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Storage changes in packaged meats

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NOTE TO USERS

STORAGE CHANGES IN PACKAGED MEATS

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I. INTRODUCTION

A recent development in the retailing of meats is self-service merchandising. This form of marketing has expanded rapidly during the years following World War II. According to a survey made by Armour and Company (Modern Packaging, 1952), in 1948 there were 178 stores using only the self-service method for selling meats, compared with 878 in 1949, 1,983 in 1950, and more than 5,000 in 1952. The report stated further that "indications are that this is the fastest-growing field in all packaging". Although markets offering self-service sold more than 10 per cent of the total amount of meat purchased by consumers in 1951, these stores constituted less than 2 per cent of the total number of markets retailing fresh meats. From these observations it may be expected that the trend toward self-service merchandising will continue in the future.

With the growth of self-service marketing, several problems have come to be recognized. Among these, discoloration of packaged meats has been emphasized as a major technical difficulty associated with the retailing of these products. Another problem, that of spoilage, is of particular importance because pre-packaged meats are liable to receive greater contamination than that occurring on meat cut at the time of sale. The present work is concerned principally with studying discoloration and microbial spoilage of packaged meats held at refrigeration temperatures.

Although many studies have been made concerning the properties of

various packaging materials, relatively few of these have dealt with the performance of materials when used as wrappers for refrigerated meats. The role of packaging materials in producing quality changes in self-service meats was described by Garnatz (1949,p.57).

> Those [materials] which were moisture-proof permitted the development of sliming, off-odor, and discoloration. Others which were transmissable to moisture vapor permitted dehydration and consequent undesirable color development.

Films and foils vary widely in those properties affecting quality of the packaged product, and one of the objectives of the work presented here is to study the effects of materials on changes in packaged meats during storage. Consideration is also given to the influence of display case lighting in adding to, or detracting from, quality of self-service meat items. Also receiving attention is the problem of preservation of color by packaging with various gases, or by exclusion of air from packages; further, the relation of these practices to spoilage of packaged meats is considered. In addition, some information is given pertaining to the effect of relative humidity on color changes and spoilage of packaged meats.

It is hoped that the information presented here may be of value in future investigations designed to study means of improving keeping quality of meats packaged for self-service merchandising. It is recognized that the present work is exploratory in nature; the need exists for further studies concerning problems associated with changes in quality of packaged meats during storage.

II. REVIEW OF LITERATURE

A. General

A knowledge of the factors affecting the keeping quality of meats is requisite to an understanding of the role of these agents in the storage life of the packaged product. For this reason, consideration has been given in the literature review to quality changes in meats stored in the unpackaged state.

The properties of films and foils that may be used for wrapping meats received much attention, inasmuch as the ultimate keeping quality of the product depends to a large extent upon the characteristics of the packaging material.

B. Types and Properties of Packaging Materials

A survey of the literature revealed that considerable attention has been given to the passage of water vapor through films. According to Throckmorton (1942), maintenance of the original quality of a packaged food depended also upon the resistance of the wrapper to the transfer of gases. Moisture vapor transmission and gas transfer are related and both are involved in the preservation of packaged meats; for simpli-

city, however, they will be considered separately in this review.

1. Types of flexible films and foils

A recent general review of plastic films was made by Southwick (1953) who emphasized the difficulties in classifying packaging materials. For example, the word "flexible" did not properly describe this class of materials, since in some cases rigidity was demonstrated by these substances. Schack (1950, p.345) defined a "plastic" as a polymeric organic compound capable of being shaped by heat and pressure to a rigid form. Southwick (1953) believed that cellulosic materials did not conform to the definition given for plastics, but felt that popular usage of the term and the widespread application of cellulose derivatives justified their inclusion in this category.

Regenerated cellulose films were described in detail by Hyden (1929). Schack (1950, p.80) listed the composition of cellophane as a three-component system: 60 to 85 per cent regenerated cellulose, 10 to 25 per cent plasticizer or softener, and 5 to 15 per cent water. For food packaging, cellulose films were rendered moistureproof by the addition of a lacquer consisting principally of nitrocellulose.

Other films manufactured from cellulose were enumerated by the American Paper and Pulp Association (1940). Of these, cellulose acetate was considered by Southwick (1953) to be well adapted to food packaging.

The composition of rubber hydrochloride films was reported by Coulter and Vaughan (1950). In brief, these workers defined Pliofilm, the trade name for these materials, as the product resulting from the action of

gaseous hydrogen chloride on rubber. Twelve compositions of transparent Pliofilm were listed as available for commercial use.

Plastic films made from vinylidene chloride polymers were investigated by several workers. Saran film was described by Wakeman (1947, p.393) as the copolymer of vinylidene chloride and vinyl chloride in which the former predominates. According to Wakeman, molecular weights of Saran films were in the order of 20,000. Southwick (1953) mentioned that vinylidene chloride resins were combined with other materials to produce Cry-O-Rap, a film which found particular usage in wrapping irregularly shaped food items.

Vinyl films and vinyl-rubber films were considered by Southwick as representative of a broad range of materials that were adaptable to many specific uses in packaging. He described the basic vinyl-rubber material as an equal mixture of Buna N synthetic rubber and vinyl chloride. This formulation has been used extensively for oleomargarine coloring-bags and for packaging certain meat products. The function of nitrile rubbers used in combination with polyvinyl chloride and with vinyl chloride-acetate copolymers was stated by Schack (1950, p.408). Such compositions combined the resistance of polyvinyl chloride to oils, chemicals, and aging, and the flexibility and solvent resistance of nitrile rubber.

Wakeman (1947, p.575) stated that polyethylene was a microcrystalline polymer of ethylene, with molecular weights ranging from 3000 to 50,000.

Schack (1950,p.356) classified the film as occupying a position between the rigid and non-rigid plastics because of its stiffness.

Combinations of materials, either as coatings or laminated films, were described by Hanmer (1946), Southwick (1953), and Cowen (1953). The general opinion shared by these workers was that improvement in functional characteristics could be obtained by combining two or more of the individual films.

The prominence of aluminum foil in commercial packaging was mentioned by Hanmer (1946), Edwards and Strohm (1948), and Schmied (1953). Edwards and Strohm (1948) defined the reduction of sheet aluminum to foil as a process by which a final foil thickness of less than 0.006 inch was obtained. For packaging, the most common gauges were specified as 0.0015 to 0.00035 inch. Bonding of aluminum foil to other materials to obtain laminates of desirable characteristics for food containers was discussed by Edwards and Strohm, Hanmer (1946), and Schmied (1953).

2. Properties of materials

a. Influence of product. Elder (1949) believed that the degree of protection given a packaged product by its wrapping material was dependent on the properties of the product and the conditions encountered in merchandising channels. Elder outlined some important product properties as follows: (1) the tendency of a product to gain or lose moisture as influenced by external humidity conditions; (2) susceptibility to

spoilage by atmospheric oxygen; (3) the tendency to lose volatile flavors or to acquire foreign odors as influenced by temperature, ventilation, and other conditions of storage; (4) seepage of fat or oil; (5) susceptibility to insect infestation. The temperature and physical form of the product at the time of packaging were considered by Hayhurst (1952) as additional factors affecting the performance of the wrapping material. Callow (1934) and Urbain and Ramsbottom (1948) emphasized that cured meats were particularly susceptible to atmospheric oxidation. Since cured meat items contain high levels of moisture, Urbain and Ramsbottom indicated that protection against dehydration was essential for maintenance of quality. Similar observations were made by Brooks (1933a) from studies with fresh meats.

b. Transmission of moisture. Early work on the permeability of plastics and other protective materials to water vapor was summarized in a review by Carson (1937).

As given by Carson, the diffusion of moisture through membranes was believed to obey the relation:

$$W = KtAp/s$$

in which W is the mass of moisture that passes in time, t, through a membrane of area, A, and thickness, s, when the partial pressure of water vapor on the two faces of the membrane is p, and the coefficient of diffusion is denoted by K. However, Carson believed that this relation did not hold when cellulosic materials were tested. The sorption of water by cellulose derivatives was considered by Carson, Barrer (1941, p.443)

and Doty et al. (1944) to result in deviation from the permeability expression. Doty et al. concluded that films which contained hydrophilic groups that sorbed water molecules could not easily be evaluated in terms of the vapor pressure difference across the film faces. Resistance to moisture vapor was emphasized by Halladay (1942) as depending on the hydrophilic or hydrophobic quality of the material.

Edwards and Wray (1936) studied the influence of temperature on water vapor permeability. They determined the moisture "impedence" (the reciprocal of permeability) of two paint films at four temperatures from 15.6° to 32.2° C. under constant conditions of air moisture content. As the temperature was increased through intervals of 5.6° C., a slight decrease in the permeability of the films was noted. Edwards and Wray concluded that temperature did not appreciably influence the passage of moisture through films. On the other hand, Doty et al. (1946) found that temperature affected the diffusion and solubility of moisture in certain organic materials. Measurements were made of the permeability rates for polystyrene, polyvinyl chloride acetate, rubber hydrochloride, and polyvinylidene chloride films. No effect of temperature was observed for polystyrene, but a doubling of the permeability constant with a 5° C. increase occurred with polyvinylidene chloride. Since wide differences in the effect of temperature on permeability were noted, Doty et al. concluded that the performance of a given packaging film could not be predicted at one temperature on the basis of measurements made at another temperature.

The Von Schroeder effect was described by Carson (1937) as an increase in the absorption of water by colloidal materials in contact with liquid as compared with saturated vapor. Cellulosic materials demonstrated this effect. Halladay (1942) cautioned against the evaluation of films in terms of water vapor resistance when measurements were actually made of resistance to water in the liquid form. He stressed the need for distinguishing between the two types of measurements since water obeyed the laws for fluid flow, while passage of moisture vapor was concerned with laws relating to gases. Many of the criteria for permeability determinations listed by Carson (1937) were not clearly differentiated with respect to the two physical states of water. Other workers found physical changes in cellulosic films that were attributed to the wetting of the material and that might be expected to influence permeability. For example, Morton (1935) estimated that the pore size of regenerated cellulose was 5 Angstrom units (A) in the dry state, and between 20 and 30 A when wet; Oswin (1943) observed that there was an increase in linear expansion of about 5 per cent in wet cellulose.

Doty et al. (1946) agreed with Carson (1937) that the process of permeation of water through an organic film consisted of solution of the vapor in the membrane on the side exposed to the vapor, followed by diffusion through the material and evaporation from the other side. Flow occurred through pre-formed capillaries only as a result of mechanical injury or imperfections in the film. Doty et al. concluded that the permeation

mechanism involved both diffusion and solubility. In an earlier report, the same workers (1944) considered activated diffusion as playing an important role in the transfer of water through organic polymers. Barrer (1941, p.71) stated that activated diffusion predominated in the permeation of water through compact solids. In this type of diffusion, the water molecules present in the film acquired an activation energy due to the effect of other molecules striking the film surface, with subsequent diffusion through the material.

According to Elder (1949), cellulosic films did not conform to Fick's law for diffusion of vapors and gases. Barrer (1941, p.430) believed that the law held for the transfer of gases through organic solids, but not for the diffusion of water vapor.

The mechanisms by which water vapor penetrates aluminum foil and plastic films were considered by Edwards and Strohm (1948) to be different. In the case of foil, water vapor was demonstrated to diffuse through openings in the material and not by the process involving solution, characteristic of plastic films. Because of this difference in the methods of penetration, Edwards and Strohm believed that laminates of foil and plastics offered effective barriers to the transfer of water vapor.

c. Permeability to gases. The permeability of films to gases has been discussed to some extent in the preceding sections. Carson (1937) believed that no definite relation existed between the water vapor permeability and air permeability of films. He concluded that the mechanism

for the passage of moisture was different from that for the passage of air. Halladay (1942) thought that resistance of materials to water vapor could be expected to parallel the resistance to gases. Barrer (1941, p.406) pointed out that the permeability of membranes to air was due partly to pin-holes, or orifices, a condition described by Edwards and Strohm (1948) which accounted for the transfer of gases through aluminum foil. Shuman (1944) apparently disagreed with Carson (1937) in that Shuman (1944) attributed the transmission of gases through films to differences in total pressure on the two sides of the film and to differences in the partial pressure of the gas on the film faces. Elder (1944) demonstrated that Dalton's law of partial pressures applied to the diffusion of gases across permeable barriers; the diffusion of any gas was independent of the presence or pressure of any other gas in the mixture.

In studies with many different types of sheet materials, Davis (1946) found that permeability to carbon dioxide was higher than permeability to oxygen; a ratio of approximately 4 to 1 was demonstrated for the respective diffusion rates of these gases by Landrock and Proctor (1952). According to Barrer (1941, p.447), differences in permeation velocities of gases were not considered to be due to the molecular weight of the diffusing gas, but largely to the solubility of the gas in the membrane. Nitrogen permeability was observed by Davis (1946) to be slightly lower than permeability to oxygen for many packaging materials.

Davis demonstrated that the permeability of Parafilm, a rubber-wax

composition, to carbon dioxide was approximately tripled by increasing the temperature from 16° to 32° C.

With materials that were not moisture-absorbent, such as Saran films, Pliofilm, and polystyrene, Davis observed no increase in gas permeability when the relative humidity was varied between 0 and 75 per cent. However, when tests were conducted with cellophane, appreciable increases in permeability to carbon dioxide and oxygen were noted as the relative humidity was increased.

From a consideration of the references cited, it appears that many of the factors which affect the transmission of moisture vapor through films are also operative in the transfer of gases. The degree of solubility of the particular gas in the packaging material was shown to play an important role in gas transfer.

d. Transfer of odors and flavors. Crocker (1945a) believed that wraps which were impermeable to moisture also served to retain flavor. Urbain and Ramsbottom (1948) agreed that prevention of moisture loss aided in preserving the flavor of cured meats. With frozen meats, however, Winter and Hustrulid (1951) found that desiccation was not a reliable criterion for evaluating the protective value of a packaging material with respect to flavor retention.

In tests of the transmission rate of methyl furoate through several wrapping materials, Muldoon and Sylvester (1951) demonstrated that the effect of relative humidity varied with the type of packaging material employed. An increase in relative humidity from 34 per cent to 84 per cent caused about a ten-fold increase in the permeability of glassine to

methyl furoate; for cellophane, the transmission rate was increased about eight times. Sulfite paper also showed greater permeability at the higher humidity, but the per cent increase in transmission rate was less than it was for glassine or cellophane. On the other hand, polyethylene exhibited an inappreciable difference in transmission rate of the test substance. Whether an analogy may be drawn between the transmission of methyl furoate through packaging films and the loss of aroma constituents of packaged meats is questionable. Muldoon and Sylvester did not know how well methyl furoate represented the odorous components of foods.

Polyethylene was shown by Tauber et al. (1949) to have a differential ability to transmit essential oils. Of the oils tested, lemon oil and orange oil were transferred through the material to the greatest degree, while vanillin was transmitted in the least quantity. Crocker (1945a,b) stated that orange oil and lemon oil belonged to a class of compounds having vapor pressures higher than that of vanillin, and, consequently, transferred their odor more readily. Elder (1947) reviewed Crocker's work and pointed out that the aroma constituents of foods had very low vapor pressures. He believed that odor transmission could not be attributed to extreme volatility, but rather to the unique properties of the aroma components in stimulating the sense of smell. For food packaging, Elder concluded that the greatest protection against odor transfer was obtained when metal foil was utilized as the packaging material. Saran, cellophane, and glassine were ranked as the next most desirable materials.

Changes in aroma and flavor of foods which could be attributed to

the packaging material were considered by Carlin (1948), Sjostrum (1950), and Cartwright and Kelley (1952). Cartwright and Kelley employed subjective evaluation methods to determine the transfer of odors and flavors from packaging materials held in contact with foods. Common odors found in wrapping materials were described as: cardboard, paper, ink, sweet, glue, oil, musty, turpentine, solvent, and fruity. Practically all paper containers used as packages for foods were believed by Sjostrum (1950) to transfer odor to the enclosed products. He attributed the acquisition of odors largely to the manufacturing processes employed in the paper industry. Musty or moldy odors were believed to be due to the use of "poor" waters, while sour and putrid odors were considered to be caused by slime-forming organisms. The elimination of these organisms contributed to another type of odor, described as "chemical" or "medicinal", when fungicides were used as a means of control. Off-odors classified as mercaptans, amines, or sourness were believed to be caused by garbage, residues in used paper stock, or by adhesives. Carlin (1948) indicated that paper packaging materials contained oxidation catalysts which hastened rancidity development and the consequent production of undesirable flavors in foods of high fat content. Of the plastic materials, polyvinylidene chloride films were described by Hammer (1946) as imparting an undesirable odor to foods, which he believed to be caused by the plasticizer used in the manufacture of these materials. However, in a later review, Southwick (1953) pointed out that the more recent vinylidene chloride films were free from odors and tastes.

e. Sanitary aspects. Barail (1947) expressed the belief that practically all packaging materials were attacked by bacteria, yeasts, and molds under conditions favorable for their growth. He indicated that the moisture content of the food and the tensile strength of the wrapping materials determined the extent of contamination of the product. Materials having high permeability to moisture promoted conditions conducive to the growth of microorganisms on the packaged food. Increase in moisture content of the wrapper resulted in a loss in tensile strength; consequently, the chance of breakage of the package was increased. The need for rendering packaging materials bacteriostatic and fungistatic by chemical treatment was emphasized by Barail. He believed that plastics of the vinyl type were well adapted to chemical treatment and that such treated materials offered the best possibilities for food protection. In regard to treatment of wrapping materials, Barail concluded that propionic acid and sodium propionate did not give as lasting preservative effects as did long-chain mercury compounds. The latter compounds, employed as coatings for packaging films, were useful in the preservation of foods containing high levels of moisture.

