2}{\underline{k}}$

Table 14A. Bacterial counts from stored fresh, commercially dressed chicken thighs[a] (Run 5A)

Storage temp. (° F.)	Storage time (days)	CO_2 levels (%)					
		0	5	10	15	20	
40	0	1.43×10^4	8.7×10^3	7.6×10^3	1.0×10^4	4.5×10^4	1
	2	1.44×10^4	1.04×10^4	6.0×10^3	1.9×10^4	2.02×10^4	1
	4.4	8.9×10^5	2.0×10^4	2.3×10^4	3.5×10^4		
	5					3.3×10^4	1
	6.2	2.4×10^7	4.0×10^5	8.6×10^4	1.8×10^5		
	7.2	6.5×10^8	3.3×10^5				
	8.2		6.6×10^7	2.2×10^6	3.4×10^6	2.1×10^5	6
	9		2.8×10^8				
	10.2			6.1×10^7	4.8×10^7	5.6×10^6	
	11.2			2.2×10^8	1.95×10^8		1
	12.2					1.5×10^7	
	13						4
	15					3.0×10^8	4
	18						
32	0	3.7×10^4	1.6×10^4	1.2×10^4	7.7×10^3	1.4×10^4	2
	7	5.6×10^4	1.8×10^4	1.1×10^4			
	10	1.6×10^5	2.8×10^4	4.2×10^4			
	12	2.1×10^6					
	13		2.3×10^5	2.8×10^5	1.2×10^4	1.3×10^4	
	15	9.3×10^6					
	17	2.7×10^8					
	18		8.8×10^6	5.7×10^6	9.8×10^3	2.1×10^4	
	22		1.2×10^8	6.6×10^7		8.0×10^4	2
	27				2.3×10^6		2
	30				7.6×10^6		4
	34				1.3×10^8	7.9×10^6	1
	38				9.5×10^8		5
	41					1.2×10^8	1

[a]Number of bacteria per square centimeter of muscle surface

Bacterial counts from stored fresh, commercially dressed chicken thighs[a] (Run 5A)

CO_2 levels (%)					
0	5	10	15	20	25
1.43×10^4	8.7×10^3	7.6×10^3	1.0×10^4	4.5×10^4	1.65×10^4
1.44×10^4	1.04×10^4	6.0×10^3	1.9×10^4	2.02×10^4	1.01×10^4
8.9×10^5	2.0×10^4	2.3×10^4	3.5×10^4		
				3.3×10^4	1.03×10^4
2.4×10^7	4.0×10^5	3.6×10^4	1.8×10^5		
6.5×10^8	3.3×10^5				
	6.6×10^7	2.2×10^6	3.4×10^6	2.1×10^5	6.0×10^4
	2.8×10^8				
		6.1×10^7	4.8×10^7	5.6×10^6	
		2.2×10^8	1.95×10^8		1.13×10^6
				1.5×10^7	
					4.6×10^6
				3.0×10^8	4.0×10^7
3.7×10^4	1.6×10^4	1.2×10^4	7.7×10^3	1.4×10^4	2.3×10^4
5.6×10^4	1.8×10^4	1.1×10^4			
1.6×10^5	2.8×10^4	4.2×10^4			
2.1×10^6					
	2.3×10^5	2.8×10^5	1.2×10^4	1.3×10^4	
9.3×10^6					
2.7×10^8					
	8.8×10^6	5.7×10^6	9.8×10^3	2.1×10^4	
	1.2×10^8	6.6×10^7		8.0×10^4	2.8×10^5
			2.3×10^6		2.2×10^6
			7.6×10^6		4.3×10^6
			1.3×10^8	7.9×10^6	1.8×10^7
			9.5×10^8		5.2×10^7
				1.2×10^8	1.4×10^8

[a] teria per square centimeter of muscle surface

Table 15A. Calculation of growth rates of bacteria on chicken in terms of velocity coefficient, $\underline{k}$, and average generation time, $\underline{g}$[a,b] (Run 5A)