From the standpoint of public health, Parker (1948) considered that the primary purpose of a food package was to protect the contents from contamination. He reviewed the characteristics of several materials with regard to sanitary properties. Regenerated cellulose films were indicated as being inherently free from bacterial contamination, a quality that

resulted from the manufacturing processes and from the impermeability of the film to bacteria. Parker stated that the process employed for the manufacture of regenerated cellulose films included an evacuation procedure and treatment with acid to precipitate the cellulose; this process resulted in destruction of microorganisms originally present in the pulp. In subsequent processes, the film was coated with a lacquer and dried at high temperatures, effecting further reduction in bacterial numbers. Rubber hydrochloride films were described as receiving manufacturing treatments that were considered adequate to destroy microorganisms. The manufacture of Pliofilm included treatment with strong acid, organic solvents, and heat. According to Parker, conditions unfavorable for bacterial growth were promoted by the incorporation of plasticizers in these films. Edwards and Strohm (1948) believed that aluminum foil was free from surface microorganisms at the time of manufacture by virtue of an annealing process carried out at 371.1°C.

3. Adaptability of materials for packaging meats

A special type of cellophane, designated as MSAT-80, for use in packaging fresh red meats was described by Allen (1949). Properties of this material that Allen considered desirable included low water vapor permeability, ability to absorb water, and low oxygen permeability. At a relative humidity of 60 per cent, which Allen believed to be representative

of the humidity prevailing in self-service display cases, fresh beef packaged with MSAT-80 cellophane lost 2 per cent of its original weight in 69 hours at 4.4° C. Under the same conditions of storage, cellulose acetate permitted dehydration of the meat to the extent that approximately 15 per cent of the initial weight was lost. MSAT-80 cellophane was effective in retaining the bright red color of oxygenated beef for periods as long as 72 hours when storage was conducted at 1.1° to 4.4° C. No significant bacteriostatic action was observed when MSAT-80 cellophane was used as a wrap for boneless round steaks. At 1.1° to 4.4° C., a rapid increase in bacterial numbers was observed on meat held for 9 days in a chill room.

Allen indicated that a film of higher moisture vapor permeability was required for packaging cured meats than that used for wrapping fresh meats, since he considered that cured meats were more susceptible to sliming and molding. LSAT cellophane was believed to delay the appearance of slime and mold on cured meats without allowing appreciable weight loss of the product. Southwick (1953) listed the hygroscopic property of cellophane as a desirable characteristic for packaging foods having a high content of fat.

Pliofilm FM-1, designed for packaging fresh meats for self-service merchandising, was characterized by Coulter and Vaughan (1950). The ability of the film to stretch and its durability were believed to contribute greatly to its application in packaging heavy and/or bony cuts of meat. Increased strength and resistance to impact were demonstrated by a "tensilized" type of Pliofilm. "Tensilized" Pliofilm was described by

Coulter and Vaughan as a rubber-base film that was stretched at temperatures slightly below 107.2° C and then allowed to crystallize. The process produced a film that was dimensionally stable at "normal" temperatures. Further application of heat caused the film to retract to its original dimensions. Southwick (1953) mentioned that Pliofilm, by virtue of its water-resistant properties, was well adapted to the packaging of meat products.

Vinylidene chloride films were stated by Southwick to have the lowest permeability to water of any of the plastic films. Holbrook (1952) mentioned that Saran films also had the highest resistance to gas penetration of all transparent wrapping materials. The ability of Saran films to cling to the enclosed product was considered to be a desirable property for packaging meats. Cry-O-Rap, a material containing Saran, was stated by Holbrook as being well suited for packaging luncheon meat loaves because of the ability of the material to shrink tightly around the product and thereby virtually effect a vacuum-type package. Advantages listed by Modern Packaging (1953) for this type of package for use with meat loaves included improved appearance, retention of flavor, protection against weight loss, and maintenance of color of the meat under fluorescent lights. A disadvantage in the use of this film was the necessity for special equipment to obtain the desired vacuum and to shrink the material during packaging operations.

Tauber et al. (1949) demonstrated that polyethylene was highly effective in preventing loss of moisture from liver sausage. They stated that this meat product is normally stored for 10 to 14 days at 4.4° C.,

and that in the time stipulated, weight losses of 10 to 12 per cent occurred when cellophane casings were employed as wraps. However, liver sausage that was over-wrapped with polyethylene film lost only 0.2 per cent of its original weight after three weeks' storage in a refrigerator. Polyethylene was described as having high permeabilities to oxygen and carbon dioxide, although it provided an effective barrier against the transfer of moisture vapor. Holbrook (1952) and Southwick (1953) enumerated other desirable characteristics of polyethylene; namely, inertness to chemicals and flexibility at low temperatures.

Parafilm was described by Abrams (1938) as the film form of a composition known as Paraweld. The composition was characterized as tasteless, odorless, flexible, and waterproof. Parafilm was highly resistant to water and, for this reason, the material found particular application in cheese packaging. Abrams stated that Parafilm sheets could be sealed directly against foodstuffs which were to be kept free of mold growth and loss of moisture.

In tests conducted with laminates of aluminum foil and various plastic films, Edwards and Strohm (1948) observed values for the permeability of water vapor as low as 0.00, expressed as grams of water transferred per 100 sq. in. of material at 37.8° C and 100 per cent relative humidity in 24 hours. From the standpoint of prevention of dehydration, these materials might be considered as ideal. However, as Garnatz (1949), Holbrook (1952), Hayhurst (1952), and Hockman (1946) pointed out, transparency is requisite for self-service merchandising, and in this respect aluminum foil used

alone or laminated with other materials did not qualify as a wrapping material. With regard to color retention and keeping quality, Greenwood et al. (1945) took an opposite stand and advocated the use of a package for bacon that would exclude light, air, and microorganisms.

In connection with control of weight loss of packaged meats, Garnatz (1950) stated that the use of materials of very high moisture vapor resistance resulted in "weeping", or condensation of moisture on the surface of the cut, which presented an unsightly appearance. Under some conditions, on which Garnatz did not elaborate, condensation contributed to deterioration of the meat in the forms of discoloration, sliming, off-odors, and off-flavors. On the other hand, he also indicated that, if the moisture vapor transmission were too high, discoloration would result from localized dehydration of the meat surface. In regard to these considerations, Garnatz concurred with Allen (1949) in the opinion that MSAT-80 cellophane was satisfactorily adapted for packaging fresh meats, and LSAT cellophane served well for wrapping cured meats.

Visten, a vinyl halide nitrile rubber composition, was observed by Signer and Tauber (1948) to inhibit surface mold growth on foods packaged with the material. Retardation of molding was believed to be due to the elimination of air pockets in the package, a property resulting from the "cling" of the film to the food. No off-odors or undesirable tastes were imparted to foods packaged with Visten. Meat did not adversely affect the film during storage. When used as an outer wrap for liver sausage, Visten tubing provided a barrier to the transfer of moisture to the extent

that the meat lost less than 0.1 per cent of its initial weight after several weeks in storage. The temperature and humidity of storage were not specified by the authors. After storage for 30 days at 4.4° C, meat loaves lost less than 0.4 per cent moisture when Visten was employed as an over-wrap. Organoleptic tests made on the loaves showed the meat to be in satisfactory condition after a period of 60 days at 4.4° C.

Among laminated materials, Film 390, a combination of polyethylene and cellophane, was listed by the Modern Packaging Encyclopedia (1952) for vacuum-packaging cured meats. Another material mentioned by Holbrook (1950) for this purpose was a laminate of cellophane and Pliofilm. He stated that Flexvac, the trade name for this material, was successfully applied to vacuum-packaging sliced bacon and luncheon meats. Holbrook concluded that the combination of cellophane and Pliofilm provided high resistance to transmission of moisture and gases.

Winans (1950) outlined the essential elements in vacuum-packaging as: (1) drawing an initial minimum vacuum of 29 in. Hg and, (2) retaining a minimum vacuum of 24 in. Hg in the package. He believed that films for vacuum-packaging should have a maximum oxygen permeability of 0.0015 cc. per sq. in. per 24 hours at 4.4° C and 85 per cent relative humidity.

Data for the transmission rates of carbon dioxide and oxygen presented in the following table were compiled from various sources in the literature.

Table 1. Carbon dioxide and oxygen permeability of materials

Film thickness inches	Relative humidity per cent	Packaging material	Permeation rate in cc./sq. meter/24 hours: Carbon dioxide	Oxygen
0.0009	0	300 MSAT Cellophane[1]	24	1
0.0009	100	300 MSAT Cellophane[2]	—	240
0.0009	0	Cellulose acetate[1]	17,000	2,300
0.0009	75	Cellulose acetate[1]	14,000	1,200
0.0009	100	Cellulose acetate[2]	—	3,400
0.0050	0	Parafilm[1]	640	120
0.0050	75	Parafilm[1]	550	120
0.0006	0	Tensilized Pliofilm[1]	1,500	—
0.0012	50	FF-Pliofilm[3]	13,000	1,600
0.0013	0	Saran[1]	48	—
0.0010	50	Saran[3]	49	8
0.0005	50	Saran[3]	100	19
0.0015	50	Cry-O-Rap[3]	480	81
0.0010	0	Polyethylene[1]	14,000	3,500
0.0020	50	Polyethylene[3]	18,000	4,000
0.0030	0	Polyethylene[1]	6,100	1,000
0.0010	0	Polystyrene Q-641[4]	7,752	4,573
0.0010	75	Aluminum foil[1]	79	91
0.0010	—	Visten[5]	5,891	698

[1]Davis (1946)

[2]Platenius (1946)

[3]Winter (1951)

[4]Dulmage (1951)

[5]Signer and Tauber (1948)

By converting the value given by Winans (1950) for the maximum oxygen permeability permissible for vacuum-packaging into cc. per sq. meter per 24 hours, a figure of 9.675×10^{-7} is obtained. Examination of the tabulated values indicates that none of the materials listed are satisfactory for vacuum-packaging when allowances are made for the differences in temperature, gas pressure, and humidity of the tests conducted by different investigators. Comparison of the value given by Platenius (1946) for oxygen permeability of MSAT cellophane with that presented by Davis (1946) indicates that high humidities greatly increase the permeation rate of oxygen through the film. As stated previously, Davis believed that the permeability of cellophane to carbon dioxide and to oxygen was appreciably increased as the relative humidity became greater.

C. Factors Affecting Color of Meats

1. Pigments

a. **Fresh meats.** Early work on the muscle pigments dealt largely with blood hemoglobin, rather than with the pigment components of muscle. Difficulties in obtaining and preparing muscle myoglobin as contrasted with blood hemoglobin were cited by Hektoen *et al.* (1928), and Urbain and Jensen (1940) as the principal reasons for study of the blood pigment instead of that of muscle. DeDuve (1949) pointed out that the main difficulty in the determination of myoglobin in muscle tissue was brought about by the presence of blood hemoglobin. He believed that blood pigment

was always present in muscle extracts and could not be removed quantitatively without the loss of some myoglobin. Kennedy and Whipple (1926) made use of spectrophotometric curves for differentiating between myoglobin and hemoglobin, and concluded that the two pigments were not identical. Solutions of myoglobin were observed by Hektoen et al. (1928) to give a specific precipitin reaction that distinguished the pigment from the hemoglobin of dogs' blood. Shenk et al. (1934) demonstrated that myoglobin comprised almost all of the tissue pigments of beef. They extracted the myoglobin from chilled beef by grinding portions of muscle and macerating small quantities with ice water in a pebble mill. Spectrophotometric measurements were employed to determine the relative concentrations of pigments in solutions of myoglobin. The content of myoglobin was calculated to be between 89.50 and 96.75 per cent of the total pigment of the ribeye muscle, while blood hemoglobin made up the remainder.

Differences between hemoglobin and myoglobin with regard to molecular weight, ease of oxidation, oxygen-combining capacity, and spectroscopic properties were described by Millikan (1939). Wyman (1948) believed that the pigments had separate functions; myoglobin acted as a storage compound for oxygen, while hemoglobin was involved chiefly in the transport of respiratory gases.

Although it was recognized that the true meat pigments are principally derivatives of myoglobin and are not identical with blood pigments, Urbain and Jensen (1940) and Lavers (1948) indicated that the close relationship between blood hemoglobin and muscle myoglobin permitted correlation

of the reactions of hemoglobin derivatives with those of meat pigments. Lavers stated that essentially three forms of hemoglobin were related to color changes in meats. Reduced hemoglobin, or more simply, hemoglobin, was thought to be responsible for the dark red or purple color of freshly cut meat. Oxyhemoglobin was formed by combination of reduced hemoglobin with the oxygen of air. The iron in reduced hemoglobin and oxyhemoglobin was in the ferrous state. Oxygenation caused the formation of oxyhemoglobin from reduced hemoglobin, whereas methemoglobin resulted from oxidation of reduced hemoglobin. Methemoglobin was described as dark brown in color.

Peters (1912) hypothesized that oxygen attached to the iron of the hemoglobin molecule. He obtained similar values for the ratio of oxygen to iron in hemoglobin solutions prepared from beef, sheep, hog, and cat blood. Peters proposed that a chemical combination of iron and oxygen existed as described in the reaction:

$$Fe + O_2 \rightleftharpoons FeO_2 \qquad \text{when:} \quad Hb + O_2 \rightleftharpoons HbO_2$$

Conant (1923) followed the oxidation of hemoglobin to methemoglobin by electro-chemical methods and found that the reaction involved one hydrogen equivalent. He also observed that the oxygenation of hemoglobin was not an oxidation in the electronic sense, whereas the formation of methemoglobin from reduced hemoglobin was accompanied by a loss of one electron. Oxyhemoglobin was later demonstrated by Conant and Fieser (1925) to be a ferrous compound containing two atoms of oxygen. They postulated three mechanisms for methemoglobin formation:

(1) $2H_3(HbO_2) \longrightarrow 2H_2(MHb) + O_2 + H_2O_2$

(2) $2H_3(Hb) + O_2 \longrightarrow 2H_2(MHb) + H_2O_2$

(3) $2H_3(Hb) + 2H_3(HbO_2) \rightarrow 4H_2(MHb) + 2H_2O_2$

However, Conant and Fieser felt that the first reaction was not valid because it did not fit many cases of the formation of methemoglobin. They concluded that methemoglobin formation was not dependent upon the degradation of oxyhemoglobin, but was due to the oxidation of hemoglobin by oxygen (equation 2) or by oxyhemoglobin (equation 3). The first and third mechanisms were ruled out by Brooks (1935b) who believed that methemoglobin was formed only by oxidation of reduced hemoglobin.

Brooks and Lea (1934) indicated that hemoglobin comprised a portion of the pigments of the external fatty tissue of beef; the role of blood pigments in catalyzing the oxidation of fats in tissues was presented by Robinson (1924). Robinson found that the iron present in hemoglobin, methemoglobin, and hemin functioned as a catalyst for oxidation in much smaller amounts than that required for iron in the form of an inorganic salt. As a result of the oxidation of linoleic or linolenic acids, Haurowitz et al. (1941) noted the destruction of hemoglobin and hemin.

Chang and Watts (1949) demonstrated that the action of hemoglobin or nitroso-hemoglobin in catalyzing the oxidation of lard was inhibited by ascorbic acid and small amounts of tocopherol.

b. Cured meats. Haldane (1901) observed that cured meat had a characteristic red color when cooked. He attributed the red color to nitric oxide hemochromogen; reduced hemoglobin reacted with nitrite to

give nitroso-hemoglobin, and this product became denatured to yield the hemochromogen during heat processing. The reactions of nitrites with oxyhemoglobin, reduced hemoglobin, and methemoglobin were explained by Haldane as follows: (1) hemoglobin readily combined with nitric oxide to form nitric oxide hemoglobin, or, in combination with oxygen, hemoglobin was converted to methemoglobin.(2) nitrous acid yielded nitric oxide and oxygen (3) nitric oxide combined readily with either oxygen or hemoglobin. Haldane thought that the nitrate in cured meat was reduced to nitrite by bacterial action or by reducing substances present in the tissue itself. On prolonged cooking, the nitrite of the meat was destroyed. Further investigations of the action of nitrites and nitrates in producing the color of cured meats were made by Hoagland (1914). He confirmed Haldane's observations by spectroscopic examination of extracts of cured beef, and differentiated between nitric oxide hemoglobin and nitric oxide hemochromogen on the basis of the chemical structure of the compounds. Haldane stated that the hemochromogen did not contain the proteid group, globin, whereas globin was present in nitric oxide hemoglobin. Treatment with alcohol reduced the coloring matter of uncooked cured meats to nitric oxide hemochromogen. From a study of the solubility reactions of the pigments, Hoagland suggested that the hemochromogen was present before the material was extracted with alcohol.

The optimum concentration of nitrite for color formation in bacon was expressed by Callow (1934) as 2 to 5 parts of nitrite in 10,000 parts of meat in the presence of 0.3 per cent nitrate and 5 per cent

sodium chloride. Brown and green discolorations occurred when the nitrite concentration was increased to 10 and 20 parts respectively in 10,000 parts of meat.

The color-producing reactions of the curing process were summarized by Urbain (1944) as follows:

$NaNO_3$ + reducing conditions $\longrightarrow$ $NaNO_2$

$NaNO_2$ + acid conditions $\longrightarrow$ HONO

HONO + reducing conditions $\longrightarrow$ NO (nitric oxide)

NO + Hb $\longrightarrow$ NO-Hb

Urbain emphasized that reducing and acid conditions were necessary for color-fixation. Reducing conditions were brought about by bacterial action and the acidity requirement was provided by the meat itself, since the normal pH range of meat was stated to be between 5.5 and 6.4. Brooks (1938) noted that a low oxidation-reduction potential, in the order of -0.2 volt, was maintained in muscle tissue during curing.

2. Gases

a. Oxygen. The oxidation of hemoglobin to methemoglobin was studied by Neill and Hastings (1925) using several oxidizing agents. They observed the effect of oxygen tension on hemoglobin oxidation in sterile extracts of pneumococci cells, suspensions of anaerobic bacilli, turpentine, cod liver oil, linseed oil, sterile extracts of the alcohol-soluble constituents of potato juice, and sterile blood. Neill and Hastings

grouped all of the oxidations under the heading "oxygen activation"; oxygen activated some easily oxidizable substance to form an oxidizing agent which, in turn, oxidized the iron of hemoglobin to produce methemoglobin. The optimum oxygen tension for formation of methemoglobin was approximately 20 mm. At this tension of oxygen, more than half of the total hemoglobin existed in the reduced state, while the oxidizing agent was still allowed to form. Neill and Hastings concluded that reduced hemoglobin was the substance that oxidized to form methemoglobin. Their findings were reported at about the same time that Conant and Fieser (1925) postulated three possible mechanisms for the formation of methemoglobin, as stated previously. Neill and Hastings (1925) demonstrated that the addition of molecular oxygen or carbon monoxide changed the hemoglobin molecule to a substance more resistant to oxidation.

In order to determine the effect of oxygen diffusion on the formation of methemoglobin, Brooks (1929) made spectroscopic examinations of slices of beef tissue that were placed between glass plates. He observed that beef tissue had a small oxygen uptake; the depth of penetration of oxygen was approximately 2 mm. after a few hours at 0°C; after 100 hours, it was about 4 mm. Methemoglobin was formed only in the presence of oxygen and appeared most rapidly in parts of the tissue at some distance from the air-tissue interface. The tissue nearest this surface was still red, while the zone of methemoglobin formation was yellow-brown in color. Methemoglobin was observed in an area closely adjoining the region of reduced hemoglobin. The greatest depth of penetration of oxygen was calculated by

Brooks from an expression previously derived by Hill (1928) for the diffusion of oxygen in frog muscle. Hill calculated that oxygen dissolved in the amount of 1.95×10^{-4} cc. per sq. cm. of muscle surface when the muscle was too thick to have its whole volume penetrated by the oxygen of the air.

In further studies on the oxidation of hemoglobin by oxygen, Brooks (1931, 1935a) was able to account for the decrease in hemoglobin of oxblood by the formation of methemoglobin. At each oxygen pressure studied, the rate of oxidation was monomolecular with respect to the concentration of reduced hemoglobin. At 30° C, a well defined maximum rate of oxidation occurred at approximately 20 mm. oxygen pressure. The rate decreased rapidly as the oxygen pressure approached zero. Increase in oxygen pressure above 200 mm. had little effect on the rate of oxidation. Brooks suggested that oxygen acted in three ways: (1) to determine the concentration of the other reactant, (2) to oxidize the unoxygenated ferroradicals, and (3) to inhibit the oxidation. An equation for the oxidation of hemoglobin was given as:

$$(H_3Hb)_4 + 2O_2 \longrightarrow (H_2Hb)_4 + 2H_2O_2$$

Brooks (1935b) reasoned that oxyhemoglobin did not decompose spontaneously to give methemoglobin, since the oxidation rate would then increase with an increase in oxygen pressure. He also pointed out that if reduced hemoglobin were oxidized by oxyhemoglobin as suggested by Conant and Fieser (1925), the reaction rate would be bimolecular.

The influence of oxygen-rich atmospheres on the color of stored beef was studied by Brooks (1935b). He found that an increase in oxygen pressure from 152 mm. (air) to 723 mm. (99.2 per cent oxygen) caused a decrease of 28 per cent in the rate of formation of methemoglobin when the storage temperature was 30° C. With beef stored at 0° C, the effect was considered to be less; Brooks thought that the decrease in the rate of formation of methemoglobin amounted to 15 per cent. An increase in the time required for superficial discoloration of beef muscle was attributed also to the greater depth of penetration of oxygen when beef was stored in atmospheres containing high concentrations of the gas.

At 0° C, Brooks (1938) observed that the maximum rate of oxidation of hemoglobin occurred when the oxygen pressure was approximately 4 mm. He stated that fresh meat became brown in color when about 60 per cent of the hemoglobin present in the superficial tissues was oxidized to methemoglobin.

The effect of packaging materials on the color of fresh meats was summarized by Hockman (1946). He indicated that the permeability of materials to oxygen determined whether the desirable red color would be developed or maintained in packaged meats. Hockman believed that the use of some wrappers prevented the formation of oxyhemoglobin when fresh meat was packaged before oxygenation of hemoglobin was permitted to take place. Materials that were slightly permeable to oxygen allowed the oxygenation

reaction to occur after some period of time, while other packaging materials reacted with the meat to produce brown discoloration. Lavers (1948) observed that brown discoloration was not caused by one specific film, but resulted when several materials were held in contact with beef. He placed small patches of different packaging films on the surface of beefsteak and stored the meat in a refrigerator at 7.2° C. Considerable discoloration of the beef occurred beneath the patches within 24 to 48 hours. Lavers concluded that discoloration was not a function of a particular material, but was due to a general lowering of the oxygen pressure at the meat surface. He tested this assumption by placing patches of vegetable parchment, a material having high permeability to oxygen, on fresh meat and noted the color changes. The area beneath the vegetable parchment became discolored at the same rate as that of the uncovered surface of the meat. Lavers believed that packaging materials with slow rates of oxygen transmission permitted rapid discoloration of fresh beef.

In a review of the color changes characteristic of fresh beef, Allen (1948) stated that the maximum change from purple to red color occurred when the meat surface was exposed to the oxygen of air for a period of 15 minutes. Allen believed that the red color of oxygenated beef would be retained for about 2 days by contact with air, if dehydration of the meat were prevented. He thought that brown discolorations resulting from contact of meat surfaces with certain packaging materials could not be

changed to the desirable red color. According to Greenwood et al. (1940), the reactions leading to the formation of methemoglobin in meats were reversible; reduction of methemoglobin gave hemoglobin, which could then be oxygenated to form oxyhemoglobin.

Derby (1951) considered that the oxygen demands of ground beef for maintenance of red color were greater than those of other fresh meats. He proposed a method for wrapping ground beef whereby air was trapped in the package in order to maintain the oxygen pressure sufficiently high for retention of red color.

The stability of the pigments of cured meats to oxygen was observed by Urbain and Jensen (1940) to be different from that of fresh meat pigments. Solutions of nitric oxide hemoglobin were very susceptible to oxidation by atmospheric oxygen; an oxygen pressure as low as 0.072 mm. caused appreciable oxidation of the pigment. No optimum oxygen pressure was found to exist for the formation of methemoglobin; the rate of oxidation was proportional to oxygen pressure. Nitric oxide hemoglobin remained stable in the absence of atmospheric oxygen.

From measurements of the oxygen uptake of bacon at 0° C, Brooks (1936) found that the effect of oxygen in causing discoloration was limited to the surface of cured meat, since oxygen was absent in the bulk of the tissues.

b. Carbon dioxide. Studies relating to the effect of carbon dioxide on color changes in lean meat were conducted by Brooks (1933b). Carbon

dioxide in concentrations ranging from 0 to 30 per cent of the atmospheric gases caused no marked decrease in the depth of penetration of oxygen in muscle tissue at 0° C. Although 30 per cent CO_2 accelerated the onset of discoloration to a slight extent, Brooks thought that moderate concentrations of the gas were not instrumental in effecting undesirable color changes. However, with commercial CO_2, oxidation of hemoglobin was complete in less than 3 days, as compared with 6 to 8 weeks in air. Brooks concluded that carbon dioxide functioned to lower the pH of the meat and to decrease the oxygen pressure; both effects resulted in an increase in the rate of oxidation of hemoglobin.

Empey and Vickery (1933) observed that 10 to 12 per cent carbon dioxide did not adversely affect the color of beef stored at -1° C. Brooks (1935b) claimed that when beef was stored in 10 per cent CO_2, no significant improvement in storage life from the point of view of discoloration would be attained unless concentrations of oxygen in the order of 60 per cent were added. The partial pressure of oxygen at which maximum oxidation of hemoglobin occurred was considered by Brooks (1938) to be of utmost importance in fixing the upper limit of carbon dioxide. Ogilvy and Ayres (1951a) found that CO_2 in concentrations greater than 25 per cent caused rapid discoloration of the flesh of cut-up chicken.

Adair (1925) studied the reactions of CO_2 with hemoglobin. He believed that the combination of the two compounds differed from that of oxygen and hemoglobin in that more than one molecule of CO_2 united with a molecule of

hemoglobin. Adair concluded that CO_2 either formed an adsorption compound with hemoglobin, or formed bicarbonate which existed in the ionized state. The relationships between carbon dioxide, oxygen, and hemoglobin were reviewed by Wyman (1948) who stated that the affinity of hemoglobin for oxygen was reduced by acidification with carbonic acid. If these findings hold for the myoglobin of meats, an explanation may be offered for the action of CO_2 in producing the discolorations observed by Brooks (1933b) and by Ogilvy and Ayres (1951a).

Bacon stored in an atmosphere provided by commercial CO_2 was considered by Callow (1932) to have a brighter color than that of bacon stored in air. Callow attributed color retention to the increased stability of nitroso-hemoglobin in the absence of air. Carbon dioxide at a level of 96 per cent did not cause discoloration of frankfurters in experiments conducted by Ogilvy and Ayres (1951b). They found that the color of frankfurters sometimes became faded when the meat was stored in air or in low concentrations of CO_2; the stabilizing effect of high CO_2 concentrations on color was believed to be due to exclusion of oxygen.

c. Nitrogen. Very few reports were found in the literature relative to the influence of nitrogen on color of meats. Callow (1932) compared the effects of air, of carbon dioxide, and of nitrogen on the keeping quality of unsmoked bacon. He showed that nitrogen preserved the bright red color of bacon, although not to as great a degree as that observed when carbon dioxide was used. Callow concluded that both CO_2 and

nitrogen were effective in retaining the color of bacon primarily because air was excluded. Urbain and Jensen (1940) noted that the replacement of oxygen by small amounts of nitrogen delayed, but did not prevent, the oxidation of nitric oxide hemoglobin.

Since methemoglobin formed most rapidly at low pressures of oxygen, Moran (1935) stated that discoloration of fresh meats occurred more rapidly in high concentrations of nitrogen and carbon dioxide than it did in the presence of air.

Nitrogen was shown by Vickery (1932) to prevent yellowing of rabbit fat stored at -10° or -5°C. for one year; yellow discoloration associated with a stage of rancidity occurred rapidly when the fat was exposed to air.

d. Other gases. Fisk (1936) cited a German patent which claimed that discoloration of frozen meat was prevented by treatment with carbon monoxide or with hydrogen prior to freezing.

Slices of beef muscle treated with 3 to 5 ppm. of ozone daily for 3 hours were demonstrated by Kefford (1948) to become discolored after 5 or 6 days in storage at 1° and 5°C. Control samples, held in air, remained bright red in color until the 12th or 13th day of storage.

3. Vacuum packaging

Investigations concerning the influence of vacuum packaging on color retention in cured meats and in sliced dried beef were carried out by Urbain and Ramsbottom (1948). In studies with sliced bacon, they compared

the effects of a cellophane package containing air and a vacuumized package composed of a material considered to be impermeable to oxygen. Color retention was greater in the case of the package in which air was excluded. Several packaging materials were compared with respect to their ability to preserve the color of sliced dried beef. In these tests, the meat was held under conditions approximating those encountered in merchandising. Color was evaluated by visual inspection. Ratings for color retention were given as: "fair" for normal cellophane, Pliofilm, and polyethylene; "poor" for aluminum foil; and "good" for a vacuumized package.

Sliced boiled ham was packaged in semi-moisture proof cellophane and in a packaging film described as a "transparent wrapper". Packages made up of the latter material were evacuated; some packages of both types were exposed to fluorescent lights while control samples were stored in the dark. With storage under lights, greater color retention was observed in the case of the vacuumized packages. The beneficial effect of exclusion of air was not as pronounced when the meat was held in the dark. The color of vacuum-packaged ham was rated higher at the end of 3 and 5 days than it was after storage for 24 hours. Urbain and Ramsbottom attributed the improvement in color to the biological oxygen demand of the meat; the pigment became reduced over a period of time and then combined with the excess of curing agents to form the original nitric oxide myoglobin.

Winans (1950) stated that sliced bacon and sliced luncheon meats packaged under vacuum retained their color for longer periods of time than

did similar items packaged in air. The deleterious effect of light on the color of these products was minimized when vacuum packaging was employed. Garnatz (1950) confirmed that vacuum packaging was effective against color fading in cured meats.

4. pH

The significance of pH on the color properties of fresh meats was reviewed by Bate-Smith (1948). He pointed out that the condition of the muscle fibrils depended in large measure upon the pH of the tissue; shrinkage of fibril myosin resulted when the pH decreased after slaughter. At pH values between 6.0 and 6.5, the fibrils scattered light and caused myoglobin to appear paler than it did at higher pH values. Bate-Smith stated that myoglobin was dark red when the pH of muscle was high; he thought that this was due to greater penetration of light through the muscle.

Brooks (1931) observed that the oxidation of hemoglobin to methemoglobin was accelerated as the pH of fresh ox-blood was decreased. A linear relationship existed between pH reduction and the values of the velocity constant for the oxidation in the range of pH from 7.04 to 5.70.

The effect of carbon dioxide on lowering the pH of stored lean meat was considered by Brooks (1933b) to be negligible when the concentration of the gas was less than 30 per cent of the atmosphere.

Studies of the color of pork, beef, and mutton at various pH values were made by Winkler (1939a). From about pH 4.5 to pH 5.5, the color of all of the meats appeared to be lighter than that observed at pH values in excess of 5.5. Winkler's observations apparently may be explained in accordance with the cahnges in muscle fibrils described by Bate-Smith (1948), and not in terms of the oxidation of hemoglobin.

Urbain and Jensen (1940) compared the air-oxidation of nitric oxide hemoglobin at pH 5.75, 6.75, and 8.25. Oxidation of the pigment occurred more rapidly at the lower pH values; appreciable retardation was observed at pH 8.25. Urbain and Jensen concluded that raising the pH of cured meat was not a feasible means of controlling oxidation of nitric oxide hemoglobin, since the meat itself was buffered against pH change.

With regard to color development during the curing process, Brooks (1938) stated that the reaction of nitrite with hemoglobin was slow at pH 7.8 to pH 7.2, but the reaction rate increased as the pH was lowered. Brooks concluded that the time required for color-fixation depended upon the rate of diffusion of nitrite into the tissue; the influence of pH was confined to the diffusion of nitrite, and the pH of the tissue did not affect the actual combination of nitrite with hemoglobin. Duisberg and Miller (1943) studied the relationship between pH and the development of color in pork during curing. The pH of the curing solution was varied from 2.75 and 9.95 by means of buffering agents. Between pH 4.4 and 4.9,

insufficient color-fixation was observed, while above pH 5.0 no apparent relation between pH and color was demonstrated. Optimum color development occurred at pH values between 5.2 and 6.0. Above 7.0, the pork became gelatinous and was considered to be undesirable from the point of view of texture. Duisberg and Miller agreed with Urbain and Jensen (1940) that alteration of the pH of the curing solution was not a practicable means of enhancing color development.

"Dark-cutting" beef is a serious problem in the meat industry, and has been the subject of numerous investigations. The National Livestock and Meat Board (1949) described this condition as resulting from failure of myoglobin to take up oxygen normally on exposure to air; hence the pigment remained in the reduced, or only partly oxygenated, state. "Dark-cutting" beef was characterized as having a lower acidity and a higher requirement for oxygen than was the case with normal beef. Bate-Smith (1948) cited some examples of the relation between pH and the depth of color of "dark-cutting" carcasses. Beef that was graded "dark", "shady", and "light" had average pH values of 6.53, 5.68, and 5.58 respectively.

5. Desiccation

Changes in muscle pigments due to drying appear to be related to the degree of moisture loss. Brooks (1933a,b) and Scott and Vickery (1939) believed that moderate desiccation was beneficial to the "bloom", or color, of the superficial muscle tissues. Brooks (1933a,b) stated that an increase in the depth of color resulted when beef was allowed to

lose moisture, due mainly to a decrease in opacity of the partly-dried surface tissue. The net effect of drying corresponded to an increase in concentration of pigments. In addition, Brooks observed that the formation of methemoglobin was slower in partly-dried muscle than it was in normal tissue. However, when considerable drying occurred, the meat became very dark and presented an objectionable appearance. Empey and Vickery (1933) noted that a loss in "bloom" of chilled beef resulted when weight losses exceeded 5.5 per cent.

The effect of drying on the color of cured pork was investigated by Winkler (1939b) who thought that color changes due to drying were reversible. In the absence of air, a linear relation was observed between discoloration and moisture loss. The change in the color of cured pork was mainly one of intensity when air was excluded; in air, changes in both intensity and quality of color were noted. Color changes were more rapid in saturated air than they were in air of lower humidity. Winkler concluded that methemoglobin formation was responsible for the discoloration observed early in the storage period, but that drying exerted an important effect during the later phases of storage. Winkler believed that the discoloration caused by drying was due to a concentration of pigments.

Proper selection of packaging materials for cured meats was considered by Urbain and Ramsbottom (1948) to be an effective means of preventing discoloration due to dehydration. However, susceptibility of meat products to

bacterial action at high humidities precluded the use of wrappers that were highly effective in retaining moisture.

In studies conducted by Allen (1949) with fresh beef packaged with MSAT-80 cellophane and with cellulose acetate, a direct relationship was observed between moisture loss and discoloration.

Tauber et al. (1949) demonstrated that liver sausage processed in Visking casing and overwrapped with polyethylene did not develop a type of discoloration known as "brown ring" during refrigerated storage, while this discoloration occurred frequently when no overwrap was used. "Brown ring" was caused by dehydration of the meat surfaces.

6. Microorganisms

Early observations of discoloration produced by bacteria growing on the surface of stored meat were reported by Glage (1901). Glage's "Aromobakterien" formed a gray coating on fresh meats; this film later changed to yellow as storage progressed.