Temp. (° F.)	CO_2 level (%)	b (no./cm.^{2})	B (no./cm.^{2})	t (hours)	k	g (hours)
40	0	3.9×10^7	5.1×10^5	48	0.0884	7.85
	5	2.7×10^7	5.0×10^5	48	0.0831	8.35
	10	7.3×10^8	1.2×10^5	144	0.0605	11.5
	15	4.6×10^8	2.4×10^5	144	0.0525	13.2
	20	1.4×10^8	3.3×10^5	144	0.0420	16.5
	25	8.2×10^7	3.4×10^5	144	0.0381	18.2
32	0	2.3×10^7	1.6×10^5	120	0.0414	16.75
	5	3.2×10^7	3.2×10^4	240	0.0288	24.1
	10	1.8×10^7	4.2×10^4	240	0.0252	27.5
	15	1.8×10^8	6.5×10^5	240	0.0243	29.7
	20	6.0×10^8	1.3×10^7	240	0.0160	43.3
	25	1.1×10^8	4.4×10^6	240	0.0134	51.8

[a] $\underline{k} = \frac{2.3}{t} (\log \underline{b} - \log \underline{B})$

[b] $\underline{g} = \frac{\ln 2}{\underline{k}}$

Table 16A. Bacterial counts from fresh, commercially dressed chicken thighs and legs stored at 40° F. (4.4° C.)[a] (Run 6A)

CO_2 level (%)	Sample	0	2	3	4	5	6	7
0	Thigh 1	6.4×10^4	1.7×10^6		1.7×10^8	6.8×10^8		
	Thigh 2	5.9×10^4	9.6×10^5		2.1×10^8	5.5×10^8		
	Leg	7.2×10^4	6.9×10^6		9.0×10^8	3.1×10^9		
	Log. av.	6.5×10^4	2.24×10^6		3.2×10^8	1.05×10^9		
5	Thigh 1	1.4×10^5	1.43×10^5		1.6×10^7	2.3×10^7	4.0×10^8	
	Thigh 2	1.0×10^6	3.3×10^5		5.2×10^7	1.18×10^8	7.4×10^8	
	Thigh 3	2.75×10^5	3.6×10^6		6.0×10^7	3.3×10^8	9.7×10^8	
	Leg	9.0×10^4	4.8×10^5		3.4×10^7	3.2×10^7	5.0×10^8	
	Log av.	2.4×10^5	5.3×10^5		3.6×10^7	7.3×10^7	6.15×10^8	
10	Thigh 1	1.3×10^5		5.1×10^5		5.7×10^6		1.25×10^8
	Thigh 2	1.67×10^5		1.52×10^6		2.0×10^7		4.4×10^8
	Thigh 3	1.51×10^5		9.0×10^5		1.87×10^7		7.1×10^8
	Leg	6.3×10^4		1.67×10^6		2.9×10^7		1.15×10^8
	Log av.	1.2×10^5		1.04×10^6		1.6×10^7		2.6×10^8
15	Thigh 1	6.0×10^5		4.6×10^5			2.9×10^7	2.
	Thigh 2	7.0×10^5		1.74×10^6			5.5×10^7	5.
	Leg	9.4×10^4		2.1×10^5			1.22×10^7	1.
	Log av.	3.4×10^5		5.5×10^5			2.7×10^7	2.
20	Thigh 1	2.59×10^5		5.2×10^5			2.3×10^7	9.
	Thigh 2	1.18×10^5		4.6×10^5			8.4×10^6	1.
	Thigh 3	9.4×10^4		1.6×10^5			1.8×10^7	1.
	Leg	2.3×10^5		4.6×10^5			9.5×10^6	1.
	Log av.	1.6×10^5		3.64×10^5			1.35×10^7	6.
25	Thigh 1	1.0×10^5			4.6×10^5			1.13×10^7
	Thigh 2	2.67×10^5			5.0×10^5			2.2×10^7
	Thigh 3	2.06×10^5			2.8×10^6			3.1×10^7
	Leg	1.51×10^5			8.7×10^5			1.1×10^7
	Log av.	1.7×10^5			8.7×10^5			1.7×10^7