Jensen (1945, p.73, 157) reviewed the causes of green and purple discolorations in beef carcasses. He believed that greenish discolorations of beef rounds were due to several types of bacteria: hydrogen sulfide-producers, oxidizing streptococci, and pigment-producing members of the genus Pseudomonas. Bacteria capable of oxidizing meat pigments were listed by Jensen as follows: species of Pseudomonas, Bacillus, Micrococcus, Lactobacillus, Flavobacterium, Achromobacter, enterococci, and

alpha types of cocci. Jensen and Urbain (1936) distinguished between green oxidation products and brown methemoglobin by means of spectrophotometric measurements of the green pigment.

Discolorations of sausages were classified by Niven (1951a) as: (1) surface greening, (2) green cores, and (3) green rings. The first type resulted from contamination of the product after heat processing; the organisms responsible for surface greening were studied earlier by Niven et al. (1949) and were identified as heterofermentative lactobacilli and members of the genus Leuconostoc. Hydrogen peroxide produced by these bacteria caused greening of the meat. Green cores, found primarily in larger sausages and in meat loaves, were believed to result from faulty heat processing followed by inadequate refrigeration. Bacteria implicated in this type of greening were similar to those observed in surface greening, with the exception that green cores were caused by lactobacilli of greater thermal resistance than those isolated from the green surfaces of sausages. Niven (1951a) confirmed the findings of Jensen (1945, p.176) who stated that green rings, another form of internal discoloration, were associated with unusually high bacterial loads in the sausage mix prior to processing. In both types of internal greening, oxygen tension was a determining factor in establishing the location of the green areas. Steinke and Foster (1951a) observed green discolorations beneath the casing of liver sausage after the product was stored for several weeks at 5°C. They attributed the greening to the action of oxygen on the cured meat pigment in accordance with the explanation given by Jensen

(1945, p.8) for this type of discoloration.

The effect of different artificial casings on the development of green discoloration in refrigerated liver sausage was noted by Steinke and Foster (1951b). Liver sausage packed in 200 gauge Saran casing did not exhibit greening after storage for 79 days at 5°C., but greenish discoloration was observed when Pliofilm or Visking overwrapped with Pliofilm were used as casings. Steinke and Foster concluded that greening was due to oxygen absorption, and not to the action of microorganisms.

Bulman and Ayres (1952) inoculated comminuted pork with spores of Clostridium spp. P. A. 3679 in the presence of sodium nitrate and observed a reddening of the meat after 4 weeks' storage at 37°C. Prior to that time, a type of spoilage, described as "atypical", was characterized by a change in color to gray-green. Bulman and Ayres believed that the greenish discoloration was caused by an aerobic or facultative spore former which was capable of reducing nitrate to nitrite. In studies of the spoilage of thermal processed cured pork, Stumbo et al. (1945) employed color changes in the meat as an index of keeping time. Adams et al.(1952) noted that change in color of heat processed beef was a useful criterion of spoilage; red discoloration preceded darkening of spoiled meat.

Not all of the color changes produced by bacteria are undesirable. As noted previously, the action of microorganisms in the reduction of nitrate to nitrite during the curing process was considered by Haldane (1901), Hoagland (1914), Callow (1934), and Urbain (1944, p.454) to be

an essential step in the color-fixation reactions. Niven (1952) elaborated on the role of nitrate-reducing organisms in the color formation of fermented sausages, a product which was cured without added nitrite and which depended upon nitrate reduction for its color.

7. Light

The significance of light in the fading of color of cured meats has been recognized for many years. However, a survey of the literature indicated that comparatively little work has been done on the effect of light on color changes in fresh meats.

General conclusions derived from the literature survey are as follows:

(a) Fresh meats and cured meats differ in their susceptibility to the action of light with regard to the type of light and to the intenisty of illumination.

(b) Filtering out the ultraviolet portion of visible light does not appreciably reduce the rate of fading of cured meats.

(c) With the same light intensity, different portions of the visible spectrum exert approximately equal effects in the discoloration of cured meats.

(d) As the light intensity and time of exposure are varied, the fading produced by a single type of light source appears to be constant when the product of intensity and time remains the same.

Hasselbalch (1909) investigated the change of hemoglobin to methemoglobin that occurred when defibrinated ox blood was exposed to light of

various wavelengths. Oxidation of hemoglobin was not sensitive to light of wavelengths greater than 310 millimicrons. Hasselbalch thought that in the absence of air, light acted to cause a reduction of methemoglobin; in the dark, oxyhemoglobin was formed by combination of hemoglobin with the oxygen split off from methemoglobin.

The effect of ultraviolet light on hemoglobin was reviewed by Ellis and Wells (1941, p.679). They stated that hemoglobin absorbed hydrogen under the influence of light and in the presence of quinones. Oxidation of the pigment occurred subsequent to the absorption of hydrogen. In sunlight, hemoglobin was degraded; the process was accelerated by the addition of glutathione.

James (1936) cited experiments conducted with ultraviolet "Sterilamps" in the meat display cases of a retail store. The lamps were described as radiating 90 per cent of their energy in a region of the spectrum that was strongly germicidal. After exposure to ultraviolet light, the surface of ground beef became dark in color. Other meat products retained their color to a greater degree than was observed for meat stored under similar conditions of temperature and humidity without exposure to ultraviolet radiations. Although James stated that meats stored at high humidity at 15.6° C. under germicidal lamps lost less weight than did similar products held at 5.6°C. and 50 to 60 per cent relative humidity in the absence of ultraviolet light, he did not indicate the effect of the light on weight loss of meat stored under the latter conditions. His findings appeared to describe changes in color brought about by

desiccation rather than by any unique property of the germicidal lamps.

In a review of the causative agents of food spoilage, Oser (1946) listed radiation as one of the most important physical factors. Ultraviolet radiation, used for its bactericidal value, was believed to introduce forms of spoilage other than those due to the action of microorganisms. Oser thought that the liklihood of methemoglobin formation was increased by exposure of meats to ultraviolet radiation.

Ramsbottom et al. (1951) confirmed Oser's statement by observations of the color changes in fresh meats exposed to a 30-watt germicidal lamp for 36 hours. Fresh beef was not discolored after a similar period of exposure to fluorescent light at an intensity of 60 footcandles. Pracejus (1949) found that fresh meats were highly resistant to color fading when illuminated by Soft White fluorescent lamps.

Several investigators believed that the effect of light in producing color fading in cured meats could be minimized by filtering out those wavelengths that were considered to be most harmful. For example, Urbain and Ramsbottom (1948) assumed that the use of colored wrappers for cured meats resulted in greater color retention than that afforded by the use of a material transparent to all wavelengths of light. They compared the rate of discoloration of sliced boiled ham wrapped with three different materials and exposed to fluorescent light having an intensity of 40 to 50 footcandles. Ordinary amber and red, as well as transparent semi-moisture proof cellophanes were used as second wraps for ham packaged initially with transparent semi-moisture proof cellophane. Meat

packaged with all of the materials showed discoloration after 6 hours, but after an exposure of 20 hours, the color of ham wrapped with red cellophane was improved over that of products packaged with the other materials. Urbain and Ramsbottom concluded that their assumption with regard to the effect of colored wrappers in preventing color fading was correct. They hypothesized that the light absorbed was photochemically active, and that fading was caused by all portions of the spectrum except those wavelengths in the red region (beyond 6000 A). However, Allen (1949) found that different parts of the visible spectrum produced equal fading in sliced bologna when the light intensity was held constant. Luckiesh and Taylor (1940) previously observed that for a given light intensity, wavelengths in excess of 6000 A had relatively little effect in fading many kinds of dyed textiles when compared with light in other spectral regions.

Taylor and Pracejus (1950) stated that energy must be absorbed to produce photochemical changes; however, they also indicated that the effect was not always proportional to the amount of energy absorbed and, further, that absorbed radiant energy did not necessarily produce fading of colored materials. Hockman (1946) and Allen (1949) indicated that ultraviolet light produced more rapid fading of cured meats than did light in the visible spectrum. Taylor and Pracejus (1950) commented on the reports of other investigators who believed that the ultraviolet energy radiated by fluorescent lamps was responsible for color fading.

Their observations on the amount of ultraviolet light emitted by tungsten-filament lamps, and by White, Daylight, and Soft White fluorescent lamps indicated that the fading of colored textiles was due principally to radiation in the visible spectrum. In tests with bologna, equal fading was observed when the meat was exposed either to tungsten-filament lamps or to Soft White fluorescent lamps: both types of lights were found to have the same ability to produce color fading. Taylor and Pracejus thought that the Bunsen-Roscoe reciprocity law operated over the practical range of artificial illumination. According to Ellis and Wells (1941, p.205) the reciprocity law may be defined as follows: in general, the amount of material transformed in a photochemical change is proportional to the product of the light intensity and the time of illumination. Pracejus (1949) noted that the reciprocity law was valid for the range of 20 to 200 footcandles of Soft White fluorescent light in the color fading of sliced bologna. Spectral reflectance measurements were used as the criterion for fading, and a minimum exposure of 200 footcandle-hours was found to cause perceptible fading of the product. In trials with fresh meats, color changes were not observed until an exposure of 4000 footcandle-hours was applied.

Ramsbottom et al. (1951) confirmed that tungsten-filament lamps and fluorescent lamps produced equal fading in cured meats. Sliced bacon, sliced bologna, and cooked ham showed noticeable fading after a minimum exposure of 60 footcandle-hours, a value considerably less than the 200

footcandle-hours cited by Pracejus (1949). Cured meats were observed by Ramsbottom et al. (1951) to show less discoloration after exposure to a 30-watt germicidal ultraviolet lamp than they did when exposed to fluorescent light having an intensity of 60 footcandles. Ramsbottom et al. did not specify the intensity of illumination provided by the ultraviolet lamp. Discoloration of sliced bologna occurred most rapidly when the light intensity was high. At intensities of Soft White fluorescent light ranging from 2 to 150 footcandles, the greatest amount of fading took place during the first 2 to 4 hours of display. With periods of exposure up to 6 hours, the reciprocity law apparently did not hold in regard to color fading of sliced bologna.

The influence of the angle of incidence of light on color fading of sliced boiled ham was considered by Urbain and Ramsbottom (1948). Improvement in color retention was secured when large angles of incidence were used in place of the small incidence angles which normally existed in the display of self-service meats. Data were not given for the light intensities provided by different incidence angles.

8. Temperature

As noted previously, (Brooks 1935a,b), the rate of oxidation of hemoglobin to methemoglobin in fresh meats depended upon low pressures of oxygen; a decrease in temperature caused a lowering of the optimum oxygen pressure for the reaction. The net effect of an increase in

temperature was an acceleration of the rate of oxidation.

Hartridge (1912) hypothesized that nitric oxide hemoglobin would have a higher stability to heat than would either oxyhemoglobin or carbon monoxide hemoglobin, but his experiments failed to confirm theory. At room temperature, nitric oxide hemoglobin decomposed spontaneously into alkaline methemoglobin. The effect of temperature on the atmospheric oxidation of solutions of nitric oxide hemoglobin was studied by Urbain and Jensen (1940). Oxidation of the pigment was complete at the end of 1 day at 37°C., but approximately only 50 per cent of the total hemoglobin was present as methemoglobin when the temperature of storage was 0° or 10°C. At higher pH values, the effect of temperature was less pronounced. Urbain and Jensen concluded that the high temperature coefficient of the oxidation reaction indicated a need for refrigeration in order to preserve the color of cured meats. They believed that discolorations which frequently were attributed to bacterial action actually were due to oxidation resulting from improper refrigeration.

According to Hockman (1946), color retention in packaged fresh meats was not attainable unless a temperature of about 1.7°C. was maintained in display cases. For cured meats, a range of temperature no higher than 2.2° to 4.4°C. was considered necessary for maintenance of color. The action of MSAT-80 cellophane in preserving the color of fresh beef was observed by Allen (1949) to vary with the temperature of storage; discoloration occurred more rapidly at 22.2°C. than it did at 4.4° or 10°C.

Pre-cooling of fresh meat prior to packaging was considered by Oakley (1950) to be beneficial in retaining the "bloom" of the meat. He cited examples of commercial practice wherein cuts of fresh meat held at -2.2° to 0.0°C. for short periods of time did not discolor as rapidly as did meats packaged immediately after cutting.

9. Antioxidants and Synergists

Gibson (1943) observed that methemoglobin was reduced by ascorbic acid. The reactions of ascorbic acid with oxyhemoglobin were described by Lemberg and Legge (1939, p.475-480). They postulated the occurrence of a cycle in which ascorbic acid reacted with oxyhemoglobin to form an unstable peroxide; the peroxide decomposed to yield choleglobin (a green product), and brown methemoglobin. Reduction of methemoglobin to hemoglobin by the action of ascorbic acid was followed by oxygenation to give oxyhemoglobin. Ascorbic acid was observed by Chang and Watts (1949) to protect the color of nitrosohemoglobin solutions and cured meat, but it produced fading and greening of hemoglobin. Watts and Lehmann (1952a) noted that ascorbic acid in low concentrations (0.1 to 0.5 per cent) preserved the color of hemoglobin in the absence of nitrite; when nitrite was added to hemoglobin solutions, the formation of nitric oxide hemoglobin was accelerated by the inclusion of ascorbic acid. In later studies, Watts and Lehmann (1952b) demonstrated that ascorbic acid was effective in retaining the red color of fresh pork.

The influence of metal ions in the reduction of methemoglobin by ascorbic acid was investigated by Gibson (1943) and by Weiss et al. (1953). Gibson (1943) found that salts of copper and iron catalyzed the reaction; Weiss et al. (1953) extended their studies to include zinc salts. The latter workers observed that zinc exerted the greatest effect in protecting the color of nitric oxide hemoglobin over a period of 7 days at 5°C. Weiss et al. believed that zinc offered possibilities for preserving the color of refrigerated cured meats.

According to Lavers (1948) ascorbic acid was not effective in preventing discoloration of red meats packaged with cellophane after the wrapping material was treated with the reducing agent. On the other hand, cellophane previously coated with a 5 per cent solution of sodium bisulfite effectively maintained the red color of fresh meat for 72 hours at 7.2°C. Lavers showed that the sodium bisulfite functioned only as a reducing agent; it formed no red-colored product with hemoglobin, and was not capable of producing a red color in the absence of oxygen. The water extracts of peach market paper functioned in a manner similar to that of sodium bisulfite, which led Lavers to conclude that the paper owed its color-protecting properties to the presence of a reducing agent.

Nordihydroguaiaretic acid was demonstrated by Kraft and Wanderstock (1950) to inhibit color changes in fresh beef packaged with cellophane and with Pliofilm. Butylated hydroxyanisole in a mixture with citric acid and hydroquinone was less effective in retarding discoloration.

No significant color retention was observed in smoked ham treated with antioxidants and packaged with transparent materials.

10. Other variables

Differences in the color of fresh meats that are attributable to age, breed, and species of animal have long been recognized. Hockman (1946) included feeding and slaughtering operations as factors affecting the color of packaged beef. Allen (1948) added that variations in muscle were responsible, in part, for differences in the rate of change in color of packaged meats.

Some of the conditions responsible for the occurrence of "dark-cutting" beef have already been mentioned; studies sponsored by the National Livestock and Meat Board (1949) gave evidence that low glycogen concentrations contributed to this type of discoloration.

Jensen (1945, p.44) summarized the effects of "undercuring" on the color of meats. When "undercured" meat was subjected to heat, gray discolorations were observed. Rupp and Lewis (1936) listed many discolorations of meats held for too short a period in the cure, or treated without sufficient nitrite. Greenwood et al. (1940) demonstrated that sugar improved the development and retention of color in cured meats. They pointed out that the concentration of sugar was critical in fixing the color of the cured product.

The action of smoke on the color properties of cured bacon was

described by Jensen (1945, p. 194-199). He stated that smoking did not destroy the oxidizing enzymes of bacteria; prevention of discoloration was not achieved by the smoking process. Woodcock (1943) believed that smoking increased the brightness of color of bacon, as evidenced by higher reflectance values for smoked bacon than those obtained with unsmoked bacon.

Iridescence of sliced cured meats was attributed by Jensen (1945, p.8) to the structure of surface fibers of meat, and not to a pigment.

Niven (1951b) observed that luminescence of meat could be produced by many different types of bacteria, including psychrophilic and/or halophilic forms. Luminescence depended on enzymic action; 80 to 90 per cent of the energy liberated in the reaction was in the form of visible light.

Some discrepancy was found in the literature with regard to flavor deterioration accompanying color changes in cured meats. Pracejus (1949) quoted a report which stated that taste was not affected when the color of cured meats became faded upon exposure to lights. According to Hockman (1946), the "edible life" of bologna was determined by color, taste, and odor. Protection of the appearance of cured meats was believed by Urbain and Ramsbottom (1948) to result in conditions which aided flavor retention. They distinguished between alterations in appearance due to oxidation of the pigments and oxidative changes which caused loss of flavor. Urbain and Ramsbottom obtained good agreement between flavor

retention and preservation of color in sliced bacon and in sliced dried beef when air was excluded from the packaged products. Although no data were given to support their conclusions, Ramsbottom et al. (1951) claimed that adverse effects on flavor occurred simultaneously with color fading in packaged cured meats. Deterioration of flavor was limited to the slice of meat exposed to light and was less noticeable with items that were cooked after exposure.

D. Factors Affecting Storage Life

1. Microorganisms

a. Fresh meats. (1) Bacteria. Glage (1901) isolated slime-forming bacteria from meat stored in an ice box at high humidity. A characteristic aromatic odor was noted as the "Aromobakterien" proliferated on the meat surfaces. The bacteria did not penetrate into the subsurface portions of the beef. Seven species of "Aromobakterien" were isolated; Glage believed that only one predominated. These organisms were highly aerobic and grew well in chopped meat; Glage ascribed this growth partly to the incorporation of oxygen into the meat during the chopping process. The optimum temperature for growth of the organisms was between 10° and 12°C. Good growth was observed at 2°C., but the bacteria grew poorly at 37°C.

Jensen (1948) and Ogilvy (1950) thought that Glage's "Aromobakterien" were identical with, or closely related to, Pseudomonas. Jensen (1948) stated that the organisms produced a desirable "black walnut-like"

flavor in meat during aging.

Attention was given by Moran and Smith (1929) and Hoagland et al. (1917) to the penetration of bacteria into the deeper portions of the flesh as a causative factor in spoilage of meats. Moran and Smith (1929) claimed that the invasion of the deep flesh by bacteria found on the surface of beef was negligible, even after the meat was stored for 17 days. In their experiments, samplings from the connective tissue leading into the body of the muscle were avoided; the authors believed that this in some measure accounted for the relatively low bacterial counts. Hoagland et al. (1917) found micrococci present at some distance below the surface of the round of fresh and of chilled beef quarters. These organisms were considered as representative of the initial contamination. Although molds and bacteria grew rapidly on beef held in cold storage for 177 days, the greatest depth of penetration of microorganisms was about 1 inch. Spoilage caused by bacteria on the surface of meats was of greater importance than that observed in the deeper flesh; the significance of these findings is emphasized when consideration is given to bacterial spoilage of cut-up self-service meats. Bate-Smith (1948) pointed out that meat which was divided into small portions would support bacterial growth to a greater extent than would intact carcasses or quarters.

Spoilage of beef held at 0°C. by an organism of the genus Pseudomonas was described by Haines (1931). Prescott et al. (1931) noted that beef kept in cold storage often developed disagreeable odors and superficial

slime. They demonstrated that this type of spoilage was due to an almost pure culture of a bacterium capable of growth at temperatures slightly above freezing and at high humidities.

Of the bacterial flora found in the air of slaughterhouses, Haines (1933a) showed that more than half of the organisms were rods, while the remainder were cocci. The majority of the rods were soil types; in a "poor" slaughterhouse, 19 per cent consisted of intestinal types, while 9 per cent were intestinal forms in a "good" slaughterhouse. The most striking difference observed between the numbers of organisms found in the two types of slaughterhouses was in the degree of infection of the wash water used to swab the carcasses.

An investigation of types of bacteria responsible for slime formation on stored lean beef was conducted by Haines (1933b). The slime was composed chiefly of organisms of the genus Achromobacter, with lower numbers of Psuedomonas and Proteus also present. The Achromobacter produced a characteristic "taint" odor and a definite "cold-store taint" flavor in meat; Haines considered that the bacteria were identical to the "Aromobakterien" described by Glage (1901).

Studies relating to the initial contamination of beef surfaces during processing operations were made by Empey and Scott (1939). Among the organisms viable at 20°C., bacteria comprised more than 99 per cent of the flora acquired during dressing. At -1°C., four principal genera of bacteria were represented, viz., Achromobacter, 90 per cent;

Micrococcus, 7 per cent; Flavobacterium, 3 per cent; and Pseudomonas, less than 1 per cent. Empey and Scott noted that the chief sources of the superficial flora of beef were the hide and the hair of slaughtered animals; contamination of the hide depended to some extent upon the microflora of the soils from the pastures.

Ayres et al. (1950) isolated a large number of bacterial types from the skin and flesh of fresh cut-up chicken. The development of off-odor and slime on poultry stored at 0°, 4.4°, and 10°C. was associated with the proliferation of members of the genera Pseudomonas and Alcaligenes; the former predominated. Off odor was detected when bacterial counts were somewhat above 10^8 organisms per square centimeter. Slightly higher levels of organisms were associated with the onset of slime; these investigators indicated a value of approximately 10^9 bacteria per square centimeter.

Cultures of 316 microorganisms were isolated from fresh pork sausage by Sulzbacher and McLean (1951). Of the fifteen genera identified, Bacterium, Microbacterium, Achromobacter, and Pseudomonas comprised more than 50 per cent of the total numbers of bacteria in the freshly prepared product. After storage at 5° to 8°C., the proportion of Microbacterium increased markedly, while little difference was noted in the relative numbers of Achromobacter and Bacterium. Species of Alcaligenes were more prevalent in stored sausage than they were in freshly made sausage. Deterioration of flavor in sausage held at refrigeration temperatures was attributed to the production of acid by species of Microbacterium.

Packaged ground beef from retail sources was observed by Ayres (1951) to have initial bacterial counts greatly in excess of those obtained from beef which was in the laboratory.

The bacteriological quality of commercial ground beef was studied by Kirsch et al. (1952) in 1952; these workers noted that the initial numbers of bacteria present were similar to those reported by other investigators in 1914. A heterogeneous bacterial flora was observed in market hamburger; namely, Gram-negative non-sporeforming rods, various species of the family Micrococcaceae, and less frequently, lactobacilli. During storage at 0° to 2°C. in a household refrigerator, spoilage was detected by the onset of a sour odor which became evident at 8 to 12 days. Organisms responsible for spoilage were five nonpigmented species of the genus Pseudomonas.

Several investigators have recognized that the keeping time of meats depends to some extent on the original contamination of the product. Haines (1933b) demonstrated the relation between the initial bacterial population and the time for appearance of slime on beef stored at 0°C. and 100 per cent relative humidity. When the initial concentration of bacteria was 40,000 per sq. cm., the meat became inedible after 8 days, while lean beef with an original population of 43 organisms per sq. cm. kept for 18 days. A direct relationship between initial counts and the time for occurrence of slime was observed by Haines and Smith (1933) who thought that the degree of infection of lean meat was the most important

determinant of storage life.

Empey and Vickery (1933) found an increase in storage life of chilled beef amounting to 29 days when the initial contamination was about one-tenth as great.

The keeping time of chicken stored at 4.4°C. and at 10°C. was observed by Ayres et al. (1950) to depend on the amount of initial contamination. At 10°C., fryers with an original load of millions of micro-organisms per sq. cm. kept for only 2 days, but slime was not detected on chicken with an initial count slightly in excess of 100 organisms per sq. cm. until the fourth day of storage. Ogilvy and Ayres (1951a) found that the relationship between the extent of original contamination and the storage life of chicken was linear, thus confirming the findings of Haines and Smith (1933).

Slime formation as an indication of bacteriological spoilage of meats has been reported by several workers; sliming was related to a specific level of surface organisms. Haines (1933b) gave a value of approximately $10^{7.5}$ for the number of bacteria per sq. cm. of meat surface when slime became evident. Empey and Vickery (1933) observed slime formation on chilled beef when the number of surface bacteria was about 5×10^7 per sq. cm. When the number of cells per sq. cm. was 10^8 or greater, Scott (1936) observed sliming on beef muscle.

Lochhead and Landerkin (1935) believed that the development of undesirable odor on dressed poultry was essentially a surface spoilage due

to bacterial growth. Surface odor became evident on poultry stored at -1.1°C. and 0.0°C. when counts approached 2.5×10^6 bacteria per sq. cm. According to Kirsch et al. (1952), a sour odor in ground beef was associated with a count of 5×10^8 bacteria per gram.

As stated by Tanner (1944, p.885), the terms "ham souring", "bone stink", or "bone taint" are used in the meat industry to designate a condition of putrefaction and not formation of acidity. In studying the causes of bone taint in beef, Haines and Scott (1940) isolated an organism resembling Clostridium oedematiens (Cl. novyi); they estimated that the tainted quarter contained 10^{11} bacteria of this type.

Very little work has been done on the bacteriological aspects of fresh meats packaged with flexible materials. Allen (1949) cited a report which stated that microorganisms proliferated rapidly on boneless round steak packaged with MSAT-80 cellophane and with other transparent materials. In these experiments, the meat was held in a chill room at 1.1° to 4.4°C. for 15 days. No data were presented relative to the time at which spoilage occurred, nor were any values given for bacterial counts.

Halleck et al. (1951) reported that the spoilage of fresh packaged meats, as indicated by organoleptic tests, was related to the growth of the Pseudomonas group of bacteria. Of various packaging materials tested with sliced fresh beef, Kraft and Ayres (1952) found that a laminate of aluminum foil and Pliofilm was most effective in retarding bacterial spoilage. In addition to the laminate, other materials tested included:

MSAT-80 cellophane, aluminum foil, and Pliofilm FF-120. The packages of beef were stored at 4.4°C.

In studies of the chemical changes of the fat of chilled beef, Lea (1931) found that tainted fat was caused by the growth of microorganisms. Whenever taint was noted, a high value was observed for the free fatty acids. Jensen and Grettie (1937) stated that microorganisms hydrolyzed edible fats to form free fatty acids. The lipolytic activity toward beef fat of several strains of Achromobacter, Pseudomonas, and yeasts was tested by Vickery (1936a,b). He asserted that no general conclusions could be drawn regarding the power of the bacterial genera to split fats, and further, that the free acidity of fat was not a valid index of microbial spoilage. Vickery's contention seemed to be in accord with the findings of Goldman and Rayman (1952) who stated that the lipolytic powers of different strains and species of Pseudomonas varied widely in rate and degree of hydrolysis. Although 70 per cent of the Pseudomonas cultures isolated from new sausage by Sulzbacher and Mclean (1951) were lipolytic, these organisms did not contribute greatly to the deterioration of stored sausage. Greenwood et al. (1945) reported that the formation of free fatty acids in bacon was correlated with the growth of bacteria, yeasts, and molds.

The work of Lea (1931) and Haines (1931) indicated that odors and taint in beef fat originated from the action of microorganisms growing where large areas of fat and lean were in contact. Lea (1931) also

pointed out that the composition of the fat influenced the rate of bacterial development. He differentiated between the unpleasant tainted flavor caused by microbial action and the tallowy flavor and odor produced by atmospheric oxidation.

(2) Yeasts. An investigation of the yeasts contaminating refrigerated meat was carried out by Brooks and Hansford (1923) in England. They observed that only a few types of yeasts grew on meat; Torula (Torulopsis) botryoides, Wardomyces (Debaryomyces) anomala, and Saccharomyces spp. were important organisms. Empey and Scott (1939) noted that the following genera of yeasts were common contaminants of stored beef: Mycotorula, Candida, Geotrichoides, Blastodendrion, and Rhodotorula. All of these were viable at -1°C. Yeasts comprised approximately 2 per cent of the total number of microorganisms isolated by Sulzbacher and McLean (1951) from fresh pork sausage. Ayres et al. (1950) found Torulopsis and Rhodotorula on cut-up chicken.

(3) Molds. Brooks and Hansford (1923) reviewed earlier work on mold contamination of chilled beef and made a systematic examination of the molds responsible for spoilage of meat shipped to England from the southern hemisphere. Molds were identified as: Cladosporium herbarum, Thamnidium chaetocladioides, Thamnidium elegans, Mucor mucedo, Mucor lusitanicus, Penicillium expansum, Penicillium anomalum, and Sporotrichum carnis.

The most common mold genus found on chilled beef by Empey and Scott (1939) was Penicillium, followed in order by Mucor, Cladosporium,

Alternaria, Sporotrichum, and Thamnidium.

According to Brooks and Hansford (1923), the growth of molds on meat was superficial; the mycelium penetrated to a depth of only 4 mm. Hoagland et al. (1917) previously observed that molds were not found at depths appreciably below the surface of stored beef carcasses.

Geotrichum and Penicillium were isolated from fresh cut-up chicken by Ayres et al. (1950).

b. Cured meats. (1) Bacteria. Ingram (1935) analyzed the slimes on bacon from commercial sources and found yellow micrococci and a variable flora of lactobacilli and bacilli. Ingram attempted to classify the organisms with respect to their salt tolerance. He prepared plates of minced pork containing salt concentrations varying from 0 to 25 per cent and exposed these to the air of a cold storage room at 0°C. in which bacon was stored. When the concentration of salt was less than 4 per cent, Pseudomonas was predominant; with salt concentrations between 4 and 25 per cent, the bacteria isolated consisted principally of yellow micrococci.

White cocci were isolated by Norton and Roderick (1930) from the slimes recovered from contaminated sausages. Inoculation of the organisms on sterile sausages produced sliming. The cocci grew aerobically and could tolerate salt in higher concentrations than that used in sausage preparation. Since the cocci were destroyed by heating for 10 minutes at 60°C., Norton and Roderick concluded that infection occurred after heat processing.

Forty microbial types, in which micrococci predominated, were found by Garrard and Lochhead (1939) as representative of the contamination of sides of bacon before curing. Some strains of bacteria resembled those found in bacon slime. A numerically important group of Gram-negative cocci or coccoid rods demonstrated lipolytic activity. Garrard and Lochhead observed that the organisms were more resistant to salt in the curing pickle than they were in broths having the same concentration of sodium chloride.

Organisms predominant in freshly made liver sausage were noted by Steinke and Foster (1951a) as aerobic micrococci and/or aerobic spore-forming rods, neither of which grew in sausage stored at 5°C. Gram-positive micrococci were found in large numbers in the slime which developed between the casings of sausage during storage. In later studies, Steinke and Foster (1951b) reported the appearance of slime between the layers of double casings used for liver sausage when the product was packaged with single or double casings and held at 5°C. The organisms in the slime were largely acid-forming micrococci. Steinke and Foster believed that sliming resulted from the diffusion of nutrients from the sausage to the surface of the Visking casing; a double casing was conducive to slime formation because it provided a greater barrier to transfer of moisture than did a single casing.

Hall (1942) believed that anaerobic organisms did not grow under sausage casings. Since the Visking casing was permeable to water, Hall

assumed that it would also allow passage of air. He tested this hypothesis by comparing bacterial growth on peptone agar protected by squares of casing material with growth on the same medium covered with thin glass cover slips. Organisms tested included obligate anaerobes, facultative aerobes, microaerophiles, facultative aerobes, and obligate aerobes. Anaerobic organisms were inhibited by the oxygen transmitted through the casing, and oxygen transfer was sufficiently low that aerobic growth was retarded.

In recent studies of the microorganisms associated with spoilage of frankfurters, Ogilvy and Ayres (1951b, 1953) isolated micrococci resembling Micrococcus caseolyticus from the slime on frankfurters stored at refrigeration temperatures. Changes in appearance of frankfurters due to microbial growth occurred before pronounced deterioration of flavor became evident. The bacteria from fresh frankfurters were predominantly Gram-positive types. The most numerous bacteria were micrococci, bacilli, and sarcinae, while lactobacilli and Gram-negative organisms were present in smaller numbers. The majority of the lactobacilli were believed to be different from the organisms demonstrated by Niven (1949) to produce greening on sausages.

Niven (1952) described the conditions leading to spoilage of pickled sausages by acid-tolerant lactobacilli.

A detailed study of the causes of ham-souring was made by Jensen and Hess (1941). The etiological agents of this type of spoilage consisted

of a variety of bacteria, viz., members of the genera Achromobacter, Bacillus, Pseudomonas, Proteus, Serratia, and Clostridium, as well as micrococci, streptobacilli, and a miscellaneous group.

(2) Yeasts. A pasty slime obtained from weiner sausage by Mrak and Bonar (1938) was shown to be a mixture of yeast and bacteria. Without exception, the cultures of yeast were considered to be Debaryomyces Guilliermondi var. nova zeelandicus Lodder. Heavy growth of the yeast was observed on sausage stored at 10° to 28°C; in wort, the optimum temperature range was 22° to 28°C. The yeast was highly tolerant to salt; growth occurred in wort containing 20 per cent salt. Giant colonies liquefied gelatin after incubation for 30 days at 20°C.

Steinke and Foster (1951a) found very few yeasts on sausage during early storage at 5°C.; they observed a uniform increase in numbers from about 1000 per gram at 19 days to several hundred thousands per gram at 47 days. They did not identify the organisms and considered that the presence of yeasts on liver sausage was exceptional. Ogilvy and Ayres (1953) sometimes encountered appreciable yeast populations on fresh frankfurters but frequently no yeast colonies were found in the initial plates. The yeast flora was shown to reproduce to sizeable numbers in frankfurters stored at 7.2°C.

(3) Molds. The significance of molds in causing spoilage of sausage products was cited by Yesair (1936). Various species of molds encountered on sausage, bacon, and ham were identified as: Penicillium expansum, Aspergillus glaucus, Aspergillus clavatus, Aspergillus niger, Mucor racemosus,

Rhizopus nigricans, Alternaria tenuis, Monascus purpureus, and Monilia sitophila. Yesair stated that all of the molds were destroyed by heating at 60°C. for 5 minutes, and that none would survive cooking operations. Surface mold growth was attributed to contamination after cooking.

Ogilvy and Ayres (1953) stated that molds normally were the greatest cause of microbiological spoilage of frankfurters, although the organisms comprised only a small portion of the initial flora. The majority of molds isolated from stored frankfurters belonged to the genus Penicillium. Other genera found were: Sporotrichum, Zygorrhynchus, Monilia, Mucor, Aspergillus, and Alternaria.

2. Storage conditions

a. Carbon dioxide. The inhibitory effect of CO_2 on microorganisms found on fresh and on cured meats was reviewed by Ogilvy (1950) who made an extensive study of the application of the gas in the preservation of chicken and frankfurters.

Valley and Rettger (1927) believed that the bacteriostatic or bactericidal action of CO_2 was due to an increase in hydrogen ion concentration. Bacterial growth was noted in a buffered medium when the concentration of CO_2 was as high as 97 per cent. However, Haines (1933c), Tomkins (1932), and Coyne (1933) were of the opinion that the inhibitory effect of CO_2 was greater than that due to a lowering of pH alone.

In a comparison of the influence of atmospheres of air, of CO_2, and

of nitrogen on mild-cured, unsmoked bacon, Callow (1932) found that the bacon kept best in an atmosphere of commercial CO_2. At a temperature of of 5°C., the keeping time of bacon was more than doubled by the use of commercial CO_2 as compared with air.

Haines (1933c) compared the rates of growth of Proteus, Pseudomonas, and Achromobacter in 10 and 20 per cent CO_2 at 0°, 4°, and 20°C. Proteus was affected only slightly by CO_2 in the concentrations used, but a marked increase in the lag period and generation time of Pseudomonas and Achromobacter was observed at 20°C. At 0°C., both 10 and 20 per cent CO_2 caused a doubling of the generation time for Achromobacter. Haines concluded that the inhibitory effect of CO_2 was greater at lower temperatures.