[a] Number of bacteria per square centimeter of muscle (thighs) or skin (legs) surface

esh, commercially dressed chicken thighs
. (4.4° C.)[a]

4	5	6	7	8	9	10	11
1.7×10^8	6.8×10^8						
2.1×10^8	5.5×10^8						
9.0×10^8	3.1×10^9						
3.2×10^8	1.05×10^9						
1.6×10^7	2.3×10^7	4.0×10^8					
5.2×10^7	1.18×10^8	7.4×10^8					
6.0×10^7	3.3×10^8	9.7×10^8					
3.4×10^7	3.2×10^7	5.0×10^8					
3.6×10^7	7.3×10^7	6.15×10^8					
10^5	5.7×10^6		1.25×10^8				
10^6	2.0×10^7		4.4×10^8				
10^5	1.87×10^7		7.1×10^8				
10^6	2.9×10^7		1.15×10^8				
10^6	1.6×10^7		2.6×10^8				
10^5		2.9×10^7		2.9×10^8			
10^6		5.5×10^7		5.8×10^8			
10^5		1.22×10^7		1.55×10^8			
10^5		2.7×10^7		2.96×10^8			
10^5		2.3×10^7		9.1×10^7		5.8×10^8	
10^5		8.4×10^6		1.05×10^8		3.6×10^8	
10^5		1.8×10^7		1.2×10^8		4.7×10^8	
10^5		9.5×10^6		1.3×10^7		2.9×10^8	
10^5		1.35×10^7		6.2×10^7		4.1×10^8	
4.6×10^5			1.13×10^7		1.2×10^8		3.6×10^8
5.0×10^5			2.2×10^7		7.3×10^7		2.8×10^8
2.8×10^6			3.1×10^7		2.1×10^8		8.9×10^8
8.7×10^5			1.1×10^7		8.1×10^7		1.9×10^8
8.7×10^5			1.7×10^7		1.1×10^8		3.6×10^8

eter of muscle (thighs) or skin (legs) surface

Table 17A. Calculation of growth rates of bacteria on chicken in terms of velocity coefficient, $\underline{k}$, and average generation time, $\underline{g}$[a,b] (Run 6A)

Temp. (° F.)	CO_2 level (%)	b (no./cm.^{2})	B (no./cm.^{2})	t (hours)	k	g (hours)
40	0	1.8×10^8	2.3×10^6	48	0.0908	7.64
	5	7.8×10^8	5.3×10^6	96	0.0759	9.14
	10	2.9×10^8	9.4×10^5	96	0.0597	11.6
	15	3.3×10^8	5.7×10^5	120	0.0529	13.1
	20	6.9×10^8	5.1×10^5	168	0.0428	16.2
	25	4.7×10^8	1.1×10^6	168	0.0360	19.2

[a] $\underline{k} = \frac{2.3}{\underline{t}} (\log \underline{b} - \log \underline{B})$

[b] $\underline{g} = \frac{\ln 2}{\underline{k}}$

Table 1B. Effect of storage time on pH of frankfurters stored at several temperatures with 0, 25 and 25 per cent CO_2[a]

CO_2 conc. (%)	0				25				96			
Temp. (° F.)	35	40	45	50	35	40	45	50	35	40	45	50
Microbiological keeping time (days)	26	18	10	6	60	50	32	10	ca 65	ca 60	ca 40	ca 19
Time stored (days)												
3				6.2				6.15				6.2
7				6.1				6.05				6.1
8			6.15				6.05					
11				5.85				6.05				6.2
14			6.25					5.8				5.9
17			5.95				5.9					
19	6.2				6.05			5.6	6.0			
25			6.1				5.95					5.2
29	6.3				6.2				6.2		6.0	
39	6.2				6.2				6.15			
42						6.1						
45							5.3				5.1	
53						6.0				6.15		
66					5.9				5.85		4.9	
72						6.0				5.2		
86										5.3		
120									5.5		4.8	

[a]Fresh frankfurters varied in pH from 6.0 to 6.2.