Chilled beef stored in an atmosphere of 10 to 12 per cent CO_2 was observed by Empey and Vickery (1933) to have an increase in storage life of 40 per cent compared with storage in air. Meat stored in CO_2 kept for 23 days, while slime was detected on beef held in air after 16 days at -1°C.

When Smith (1934b) treated lamb with 100 per cent CO_2 for 24 hours, followed by storage in air, no delay in the appearance of molds or in the proliferation of bacteria on the surface of the meat was observed in comparison with similar meat held continuously in air.

Tomkins (1932) observed the effect of CO_2 on the growth of molds known to cause spoilage of meat. Petri dishes containing nutrient agar were inoculated with spores of Sporotrichum carnis, Cladosporium herbarum,

Thamnidium elegans, Thamnidium chaetocladioides, and Mucor mucedo. The plates were stored at 0°, 5°, 10°, 15°, and 20°C. in desiccators containing 0, 10, 20, and 30 per cent CO_2. Carbon dioxide extended the lag phase; this effect was more pronounced at higher concentrations of the gas. Retardation of growth was influenced to a greater extent than was germination of spores.

Moran et al. (1932) suspended small pieces of lean beef in gas-tight jars and sprayed the meat with an aqueous spore suspension of Thamnidium chaetocladioides. Carbon dioxide in concentrations of 0, 20, 40, 60, 80, and 100 per cent was added to the jars. After 8 days of storage, mold growth was evident on meat stored in the absence of CO_2, but samples held in the gas showed no mold growth until the 18th day. Yeasts appeared on the 18th day of storage with 20 per cent CO_2. In a second experiment, CO_2 concentrations of 0, 4, 8, and 12 per cent were employed; the respective times for the first appearance of mold were 8 or 9, 11, 15, and more than 19 days. In atmospheres containing 0, 10, and 20 per cent CO_2, growth of Thamnidium on the connective tissue as well as on the lean was inhibited by CO_2.

In another experiment, Moran et al. found that an increase in CO_2 by intervals of 10 per cent caused a reduction in the rates of growth of Thamnidium chaetocladioides, Mucor mucedo, and Cladosporium herbarum. Plates containing nutrient agar and inoculated with pure cultures of molds 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. A decrease in temperature gave more

marked inhibition of growth.

The ratio of the keeping time of cut-up chicken in the presence of CO_2 to its keeping time in air was found by Ogilvy and Ayres (1951a) to be a linear function of CO_2 concentration within the range of 0 to 25 per cent. At 10°C., an increase in CO_2 concentration from 0 to 25 per cent resulted in a two-fold increase in the average generation time of slime forming bacteria; at lower temperatures (4.4° and 0.0°C.) the generation times were prolonged. Carbon dioxide at a level of 15 per cent at 4.4°C. had the same effect on average generation time as did a reduction in temperature to 1.1°C. They believed that the use of 25 per cent CO_2 provided a greater retardation of generation time than was noted when any temperature above freezing was used. Organisms causing slime formation were similar to those which produced this defect in the absence of added CO_2; however, growth of slime producing bacteria was suppressed in high concentrations of the gas.

The effectiveness of CO_2 in prolonging the storage life of frankfurters was demonstrated by Ogilvy and Ayres (1951b). Concentrations of the gas up to 50 per cent promoted an increase in keeping time at temperatures of -1.1°, 4.4°, and 10°C. Additions of CO_2 above a level of 50 per cent made little difference in the time for appearance of microbial growth on the frankfurters. Similar to the results obtained by these investigators (1951a) with chicken, a reduction in temperature increased the beneficial action of CO_2. Storage life was prolonged by a limited holding period in 25 and 96 per cent CO_2 followed by storage in air.

In further experiments, Ogilvy and Ayres (1951b) studied the effects of CO_2 in the preservation of frankfurters packaged with LSAT cellophane. Samples were given a preliminary treatment with 96 per cent CO_2 for 2 days and then wrapped, sealed, and stored in refrigerators. Spoilage became evident on CO_2-treated frankfurters at the same time as it was noted on control samples. Ogilvy and Ayres concluded that LSAT cellophane was sufficiently permeable to CO_2 to allow the gas to escape before any advantage could be gained from its use.

Additional studies were undertaken by the same authors (1953) in an attempt to characterize the various kinds of microbiological deterioration of frankfurters stored in different concentrations of CO_2. Mold growth was retarded by moderate levels of CO_2 (5 to 25 per cent). A progressive reduction in the growth of micrococci and yeasts occurred with increasing concentrations of CO_2. However, lactobacilli developed readily on frankfurters stored in 50 to 96 per cent CO_2; high concentrations of the gas permitted more rapid growth of lactobacilli than did low levels of CO_2. Ogilvy and Ayres indicated that some of the organisms isolated may have been members of the genera Leuconostoc and Microbacterium.

Bacon stored in commercial CO_2 or in nitrogen was shown by Callow (1932) to have a more desirable taste than bacon stored in air.

The protective effect of CO_2 in retarding the occurrence of taint in beef fat was demonstrated by Lea (1933a). In a saturated atmosphere, the time required for perceptible off-flavor was approximately doubled when 10 per cent CO_2 was employed.

Lea noted that CO_2 did not adversely affect the resistance of beef fat to oxidation during a storage period of 50 days. Ogilvy and Ayres (1951b) found that frankfurters stored at -1.1°C. with CO_2 concentrations of 15 and 50 per cent developed oxidative rancidity before visible microbial growth became apparent. Frankfurters stored in atmospheres containing high concentrations of CO_2 developed in acid taste.

b. Nitrogen. An atmosphere of nitrogen was observed by Callow (1932) to prevent aerobic spoilage of uncured pork at 0°C., but to allow the growth of anaerobic organisms to proceed to the point of putrefaction. Pork stored in nitrogen had a more desirable taste than did similar meat held in air.

Walker (1951) believed that the use of nitrogen eliminated the danger of oxidative deterioration brought about by packaging fatty foods in transparent wrappers.

c. Light. In early investigations of the effect of light on bacteria, Downes and Blunt (1877) utilized the development of turbidity in tubes containing Pasteur's solution as an index of bacterial growth. They exposed four tubes containing the solution to daylight and held four similar samples in the dark, covered by thin sheet lead. The solution in the exposed tubes remained clear for "an indefinite time", while the covered tubes became turbid; the differences were most marked when the sun shone brightly. Downes and Blunt concluded that light inhibited the growth of bacteria and "the microscopic fungi associated with putrefaction and decay".

Light was considered by Rahn (1929) to be one of many agents which caused death of bacteria; he believed that the order of death was logarithmic and that the destruction of a bacterial cell was brought about by the inactivation of one molecule in the cell. In later work (1930), he explained the occurrence of exceptions to the monomolecular reaction on the basis of variations in resistance of the cells under test.

Exponential disinfection curves were obtained by Haines and Lea (1937) when Bact. coli (E. coli), spores of B. mesentericus, and mixed suspensions of organisms from slimy meat were exposed to the radiations of a lamp producing 90 per cent light of wavelength 2536 A. From measurements of the absorption of light by beef juice, they found that a thickness of 0.2 mm. of juice reduced the intensity of light one thousandfold. Haines and Lea concluded that ultraviolet light caused a decrease in the numbers of bacteria on the surface of beef tissue but did not penetrate the tissue sufficiently to bring about inhibition of growth of subsurface microorganisms.

Wyckoff (1932) and Lea and Haines (1940) observed that the destruction of several species of bacteria subjected to ultraviolet radiations was described by exponential survival curves. Lea and Haines believed that a single ultraviolet quantum absorbed by a gene in a bacterial cell did not produce a lethal mutation. Wyckoff (1932) thought that it was improbable that the absorption of a single quantum of ultraviolet energy was responsible for the death of E. coli; no injurious effects were noted in surviving organisms, each of which he considered to have absorbed

millions of quanta.

Individual variations in the resistance of microorganisms to ultraviolet light were emphasized by several workers. Suspensions of cells of Staphylococcus aureus (Micrococcus pyogenes var. aureus) were exposed to different wavelengths of light in the ultraviolet region of the spectrum by Gates (1929a) who observed the lethal action of the radiations. No bacteria succumbed to ultraviolet radiations in the initial period of exposure, but bactericidal effects were observed after 6 to 7 per cent of the total number of bacteria were killed according to an exponential relationship between energy and lethal effect. In the final period of exposure, a number of cells remained which required an excess of ultraviolet energy for destruction.

Wyckoff (1932) and Rentschler et al. (1941) were of the opinion that the amount of lethal radiation necessary for the destruction of bacteria varied with the individual organisms and with the stage of growth. The latter workers observed that E. coli was more susceptible to radiation in the ultraviolet during the logarithmic growth phase than it was at other stages of growth. Also, a sublethal dose of radiation retarded the rate of development of bacteria after irradiation. Rentschler et al. concluded that the lethal action of low intensities of ultraviolet light was not accounted for by the absorption of a single quantum of radiation. Rahn (1945) questioned the interpretation made by Rentschler et al. (1941) of the effect of sublethal radiation; he attributed the delay in bacterial growth to ionizations produced by single quanta of ultraviolet energy.

Variations in sensitivity to radiation among species of bacteria were found by Haines and Lea (1937), Koller (1939), and Whisler (1940). Luckiesh et al. (1947) noted that bacteria generally were more susceptible to the action of ultraviolet light than were yeasts or molds. They expressed the belief that higher dosages of ultraviolet energy were necessary for preventing mold contamination of foods than were the values they secured for destruction of bacteria in air. Koller (1939) reported that spores of Aspergillus niger required about 45 times as much ultraviolet radiation for complete killing as did E. coli. In a study of the application of ultraviolet light to surface disinfection of citrus fruits, Fulton and Coblentz (1929) exposed spores of 27 different species of molds to the radiations from a mercury-tungsten arc. After irradiation for 1 minute, 16 of the species were completely destroyed; less than 1 per cent of 4 other species survived, while survival of the 7 remaining species ranged from 2 to 53 per cent. Of the molds tested, those having dark spore walls were most resistant to ultraviolet radiations. Mycelium was more easily killed than were spores.

The Bunsen-Roscoe reciprocity law was re-stated by Luckiesh et al. (1947) as follows: a given result from radiation occurred by the use of any combination of intensity of radiation and time of exposure which yielded a constant product. In order to ascertain the range over which the reciprocal relationship was valid, Luckiesh et al. used intensities of 350 and 0.35 microwatts per sq. cm.; the respective times of exposure

to germicidal energy were 0.11 and 110 minutes. When Staphylococcus aureus (Micrococcus pyogenes var. aureus) was employed as a test organism, the same per cent of kill resulted at both extremes of time and intensity. Luckiesh et al. concluded that the law held in the range of time and intensity of 1000 to 1. Rentschler et al. (1941) stated that the reciprocity law was not operative when the time of exposure involved an appreciable part of the "life cycle" of bacteria. In studying the effects of several environmental conditions on the action of ultraviolet light, Gates (1929b) found that the reciprocity law was not effective in the case of young, actively-metabolizing cells of Staphylococcus aureus (Micrococcus pyogenes var. aureus).

In a review of the lethal action of ultraviolet radiation, Ellis and Wells (1941, p. 241) stated that the majority of workers found that the region of bactericidal activity ranged from 2960 to 2100 A. Gates (1929b) believed that 2250 and 3130 A were the limits of wavelength between which ultraviolet light exerted bactericidal effects: the most effective rays for killing microorganisms were stated by Lea (1938) to be those which were emitted at approximately 2536 A. According to Rahn (1945), the greatest absorption of ultraviolet energy by bacterial occurred at about 2650 A; with wavelengths shorter or longer than 2650 A, the per cent of destruction decreased markedly. Duggar (1936, Vol. II, p.1127) indicated that visible light had a negligible lethal effect on bacteria.

Evidence for the photochemical nature of the action of ultraviolet

light on bacteria was presented by Gates (1929b); he obtained a value of about one for the temperature coefficient of the reaction.

The influence of humidity on the resistance of organisms to germicidal energy was investigated by Wells (1940), Whisler (1940), and Luckiesh et al. (1947). These workers confirmed that, in general, ultraviolet radiations were ten times as effective in dry air as they were in moist air.

Storage of beef under radiation produced by "Sterilamps" was described by James (1936). The lamps were stated to emit 90 per cent of their rays in a highly germicidal region of the ultraviolet spectrum. No bacterial or mold growth was observed on meat exposed to ultraviolet rays; however, James did not indicate the microbiological condition of beef held without exposure to germicidal radiation, nor did he state the duration of the storage period. In other trials cited by James, pork chops exposed to the light emitted by "Sterilamps" in meat display cases had lower surface bacterial counts than were revealed on chops stored in cases without lamps. James believed that no deleterious effects were produced in irradiated beef fat. Griswold and Wharton (1941) noted that ultraviolet radiations did not affect the quality of beef quarters stored at 15.6°C. for 48 hours. Germicidal ultraviolet radiation was considered by Brady et al. (1949) to be unimportant in promoting the development of rancidity in beef and pork held below freezing temperatures after irradiation.

The use of ultraviolet light for control of microbial growth on

foods containing high proportions of fat was criticized by Lea (1938) and by Oser (1946). They stated that the onset of rancidity was accelerated by ultraviolet radiations. According to Lea (1931), the oxidation of beef fat at 0°C. or at 25°C. proceeded slowly in the absence of sunlight or direct sky light. Lea and Lea (1937) believed that ultraviolet light was best applied as an inhibitor of microbial growth, rather than as a germicide, because of the danger of development of oxidative rancidity when germicidal dosages were employed. Ultraviolet irradiation of skinned pork carcasses prior to freezing was observed by Volz et al. (1949) to cause rapid development of rancidity after the meat was frozen.

Coe and LeClerc (1932) showed that the role played by light in the production of rancidity in foods varied with the wavelength. They used wrappers of different colors and monochromatic sources of light to demonstrate that green light, between 4900 and 5600 A, was primarily inactive with regard to rancidity development. In later work, Coe (1941) reported that the ultraviolet, violet, and blue regions of the spectrum were most effective in promoting rancidity, while the red region at about 6600 A and the yellow region at 5700 A were intermediate between the blue and the green portions of the visible spectrum in their ability to accelerate rancidity. Coe stated that the regions which were active corresponded to the portions of the spectrum at which oils strongly absorbed light.

The effect of sunlight on deterioration of the fat of foods packaged with completely transparent and with colored cellophanes was investigated

by Davies (1934). He believed that the depth of color was important in the choice of a cellophane for preserving food fats. Morgan (1935) considered that rancidity-retarding wrappers should be of any visible color except blue. He based this conclusion on the results of tests obtained with cellulose films having a wide variety of shades and colors. Morgan cited data to show that a yellow transparent cellulose sheeting known as Sylphrap R.R. Old Gold, which absorbed only the blue of visible light, was effective in retarding the production of rancidity in bacon.

Several investigators attempted to distinguish the bactericidal action of the radiations emitted by ultraviolet lamps from the bacteriostatic effect of ozone produced by the lamps. Lea (1937) stated that an equilibrium existed between the formation and decomposition of ozone in air irradiated by ultraviolet lamps. Ozone was produced in increasing quantities as the wavelength of light was decreased from 1900 A; rapid decomposition of the gas occurred at 2536 A. Lea's observations appear to account for the low values obtained by Koller (1939), Ewell (1941) and Mallman and Churchill (1946), for the quantity of ozone produced by germicidal lamps; these workers indicated that ozone was formed in concentrations in the order of tenths of a ppm.

Mallman and Churchill reported that ozone at a level of 0.1 ppm. inhibited the development of bacteria on beef. They believed that the bacteriostatic action of ozone was enhanced by moisture present on the surfaces of fresh meat. Their findings were in disagreement with those

of Kefford (1948); Kefford claimed that ozone in concentrations of 3 to 5 ppm., applied daily for 3 hours, had no effect on bacterial growth on beef muscle having a moisture content of 290 to 315 per cent of the dry weight. At moisture contents below 150 per cent dry weight, however, storage life of beef was prolonged by treatment with 3 to 5 ppm. of ozone. Kefford attributed the discrepancy between his observations and those reported by Mallman and Churchill (1946) to variations in moisture contents of muscle and to differences in the length of time in which ozone was applied.

According to Ewell (1941), a concentration of ozone of 12 ppm. was necessary in order to accomplish germicidal action equivalent to that provided by ultraviolet radiation at a wavelength of 2537 A.

d. Temperature. The relation of the Van't Hoff-Arrhenius equation to growth of microorganisms has been debated by several workers. Crozier (1926) thought that the equation held but was subject to deviations. Fulmer and Buchanan (1929) noted that the thermal increment, μ, decreased regularly with an increase in the temperature of growth for Saccharomyces cerevisae. The equation was considered by Scott (1937) to apply to a sub-optimal temperature range of growth. He questioned the accuracy of Crozier's findings on the basis of evidence obtained from the growth of several species of bacteria and yeasts at eight temperatures in the range -1° to $30^{\circ}C$. Ogilvy and Ayres (1951a) believed that the equation applied for bacterial growth on chicken in the range of temperature from 0° to $10^{\circ}C$.

Haines (1937) contended that temperature was the most important single factor controlling the rate of growth of bacteria when an adequate nutrient medium was furnished; meat was considered to be such a medium. The rates of growth of a number of common bacteria were estimated by Haines (1938) at intervals of a few degrees from -5° to 37°C. Haines divided the organisms into four groups as follows: Group 1 — the staphylococci, optimum at 37°C., growing very slowly if at all below 10°C.; Group 2 — most strains of B. coli (E. coli), B. proteus (Proteus vulgaris), B. subtilis (optima at 37°C.) and micrococci (optima 20°to 30°C.), growing very slowly in the range 5° to 0°C.; Group 3 — some strains of B. proteus (Proteus vulgaris) growing at 0°C.; Group 4 — most strains of Achromobacter, Pseudomonas, and various yeasts showing rapid growth at 0°C. and growing at -5°C. on supercooled media. According to Haines, Pseudomonas and Achromobacter grew on frozen media at -3°C. He differentiated between the effect of temperature per se and the influence of desiccation due to freezing.

Scott (1937) observed that a definite low temperature zone existed for maximal crop production of strains of Achromobacter, Candida, and Geotrichoides. He classified these organisms as psychrophiles and regarded strains of Pseudomonas and Mycotorula as mesophiles capable of growth at low temperatures. Bergey's Manual of Determinative Bacteriology (6th ed., 1948) listed many species of Pseudomonas that do not develop at 37°C. In studies of the bacteriological flora of refrigerated ground

beef, Kirsch et al. (1952) mentioned that many members of the genus Pseudomonas were unable to grow at 37°C.

According to Haines and Smith (1933), the storage life of lean meat was not greatly affected by variations in temperature of a few degrees in the range 15° to 5°C. Slime became visible in 1 day at 15°C., in 2 days at 10°C., and in 3 days at 5°C. Meat held at 0°C. did not show sliming until the tenth day of storage, at which time the bacterial count reached 30 x 10^6 organisms per sq. cm. of surface. With regard to mold infection, a three-fold increase in storage life was obtained by reducing the temperature from 5° to 0°C. Brooks and Hansford (1923) favored the use of temperatures lower than 0°C. for cold storage beef.

Increases in soluble nitrogen in beef stored at 0°C. were ascribed by Haines (1931) to the action of microorganisms. He felt that a temperature of 0°C. was not sufficiently low to inhibit bacterial action. Empey and Scott (1939) found that yeasts and molds made up a greater proportion of the microbiological flora of beef stored at -1°C. than they did at 20°C., while the numbers of viable bacteria were greatly reduced at -1°C.

At storage temperatures of 10°C., 4.4°C., and 0°C., appreciable differences in the keeping time of commercial fresh and defrosted-frozen chicken were shown by Ayres et al. (1950). Deterioration became evident in 2 to 3 days at 10°C., in 6 to 8 days at 4.4°C., and in 16 to 18 days when the temperature was 0°C. Ogilvy and Ayres (1951a) believed that the average generation time of bacteria responsible for sliming of chicken

was a logarithmic function of temperature within the range 0° to 10°C. These workers (1953) reported that members of the genus Bacillus were unimportant in spoilage of frankfurters stored at temperatures below 10°C., but at 10°C., aerobic sporeformers were isolated on several occasions from the interior of the meat. Sporeforming anaerobes were not implicated in the deterioration of frankfurters held at refrigeration temperatures.

When frankfurters and fresh pork sausage were packaged with different types of cellophane, Allen (1949) found that slime development and the time for appearance of molds were greatly accelerated by an increase in temperature from 7.2°C. to 21.1°C.

Garnatz (1950) emphasized the need for maintaining low temperatures in cutting rooms of meat packing plants; for meat display cases in retail stores, he believed that the upper limit of temperature should be no greater than 4.4°C.

e. Relative humidity. The role of humidity in microbiological spoilage of meats has long been recognized by investigators in the field. Moran and Smith (1929) summarized the relationships between humidity and air movement in the storage atmosphere and the development of bacteria as follows:

> In addition to the growth of bacteria on the surface of the meat there is the additional possibility of their invasion into the body of the meat....invasion will be facilitated by the presence of films of moisture on the surface, sufficient

> to allow free movement from the infected area. The humidity of the air immediately surrounding the meat should therefore be low. This not only postulates a low average humidity.... but also a certain amount of air circulation. Under these conditions the surface of the meat is dried, and this dried layer not only inhibits the growth of bacteria, but also acts as a mechanical barrier to their invasion into the meat.

Scott (1936) reviewed earlier work and made an exacting study of the growth of microorganisms on beef muscle of known water content; he contended that the relative humidity at the surface of muscle was dependent on the amount of moisture in the bulk of the tissue. Scott thought that the relative humidity at the tissue surface was higher than that of the surrounding atmosphere owing to diffusion of water from the interior to the external portions of meat tissue. Thin slices of muscle tissue were dried in an incubator at 45 $\pm$ 0.5°C. until the weight of the muscle conformed to a predetermined value for the desired water content. Scott obtained an initial value for the water content of fresh muscle of 290 to 330 per cent of the dry weight. On the basis of the average value of 300 per cent moisture, corresponding to a relative humidity of 99.3 per cent, the moisture contents of dried muscle strips were determined before studies on the growth of microorganisms were initiated.

After equilibration, Scott inoculated muscle strips with two strains of Achromobacter and Pseudomonas, and one strain each of the yeasts Geotrichoides, Candida, and Mycotorula. Slime formation was evident at relative humidities of 99 per cent and above when the number of bacterial cells per sq. cm. of surface was 10^8 or greater. A lower level of yeasts

produced slime at 99 per cent relative humidity; their numbers were between 2 x 10^6 and 10^7. Although growth of bacteria was visible when a concentration of 10^8 cells per sq. cm. was attained, no slime was noted at 98 per cent relative humidity. At relative humidities of 96.5 and 97 per cent, the numbers of bacteria reached 10^9 per sq. cm. without visible growth on the meat surface. At higher humidities, the characteristic spoilage odor was more marked.

No growth of Achromobacter was observed at 96 per cent relative humidity within a period of 5 to 6 weeks; for growth of Pseudomonas the limiting level of humidity was 98 per cent. Yeasts were less susceptible to drying than were the bacteria; growth of the yeasts was inhibited at 90 per cent relative humidity. From the practical point of view, Scott concluded that drying to an extent sufficient to reduce yeast growth was not feasible, but that restriction of the development of bacteria could be achieved by maintaining the moisture contents of surface tissues below 90 per cent (corresponding to 96.5 per cent relative humidity).

In later studies, Scott (1937) found that growth of Achromobacter occurred at 4°C. at a relative humidity of 96 per cent. When muscle strips were stored in an atmosphere containing 10 per cent CO_2, Scott (1938) determined that the critical water contents for growth of Achromobacter were higher than they were in air; the rate of growth approached zero at 97 per cent relative humidity.

Haines and Smith (1933) showed that bacterial growth on the surface of lean meat stored at a relative humidity of 70 per cent and at a temperature of 1°C. was retarded only about one-tenth as compared with meat held in a saturated atmosphere.

Allen (1949) indicated that bacterial and mold growth on fresh meat packaged with MSAT-80 cellophane might be due to the high relative humidity obtaining in the atmosphere of the package. He observed that weight losses were low when fresh beef was packaged with cellophane and stored for 69 hours at 4.4°C. and 60 per cent relative humidity.

Microbial growth was not appreciably retarded on frankfurters stored at relative humidities close to 95 per cent by Ogilvy and Ayres (1951b). Since meat held at lower humidities exhibited severe desiccation, they concluded that reducing the humidity of storage was not a feasible means of controlling the growth of microorganisms.

The importance of circulation of air in conjunction with low relative humidities for preserving meats was mentioned previously. Norton and Roderick (1936) noted that dry air or adequately circulated air reduced the bacterial contamination of frankfurters. According to Yesair (1936), mold growth in sausage drying rooms was not prevented by controlling the temperature, humidity, and air movement within practical limits. He observed that various molds grew equally as well on meat products at 0 per cent relative humidity as they did at 100 per cent relative humidity.

Scott and Vickery (1939) stated that the extent of changes in the

bacterial population of the surface of beef depended upon the mean drying power of the air after the meat attained the temperature of the surrounding air. The rate of evaporation of water from beef depended on the temperature gradient between the surface and the external atmosphere; the relative humidity of the air had a negligible effect on the evaporation rate. The limiting level of humidity, below which excessive desiccation occurred, was believed to be approximately 90 per cent. The rate of evaporation of water from chilled beef quarters was stated by Hicks (1936) to decrease as the time of storage increased. He thought that evaporation was governed by the loss of water in chilling and by the drying power of the atmosphere.

Other relationships between storage temperature, humidity, and air velocity were shown by Bates and Highlands (1934). Growth of microorganisms was stimulated by variations in temperature; this was due to the deposition of moisture on meat surfaces when air of high humidity was cooled suddenly. For a given relative humidity, an increase in the rate of air flow caused a reduction in the rate of bacterial growth. Bates and Highlands believed that comparable storage conditions with respect to the development of bacteria could be obtained by decreasing the relative humidity 5 per cent for each degree C. rise in temperature.

The influence of atmospheric humidity on the rate of deterioration of bacon fat was studied by Lea (1933b). At relative humidities of 60 and 75 per cent, the fat in areas adjacent to the lean was very

susceptible to oxidation. No appreciable growth of microorganisms was observed on the fat. When the humidity of storage was 90 and 100 per cent, bacon fat oxidized more rapidly than it did at lower humidities, but reached a constant value after 12 days at 15°C. Oxidation continued to increase in bacon held at 60 and 75 per cent relative humidity; no maximum was noted up to 25 days. Lea concluded that the rapid growth of microorganisms at high humidities inhibited further oxidation and that hydrolytic and oxidative rancidity did not develop simultaneously.

f. Vacuum packaging. The few reports available in the literature pertaining to evacuation of flexible packages and microbial spoilage of self-service meat items appear to be in disagreement. Winans (1950) thought that the action of microorganisms was retarded on sliced bacon and sliced luncheon meats when these products were packaged under vacuum. However, Garnatz (1950) stated that the conditions resulting from vacuum packaging included increased levels of moisture within the package, and thus the development of bacteria was stimulated. Another report (Modern Packaging, 1950) indicated that evacuation did not affect the growth of bacteria on packaged luncheon meats.

g. Aging. Previous sections in the literature review have shown that the keeping time of meats packaged for self-service merchandising depends upon handling and storage operations prior to packaging. Aging of beef in order to attain an increase in tenderness is a common practice

in the meat industry; changes that take place in the lean during the maturation process have received considerable attention.

Hoagland et al. (1917) believed that the tenderness resulting from storage was due to enzymic action, since the bacteria and molds which grew on the surface of refrigerated meats did not penetrate the muscle to any great depth. No appreciable increase in tenderness was observed between the second and fourth week of storage at 0.0° to 2.2°C. Harrison et al. (1949) indicated that tenderness increased with aging, but the degree of tenderization varied with different muscles and with various carcass grades.

The disappearance of rigor was associated by Moran and Smith (1929) with alterations in the protein of muscle fibers; they concluded that tenderness was due to chemical changes in the proteins of the tissues. According to Haines (1931), microorganisms caused appreciable changes in the protein of beef stored at 0°C. In a review of the ripening of the ripening of beef, Bate-Smith (1948) stated that the rate of tenderizing was faster at higher temperatures, but the chance of microbiological spoilage was increased. The effectiveness of germicidal radiations in preventing spoilage of beef held at elevated temperatures was described by James (1936).

Gibbons and Reed (1930) studied the influence of autolysis of tissues on the subsequent chemical changes produced by Proteus vulgaris. Autolysis did not affect bacterial growth, but did cause a considerable

difference in the chemical changes produced by the organism.

h. Denaturation of proteins. A decrease in the solubility of myosin was observed by Smith (1934a) in beef stored at 0°C. He believed that denaturation was responsible for the loss of solubility. On the other hand, Moran (1935) noted that there was little denaturation of meat proteins at 0°C. He contended that meat should be cooled rapidly after slaughter for two reasons; namely, because denatured proteins were more easily degraded by bacteria, and because denaturation affected the texture of the product.

III. EXPERIMENTAL MATERIALS AND METHODS

A. Materials

1. Packaging films and foils

A number of flexible materials were selected for packaging fresh and cured meats; for convenience these materials were grouped as follows: (1) cellulosic films, (2) rubber-hydrochloride films, (3) vinyl and vinylidene films, (4) coated and laminated materials, and (5) miscellaneous materials. The types of films and foils used and the thicknesses of each are listed in Table 2.

2. Package-sealing equipment

An electronic bar sealer loaned by the Dow Chemical Company was used for sealing Saran films. For other packaging materials, a Wells automatic heat sealer, Style TF, was employed in making closures.

Table 2. Types of packaging materials

A. Cellulosic films

MSAT-80 cellophane, 300 gauge (0.0009 in.)*,**
LSAT cellophane, 300 gauge (0.0009 in.)*,**
Cellulose acetate, 0.0012 in. *
Cellulose acetate butyrate, 0.0013 in. *

B. Rubber-hydrochloride films

Pliofilm
1. FF-120, 0.0012 in.*,**
2. FM-1, 0.0010 in.*
3. 120 N1, 0.0012 in.*
4. F-197, 0.0009 in.*,**

C. Vinyl and vinylidene films

Saran
1. A-517, 100 and 200 gauge (0.001 and 0.002 in.)*
2. A-517, double-wound 100 gauge (0.001 in.)*
3. 517, 100 and 200 gauge (0.001 and 0.002 in.)*
4. B-517, 100 and 200 gauge (0.001 and 0.002 in.)*
5. Experimental plastic Q-684, double-wound (0.001 in.)*

Cry-O-Rap, 0.0015 in.*
Polystyrene Q-641, 0.001 in.*
Visten-C, 0.001 in.*,**

D. Coated and laminated materials

No. 450 (0.0013 in.) cellophane coated with Saran**
Tyton Tite (0.005 in. laminated and coated kraft paper)*
No. 300 (0.0009 in.) MAT-2 cellophane with wax-elastomer coating, with and without dehydroacetic acid mold inhibitor*
Flexvac (0.0025 in., moisture proof cellophane laminated to FF Pliofilm)*,**
No. 300 MAT-2 cellophane laminated to 100 gauge Saran*,**
No. 300 MAT-2 cellophane laminated to 200 gauge polyethylene*,**
Aluminum foil (0.0015 in.) laminated to 120 N1 Pliofilm (0.0012 in.)*,**

*Used for packaging fresh meats

**Used for packaging cured meats

Table 2. (continued)

E. Miscellaneous materials	
Aluminum foil,	0.0015 in.*
Waxed paper,	0.001 in.*
Parafilm-M,	0.005 in.*

*Used for packaging fresh meats

3. Meat samples

a. Fresh meats. Round and short steaks and pork loin chops were purchased from local grocery stores and were cut in the laboratory into pieces of uniform size.* Care was taken to prevent contamination from cutting tools or from the chopping block. Samples were allowed to "brighten" by exposing them to air for 20 to 30 minutes in a refrigerator at 4.4°C. and were then packaged.

b. Cured meats. Frankfurters were obtained from the following sources: (1) skinless, uncolored, dried skimmilk-added frankfurters were received from a packing house in the evening of the day following manufacture and were stored at 0°C. until the next morning; and (2) similar frankfurters in 1-pound cellophane-wrapped packages were purchased from local retail stores. Picnic ham and large bologna were purchased at local markets; picnic ham was sliced in the laboratory while bologna was cut in the store with a U.S. Slicing Machine Company meat slicer set at No. 5 (corresponding to a slice thickness of 0.115 to 0.120 inch).

*Approximate dimensions of samples: 3in. by 2½ in. by ½ in.

4. Storage chambers

Storage at 2.5°C ±1° was carried out in a Percival open-type meat display case, Model 2306, having a display-shelf capacity of approximately 5.5 cubic feet. The display case was equipped with an Intermatic time switch, Model TS 60SP-2, set for defrosting between 4 A.M. and 6 A.M. daily. During the defrosting period, the temperature rose to 10°C., but in no instance did it remain above 3.5°C. for longer than 2 hours.

Temperatures similar to those provided by the Percival display case were obtained by the use of a Victor refrigerator, opening at the top and having a capacity of about 9.75 cubic feet. This box operated at a temperature of 1.5°C. ± 1°; both refrigerators were equipped with Ranco Type O temperature regulators.

For storage at 4.4°C. and 7.2°C., a Super-Cold, Model 44 RS refrigerator with a capacity of approximately 40 cubic feet was used. Temperature was controlled to ± 1°C. by means of a Minneapolis-Honeywell temperature regulator. Storage trials at 10°C. ± 1.5° were conducted in a Herrick 45 cubic feet refrigerator with a Par Model HA5S refrigerating unit and White Rodgers Type 1609 temperature control. Circulation of air in the Super-Cold and Herrick refrigerator was provided by fans.

In initial studies on packaging in atmospheres of CO_2, 5-gallon cans and 1-quart and 2-quart Mason type jars with rubber stoppers served as containers for packaged meats. These containers were later replaced by a gas-tight storage chamber having a capacity of about 1.75 cubic

feet (Figure 1). The storage box was constructed of aluminum with a reinforcing iron frame; ¼-inch copper tubing was fitted to the box to provide for gas intake and exhaust. Forced air circulation was maintained by a Dayton blower, Model 1C180.

One-quart Mason type jars were used as containers in studies concerning the effect of relative humidity on keeping time of packaged meats.