Table 2B. Counts of microorganisms[a] on frankfurters stored at 40° F. under various CO_2 concentrations[b,c]

CO_2 level (%)						
0	Storage time (days)	7	11	14	18	26
	#1	5.2×10^3	7.2×10^5	5.3×10^6	2.6×10^7	9.7×10^8
	#2	3.8×10^3	1.2×10^5	3.4×10^6	1.1×10^8	7.5×10^9
	Log average	4.4×10^2	2.9×10^5	4.2×10^6	5.3×10^7	2.7×10^9
5	Storage time (days)	7	13	18	26	31
	#1	1.3×10^3	6.0×10^4	2.6×10^7	5.3×10^7	3.7×10^9
	#2	2.6×10^3	1.6×10^4	1.2×10^6	3.3×10^8	1.5×10^9
	Log average	1.8×10^3	3.1×10^4	5.6×10^6	1.3×10^8	2.4×10^9
15	Storage time (days)	9	14	20	29	41
	#1	1.2×10^4	1.4×10^5	9.6×10^6	9.7×10^8	8.1×10^8
	#2	4.5×10^3	8.0×10^4	1.5×10^6	3.3×10^8	5.2×10^9
	Log average	7.3×10^3	1.1×10^5	3.8×10^6	6.1×10^8	2.1×10^9
25	Storage time (days)	9	14	20	29	41
	#1	9.2×10^3	3.4×10^3	8.6×10^3	1.0×10^8	1.2×10^6
	#2	5.4×10^2	7.5×10^3	4.7×10^4	9.2×10^8	1.4×10^8
	Log average	2.2×10^3	5.1×10^3	2.0×10^4	3.0×10^8	1.3×10^7
50	Storage time (days)	9	17	25	42	53
	#1	9.2×10^2	3.8×10^4	1.1×10^5	1.0×10^9	8.2×10^9
	#2	5.4×10^3	1.1×10^4	3.7×10^6	9.2×10^8	5.3×10^9
	Log average	2.2×10^3	2.0×10^4	6.1×10^5	9.6×10^8	6.6×10^9
75	Storage time (days)	10	17	25	42	54
	#1	5.2×10^3	7.3×10^4	5.9×10^5	2.1×10^9	9.0×10^9
	#2	6.2×10^2	2.9×10^5	6.0×10^6	1.6×10^9	6.4×10^9
	Log average	1.8×10^3	1.5×10^5	1.9×10^6	1.8×10^9	7.6×10^9
96	Storage time (days)	10	17	25	45	54
	#1	3.2×10^3	3.3×10^5	8.4×10^7	1.0×10^{10}	6.0×10^9
	#2	5.5×10^3	5.0×10^5	7.8×10^7	2.4×10^9	5.0×10^9
	Log average	4.2×10^3	4.1×10^5	8.1×10^7	4.9×10^9	5.5×10^9

[a] Does not include molds
[b] Numbers of microorganisms per frankfurter
[c] Average initial count 4.0×10^3

Counts of microorganisms[a] on frankfurters stored at 40° F. under various CO_2 concentrations[b,c]

orage time (days)	7	11	14	18	26	
#1	5.2×10^3	7.2×10^5	5.3×10^6	2.6×10^7	9.7×10^8	
#2	3.8×10^3	1.2×10^5	3.4×10^6	1.1×10^8	7.5×10^9	
Log average	4.4×10^2	2.9×10^5	4.2×10^6	5.3×10^7	2.7×10^9	
orage time (days)	7	13	18	26	31	40
#1	1.3×10^3	6.0×10^4	2.6×10^7	5.3×10^7	3.7×10^9	1.2×10^{10}
#2	2.6×10^3	1.6×10^4	1.2×10^6	3.3×10^8	1.5×10^9	2.7×10^9
Log average	1.8×10^3	3.1×10^4	5.6×10^6	1.3×10^8	2.4×10^9	5.7×10^9
rage time (days)	9	14	20	29	41	52
#1	1.2×10^4	1.4×10^5	9.6×10^6	9.7×10^8	8.1×10^8	4.5×10^8
#2	4.5×10^3	8.0×10^4	1.5×10^6	3.3×10^8	5.2×10^9	3.1×10^{10}
Log average	7.3×10^3	1.1×10^5	3.8×10^6	6.1×10^8	2.1×10^9	3.7×10^9
rage time (days)	9	14	20	29	41	53
#1	9.2×10^3	3.4×10^3	8.6×10^3	1.0×10^8	1.2×10^6	6.6×10^8
#2	5.4×10^2	7.5×10^3	4.7×10^4	9.2×10^8	1.4×10^8	2.4×10^8
Log average	2.2×10^3	5.1×10^3	2.0×10^4	3.0×10^8	1.3×10^7	4.0×10^8
rage time (days)	9	17	25	42	53	72
#1	9.2×10^2	3.8×10^4	1.1×10^5	1.0×10^9	8.2×10^9	4.8×10^9
#2	5.4×10^3	1.1×10^4	3.7×10^6	9.2×10^8	5.3×10^9	2.0×10^{10}
Log average	2.2×10^3	2.0×10^4	6.1×10^5	9.6×10^8	6.6×10^9	9.8×10^9
rage time (days)	10	17	25	42	54	72
#1	5.2×10^3	7.3×10^4	5.9×10^5	2.1×10^9	9.0×10^9	1.2×10^{10}
#2	6.2×10^2	2.9×10^5	6.0×10^6	1.6×10^9	6.4×10^9	8.8×10^9
Log average	1.8×10^3	1.5×10^5	1.9×10^6	1.8×10^9	7.6×10^9	9.7×10^9
rage time (days)	10	17	25	45	54	72
#1	3.2×10^3	3.3×10^5	8.4×10^7	1.0×10^{10}	6.0×10^9	1.1×10^{10}
#2	5.5×10^3	5.0×10^5	7.8×10^7	2.4×10^9	5.0×10^9	1.1×10^{10}
Log average	4.2×10^3	4.1×10^5	8.1×10^7	4.9×10^9	5.5×10^9	1.1×10^{10}

nclude molds
microorganisms per frankfurter
itial count 4.0×10^3

Table 3B. Counts of micrococci on frankfurters stored at 45° F. (7.2° C.) under various CO_2 concentrations[a,b]

CO_2 level (%)				
0	Storage time (days)	8	14	17
	#1	7.7×10^6	5.7×10^8	5.6×10^9
	#2	4.9×10^6	1.5×10^9	8.7×10^9
	Log average	6.1×10^6	9.2×10^8	7.0×10^9
5	Storage time (days)	8	16	20
	#1	4.6×10^6	1.7×10^9	5.3×10^9
	#2	1.8×10^6	2.7×10^8	5.9×10^9
	Log average	2.9×10^6	6.8×10^8	5.6×10^9
15	Storage time (days)	9	18	24
	#1	8.4×10^5	8.0×10^7	9.0×10^8
	#2	4.0×10^5	8.8×10^7	8.6×10^8
	Log average	6.2×10^5	8.4×10^7	8.8×10^8
25	Storage time (days)	15	22	45
	#1	2.1×10^5	4.8×10^7	7.1×10^9
	#2	1.3×10^6	2.6×10^6	2.3×10^9
	Log average	5.2×10^5	1.1×10^7	4.0×10^9
50	Storage time (days)	15	23	45
	#1	5.0×10^3	1.3×10^6	7.6×10^7
	#2	1.2×10^4	7.8×10^4	6.0×10^8
	Log average	7.7×10^3	3.2×10^5	2.1×10^8
75	Storage time (days)	17	23	46
	#1	4.4×10^3	1.5×10^4	9.1×10^6
	#2	2.8×10^3	2.0×10^4	2.3×10^6
	Log average	3.5×10^3	1.7×10^4	4.6×10^6
96	Storage time (days)	14	22	46
	#1	3.0×10^3	8.8×10^2	0
	#2	8.0×10^3	3.1×10^2	0
	Log average	1.5×10^3	5.8×10^2	0

[a] Numbers of micrococci per frankfurter
[b] Average initial count 4.9×10^3

Table 4B. Counts of yeasts on frankfurters stored at 45° F. (7.2° C.) under various CO_2 concentrations[a,b]

CO_2 level (%)				
0	Storage time (days)	8	14	17
	#1	5.5×10^5	4.9×10^8	2.2×10^8
	#2	8.8×10^5	1.3×10^8	1.7×10^9
	Log average	7.0×10^5	2.5×10^8	6.1×10^8
5	Storage time (days)	8	16	20
	#1	0	4.9×10^7	1.8×10^8
	#2	0	7.8×10^6	1.8×10^7
	Log average	0	2.0×10^7	5.7×10^7
15	Storage time (days)	9	18	24
	#1	0	1.9×10^5	3.1×10^7
	#2	0	3.0×10^5	9.7×10^7
	Log average	0	2.5×10^5	5.3×10^7
25	Storage time (days)	15	22	45
	#1	5.0×10^2	0	4.6×10^5
	#2	--	0	2.1×10^6
	Log average	5.0×10^2	0	9.8×10^5
50	Storage time (days)	15	23	46
	#1	0	0	1.2×10^4
	#2	0	0	5.3×10^4
	Log average	0	0	2.5×10^4
75	Storage time (days)	17	23	46
	#1	0	0	0
	#2	0	0	0
	Log average	0	0	0
96	Storage time (days)	14	22	46
	#1	0	0	0
	#2	0	0	0
	Log average	0	0	0

[a]Numbers of yeasts per frankfurter

[b]Initial count arbitrarily chosen as 10 yeasts per frankfurter

Table 5B. Counts of Lactobacilli on frankfurters stored at 45° F. (7.2° C.) under various CO_2 concentrations[a,b]

CO_2 level (%)				
5	Storage time (days)	8	16	20
	#1	1.7×10^5	2.6×10^8	8.1×10^8
	#2	6.3×10^4	1.2×10^8	2.6×10^9
	Log average	1.0×10^5	1.7×10^8	1.5×10^9
15	Storage time (days)	9	18	24
	#1	3.6×10^4	9.0×10^7	9.0×10^8
	#2	1.6×10^4	1.0×10^8	2.0×10^8
	Log average	2.6×10^4	9.5×10^7	5.5×10^8
25	Storage time (days)	15	22	45
	#1	5.7×10^7	2.5×10^8	8.1×10^9
	#2	1.5×10^6	3.9×10^8	5.2×10^9
	Log average	9.2×10^6	3.1×10^8	6.5×10^9
50	Storage time (days)	15	23	45
	#1	1.7×10^6	4.1×10^8	1.5×10^{10}
	#2	1.2×10^5	1.6×10^8	7.3×10^9
	Log average	4.5×10^5	2.6×10^8	1.1×10^{10}
75	Storage time (days)	17	23	46
	#1	5.2×10^6	1.7×10^8	5.2×10^9
	#2	8.1×10^6	9.3×10^7	9.1×10^9
	Log average	6.5×10^6	1.3×10^8	6.9×10^9
96	Storage time (days)	14	22	46
	#1	1.6×10^6	3.5×10^8	1.5×10^9
	#2	2.5×10^5	1.8×10^8	1.9×10^9
	Log average	6.3×10^5	2.5×10^8	1.7×10^9

[a]Numbers of lactobacilli per frankfurter

[b]Average initial count 1.5×10^3

Table 6B. Weights and weight changes of frankfurters stored under various humidites at 40° F. (4.4° C.) for 15 days

Relative humidity (%)	Sample no.	Initial weight (g.)	Final weight (g.)	Change in weight (g.)	Change in weight (%)	Average weight change (%)
100	1	44.9	45.7	+ 0.8	+ 1.8	
	2	44.1	43.7	- 0.4	- 0.9	
	3	48.7	48.9	+ 0.2	+ 0.4	+ 0.7
	4	41.9	42.5	+ 0.6	+ 1.4	
95	1	50.4	49.4	- 1.0	- 2.0	
	2	44.7	44.0	- 0.7	- 1.6	
	3	42.0	41.5	- 0.5	- 1.2	- 1.3
	4	48.6	48.3	- 0.3	- 0.6	
90	1	46.4	44.6	- 1.8	- 3.7	
	2	38.6	36.8	- 1.8	- 4.7	
	3	40.6	38.8	- 1.8	- 4.4	- 4.0
	4	38.6	36.5	- 2.1	- 5.4	
	5	45.2	44.3	- 0.9	- 2.0	
85	1	40.2	36.7	- 3.5	- 8.7	
	2	44.9	42.1	- 2.8	- 6.2	
	3	45.7	41.4	- 4.3	- 9.4	- 6.1
	4	45.7	42.8	- 2.9	- 6.3	
	5	39.5	39.5	0	0	
80	1	44.4	38.9	- 5.5	-12.4	
	2	50.9	46.7	- 4.2	- 8.3	
	3	52.3	48.5	- 3.8	- 7.3	- 7.2
	4	49.3	46.0	- 3.3	- 6.7	
	5	40.9	40.3	- 0.6	- 1.5	
75	1	45.3	41.0	- 4.3	- 9.5	
	2	44.6	42.1	- 2.5	- 5.6	
	3	45.4	40.5	- 4.9	-10.8	
	4	38.3	33.0	- 5.3	-13.8	- 7.3
	5	40.9	40.3	- 0.6	- 1.5	
	6	40.0	38.8	- 1.2	- 3.0	

Table 7B. Characteristics of types of micrococcus isolated from stored frankfurters

Type	No. of cultures	Chromogenesis	Nitrates reduced	Utilizes $(NH_4)H_2PO_4$ as sole source of nitrogen	Utilizes urea as sole source of nitrogen	Liquefies gelatin	Litmus milk	Aci fro glu
1	1	Yellow	-	+	+	-	Alkaline	+
2	2	Yellow	+	+	-	+	Acid, reduced, some coagulation	+
3	1	Pink	+	+	+	+	Alkaline	-
4	3	White or buff	-	+	-	-	Alkaline	+
5	8	Buff	-	+	+	+	Acid, may be peptized	Var
6	6	White	-	-	-	-	Peptized	Var
7	5	White	-	-	-	Usually -	No change or alkaline	Usu
8	6	White	+	+	+	+	Peptized	
9	11	White	+	+	Varies	+	Usually reduced. May be sl.acid or alkaline	
10	4	White or buff	+	+	+	-	Acid, no coagulation	
11	2	White	+	+	+	-	Acid, some coagulation	
12	6	White	+	-	-	+	Acid, may show coagulation	
13	1	White	+	-	-	-	Acid	
14	1	Buff	+	-	-	-	Alkaline	

ypes of micrococcus isolated from

Utilizes urea as sole source of nitrogen	Liquefies gelatin	Litmus milk	Acid from glucose	Acid from glycerol	Acid from mannitol	Hydrolyzes fat
+	-	Alkaline	+	-	-	-
-	+	Acid, reduced, some coagulation	+	+	+	+
+	+	Alkaline	-	-	-	+
-	-	Alkaline	+	+	-	-
+	+	Acid, may be peptized	Variable	Variable	Variable	+
-	-	Peptized	Variable	-	-	+
-	Usually -	No change or alkaline	Usually -	Usually -	-	+
+	+	Peptized	+	+	Usually -	Varies
Varies	+	Usually reduced. May be sl.acid or alkaline	+	+	+	Varies
+	-	Acid, no coagulation	+	Variable	-	+
+	-	Acid, some coagulation	-	-	-	+
-	+	Acid, may show coagulation	+	+	Usually -	Usually -
-	-	Acid	-	-	-	+
-	-	Alkaline	+	-	-	-