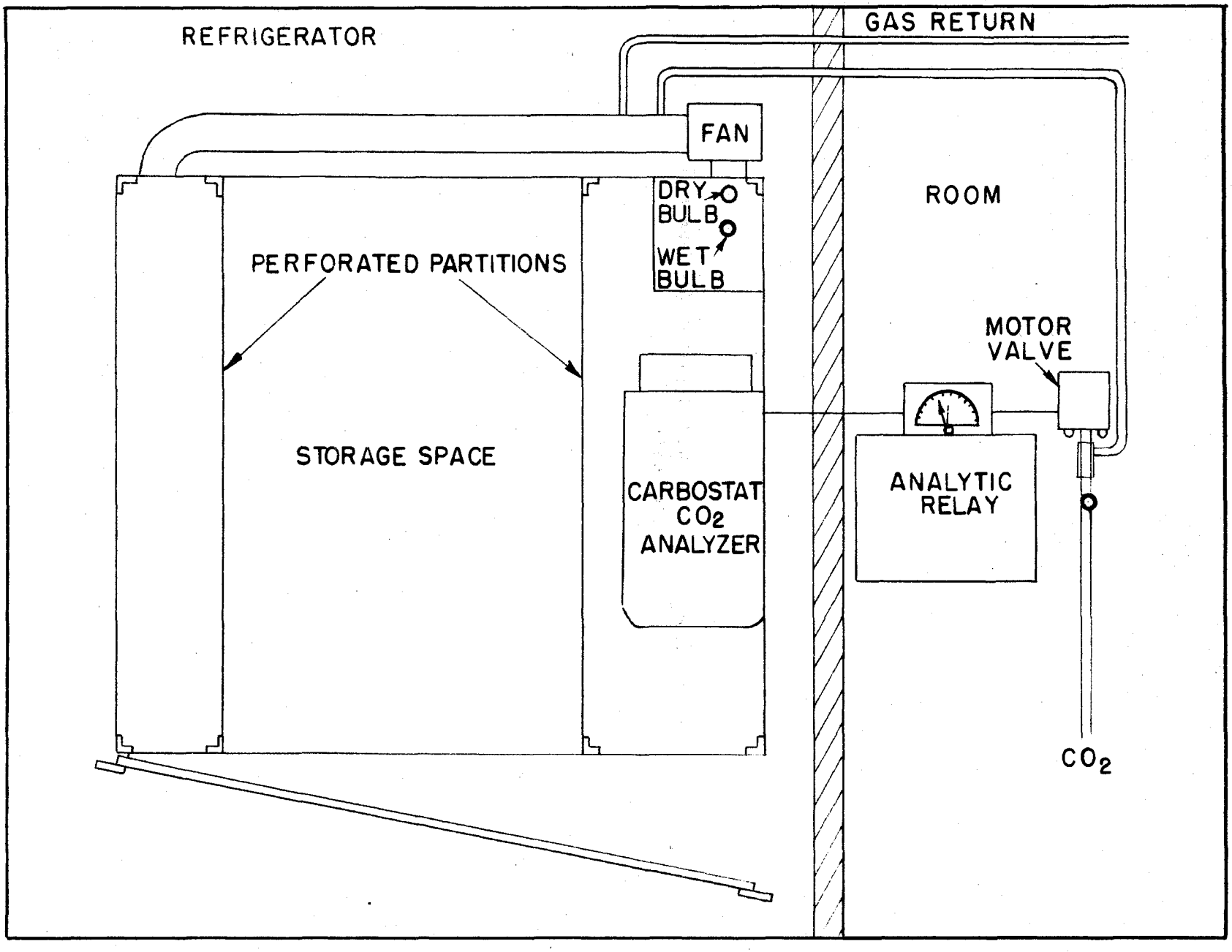
5. Gas control apparatus

Carbon dioxide concentrations in the aluminum storage chamber were controlled by means of a Carbostat CO_2 analyzer* connected to an analytic relay (Figure 1). The analyzer operated on a principle based on differences in thermal conductivity of CO_2 and of air; the presence of CO_2 caused an increase in temperature and electrical resistance which in turn resulted in deflection of the meter pointer on the analytic relay. Various concentrations of CO_2 were secured by setting an arm in the relay to the desired percent CO_2. When the meter pointer made contact with the arm the relay established electrical circuits which caused the motor valve to close and arrested the flow of CO_2 from the gas cylinder.

Analyses of CO_2 and of oxygen in the storage chambers and in packages were made with a Fisher Unitized Precision Universal Model gas analyzer (Orsat type); the analyzer was described in detail by Ogilvy (1950). For rapid determinations of CO_2 concentrations of flowing gas mixtures, a Cambridge CO_2 Indicator (Model M Exhaust Gas Tester, Modified) was employed.

*Manufactured by the White Manufacturing Company, St. Paul, Minn.

Figure 1. Storage chamber and gas control apparatus.


REFRIGERATOR
GAS RETURN
FAN
DRY BULB
WET BULB
ROOM
PERFORATED PARTITIONS
MOTOR VALVE
STORAGE SPACE
CARBOSTAT CO_2 ANALYZER
ANALYTIC RELAY
CO_2

6. Gases

Compressed carbon dioxide, nitrogen, and oxygen were obtained from commercial sources.

7. Color measurement apparatus

a. Spectrophotometer. Reflectance determinations of meat samples were conducted by means of a Beckman Spectrophotometer; Model D was used to carry out spectrophotometric measurements in the visible light range and Model DU was employed for measurements in the ultraviolet region. The spectrophotometer was equipped with a diffuse reflectance attachment; a block of U.S.P. Carbonate of Magnesia served as the standard. The spectrophotometer was also used to determine transmission of light by packaging materials.

b. Judging booth. For visual evaluation of color of meats, a judging booth having dimensions 24" x 30" x 41" was equipped with a 200-watt Sunshine Lighting Unit, No. UCD-ADD-30, manufactured by the Keese Engineering Company. The illuminant provided a light intensity of 100 footcandles on the inspection table in the booth.

8. Other control instruments

a. Temperature recorders. In order to determine fluctuations in temperature and to record operating temperatures, Foxboro recorders having temperature ranges from -17.8° to 37.8°C. and 10° to 76.7°C. were placed

at various locations in refrigerators during each run. In later trials, a Dickson Minicorder, Type 1, with temperature range 0° to 45°C. was used.

b. Humidity recorders. Records of humidity in the aluminum chamber were made by a Brown Model 625122-20 wet and dry bulb recording thermometer. Relative humidity in refrigerators was measured with a Cenco Recording Hygrograph, No. 76995. Both instruments were checked with a Mason Hygrometer having wet and dry bulb thermometers suspended in flowing air.

c. Light meter. Light intensities in the Percival display case and in the Victor refrigerator were measured with a General Electric Type DW-68 light meter calibrated in footcandles.

9. Bacteriological media

A description of culture media may be found in the section on bacteriological methods.

B. Methods

1. Packaging methods

Packaging materials were cut and formed into bags of desired size. For evacuating and gassing, bags containing meat samples* were heat-sealed to one-hole rubber stoppers fitted with glass and rubber tubing. Heat sealing was facilitated by an application of General Mills Polyamide Resin

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*Size of samples per package:
Three to five frankfurters (about 150 to 250 grams)
One slice of fresh beef or pork weighing about 50 to 100 grams
One slice of picnic ham weighing about 40 to 75 grams
Two or three half-slices of large bologna (approximately 50 to 100 grams)

95 and Cenco Tackiwax to the stoppers. Bags were connected to the manifold of the Orsat apparatus and evacuated by means of a Cenco-Hyvac vacuum pump. A mercury manometer was employed to measure the initial vacuum and that obtaining at each sampling interval; packages were considered to be gas-tight when the mercury remained at a constant level in the manometer after evacuation. Closures were made by tightening a screw clamp on the rubber tubing attached to the stopper.

Cured meats to be packaged in atmospheres of CO_2 or nitrogen were held in open bags placed in jars containing high levels (92 to 96 per cent) of the respective gases for 1 or 2 days prior to gassing and sealing the packages. Fresh meats were held in 25 per cent CO_2 for 2 days and then packaged in atmospheres approaching 95 per cent CO_2. Control packages containing either type of meat were held in air prior to packaging. These received no treatment with nitrogen or CO_2. Since the packages contained little gas space relative to the volume occupied by the meat, preliminary exposure was employed for the purpose of permitting addition of sufficient gas to the packages.

Two methods were used for controlling concentrations of gases added to the packages. In the first, bags were evacuated and gas mixtures were added at a controlled rate by employment of flow meters. The second method, which was found to be the more adaptable of the two, consisted of evacuation followed by addition of the test gas to the desired concentration as determined by pressure readings on the mercury manometer; air was then allowed to enter the packages until pressure in the bags was equal to that of the

external atmosphere. Details of evacuation and gassing procedures were described by Ogilvy et al. (1950).

When initial gas concentration was not a variable, bags without rubber stoppers were sealed simply by the use of the Wells heat-sealer or the electronic bar sealer.

2. Storage methods

a. Storage in closed containers. Prior to storage, determinations were made of the volumes of bags used for packaging stored meats. The following procedure was employed for these measurements: (1) rubber stoppers with glass and rubber tubing were heat-sealed and wired to bags, (2) bags were weighed and filled with distilled water of known temperature until the meniscus reached a graduation on the glass tubing in the stopper, and (3) bags were again weighed and their volume calculated from the weight of the water and its density. Volumes of meat samples were determined by specific gravity measurements calculated from the amount of water displaced by small pieces of meat in a 25-ml. volumetric flask. Specific gravities (corrected to 25°C.) of five cuts of round steak were as follows: 1.026, 1.048, 1.037, 1.041, and 1.039. The mean value of 1.038 was employed in calculating the volume of meat samples.

When the aluminum chamber was employed for storage of packaged meats in atmospheres of CO_2 in preliminary experiments, a constant flow of the gas was supplied to the chamber. Later studies were directed at observing

the effects of storage in air on meats packaged in CO_2; in these trials no additional CO_2 was introduced into the chamber. The analytic relay and the Orsat apparatus were used for determining CO_2 concentrations in the chamber. Relative humidity was controlled by means of a sulfuric acid solution in a tray on the floor of the chamber. Densities of the solution were determined periodically during storage and at the termination of each run. Data of Wilson (1921) were used in preparing the solutions.

With storage in Mason type jars and 5-gallon cans, similar methods were employed with the exception that the Carbostat analyzer, analytic relay, and motor valve were not used. Humidity control was maintained by passing the test gas in small bubbles through distilled water or sulfuric acid solutions in a 500-ml. bottle containing a sintered glass diffusion disc; the bottle was connected by rubber tubing to the jars or cans.

All containers were held in refrigerators during storage. Meat packages were removed from containers at intervals and analyses of the atmospheres of the packages were made for CO_2 and oxygen concentrations immediately before samples were removed for bacterial counts. Control samples (ungassed) were similarly stored and analyzed at the same time as were other meats.

In experiments dealing with absorption of CO_2 by fresh meat, beef was stored in jars containing approximately 25 per cent CO_2. Jars of known volume were used and the volume occupied by the meat was determined from specific gravity measurements made from fresh beef. Carbon dioxide concentrations were determined daily during the storage period and from

these analyses the amount of dissolved gas was calculated. Uptake of CO_2 by frankfurters was measured using methods different from those employed in fresh meat studies. Frankfurters were placed in bags made of aluminum foil laminated to Pliofilm; packages were sealed to rubber stoppers fitted with glass and rubber tubing and then were placed in jars. Frankfurters were given a preliminary treatment with 95 per cent CO_2 for 2 days prior to closing packages. Bags were evacuated and gassed in the manner previously described. Packages and jars both were analyzed periodically for content of CO_2. The amount of gas dissolved in the meat was calculated as the difference between that in the jars and in packages.

In relative humidity studies, small samples of packaged meats (approximately 1½ by 1½ by ¼ inch) were suspended over saturated salt solutions in 1-quart Mason type jars fitted with rubber stoppers and sealed with Cenco Tackiwax. A micro-slide forceps, made of nickel plated spring wire, was bent into the form of a "V" and fastened with wire to glass tubing inserted in the rubber stopper; in this way the forceps served as a shelf for holding samples. Salt solutions were selected in accordance with the data given by O'Brien (1948). In preliminary experiments, sulfuric acid solutions were employed in order to control relative humidity in jars containing unpackaged meats.

For trials wherein the effects of packaging materials were evaluated, packaged meats simply were stored on shelves in closed refrigerators.

b. Storage in open refrigerators. In studies concerning the influence of light on discoloration and keeping time of packaged meats,

samples were stored in the Percival open-type display case or in the Victor refrigerator with top open. General Electric 15-watt Soft White fluorescent and germicidal ultraviolet lamps were mounted in the display case about 30 inches from the trays used to support meat packages. Different intensities of light were obtained by placing packages at various locations on the trays.

A sheet of black paperboard served as a partition between sections of the display case illuminated by the two types of lamps. In order to prevent extraneous light from entering the case, the windows surrounding storage shelves were covered with black paper.

In experiments with packaged sliced bologna, samples designated as "good" controls were stored in the lower section of the case under a sheet of black paper. Circulation of air was maintained over the samples by supporting the paper a few inches above the upper surface of the packages. "Poor" controls were wrapped and placed in the case under Soft White fluorescent light 3 to 5 hours in advance of other packages.

Fluorescent lighting units were attached to ring stands placed in the Victor refrigerator; light intensity on meat samples was varied by altering the position of the light fixtures on the stands and by changing the settings on a Powerstat variable transformer, Type 1126, connected to the fluorescent units. In order to simulate commercial storage conditions, the door of the refrigerator remained open during storage trials.

c. Determination of storage end points. Keeping time of packaged meats was based on several criteria. Off-odor, slime formation, and

definite values for the numbers of microorganisms found on meat surfaces were the most commonly used indices of spoilage. In several experiments, the production of H_2S or CO_2 by microorganisms was considered to be additional evidence of spoilage. Hydrogen sulfide was detected by the use of a filter paper strip cut to dimensions of 1½ inches by ½ inch and impregnated with lead acetate in accordance with the directions given by the Society of American Bacteriologists, Committee on Bacteriological Technic (1949, p. 149-14). Strips prepared in this manner were placed in petri dishes and sterilized by autoclaving for 20 minutes at 121°C. One or two strips were placed in packages prior to sealing; formation of black lead sulfide on the paper strips indicated the development of H_2S. Other criteria of keeping time were desiccation and changes in color and pH of meats. Determinations of pH were made by touching the electrodes of a Beckman Model H pH meter to the surface of meats.

3. Methods for determining color changes

a. Measurements of spectral reflectance. After weights were recorded and samplings were made for bacterial counts, the pieces of meat were cut to dimensions of 1½ by 1½ by ½ inch. Samples were placed in their original wrappers, over-wrapped with aluminum foil and stored in a refrigerator at 4.4°C. until reflectance readings were made. In no instance was the time between cutting and analyzing for reflectance greater than 2 hours. For reflectance measurements, the magnesium carbonate standard was placed in the rear compartment of the drawer in the

reflectance attachment and the sample to be tested in the front compartment. The controls of the spectrophotometer were manipulated in conformance with the procedures used for transmission measurements.

Preliminary experiments with packaged bologna indicated that reflectance readings were lower than those observed when the same samples were analyzed with no wrapping material; in subsequent determinations, all samples were examined unpackaged. For measurements of spectral reflectance of picnic ham and round steak, a strip of polystyrene Q-641 film was placed over the meat in order to prevent meat particles from contacting and adhering to the reflectance attachment housing.

Examination of reflectance curves revealed that changes in reflectance were negligible between 400 and 540 mμ, but appreciable differences were noted in values from 540 to 800 mμ. The range 540 to 800 mμ was selected for reflectance analysis. The reflectance properties of meat samples were not markedly altered by the heat or light of the spectrophotometer lamp.

Color changes in cured meats were also determined by changes in the ratio of reflectance at 650 mμ to that at 570 mμ, a ratio proposed by Ramsbottom et al. (1951) as an index of color fading. Lingard (1952) found that reflectance increased at 570 mμ and decreased at 650 mμ when fading occurred.

b. Visual evaluation. A panel of six judges was selected for estimating changes in color of packaged bologna. Choice of judges was based on ability to differentiate between samples exposed for 3 hours

to 150 and to 50 footcandles of Soft White fluorescent light. The fitness of judges for evaluating color was determined by the following procedure. Judges were presented with three samples, two of which were held at the higher intensity of light while the third was exposed to the lower intensity; each panel member was asked to identify the sample having a color different from the other two. Additional tests were run wherein two samples were displayed under light having an intensity of 50 footcandles while one sample was stored 150 footcandles. Judges also ranked samples according to the amount of discoloration observed after the meat was displayed at the two light intensities. Further tests consisted of matching color of samples with colored plates in A Dictionary of Color by Maerz and Paul (1930). Those judges who made correct decisions in distinguishing between the samples held at high and low light intensities and who were in closest agreement in matching color were selected as panel members.

The reflectance ratio previously described was employed as an objective measure of the high and the low standards used for subjective estimation of color. Scores for "good" and for "poor" controls were assigned in accordance with the values obtained for the ratio of spectral reflectance at wavelengths 650 and 570 mμ. Reflectance ratios and corresponding scores for controls were designated as follows: 2.50, 10; 2.35, 9; 2.20, 8; 2.05, 7; 1.90, 6; 1.75,5; 1.60, 4; 1.45, 3; 1.30, 2; 1.15, 1. The upper limit of 2.50 for the reflectance ratio was adopted from the highest value given by Ramsbottom et al. (1951) for fresh sliced

bologna, and the lowest value of 1.15 was determined by preliminary tests on bologna stored for 9 days under Soft White fluorescent light at an intensity of 60 to 70 footcandles.

Visual evaluation of color changes in types of meat other than bologna did not include the use of a judging panel.

4. Bacteriological methods

a. Frankfurters. For microbial counts on frankfurters, a number of 500 ml. wide-mouthed bottles - each containing 10 grams of screened, washed sea sand and sufficient distilled water (approximately 94 ml.) to give a total volume of 100 ml. - were fitted with solid rubber stoppers. Bottles and contents were autoclaved for 20 minutes at 121°C. Using a flamed forceps, one frankfurter was placed in each bottle; bottles were then shaken 200 times in order to suspend surface microorganisms in the diluent. Aliquots of suitable dilutions were plated with nutrient agar for counting organisms growing on and below the surface of the media. For counting organisms growing anaerobically, similar aliquots were plated with Linden thioglycollate agar modified by the addition of 0.1 per cent soluble starch in accordance with the procedures described by Burke et al. (1950). Difco nutrient agar with 0.5 per cent yeast extract and 0.5 per cent salt added was employed for obtaining total counts. Counts were made of lipase-forming organisms using Turner's technique (Jensen, 1945, p.63) with modifications. Later, the medium and procedure described by Sulzbacher and McLean (1951) were substituted

with the exception that the modified Difco nutrient agar was added in place of the veal infusion agar employed by the authors. Hydrolysis was indicated by blue coloration of fat globules surrounding colonies of lipase-forming bacteria.

Aerobic plates were incubated 4 days at 20°C. before counting; anaerobic plates were stored for a similar length of time at 37°C. A number of colony types and sizes were found on aerobic plates; organisms were differentiated by means of Gram stains followed by microscopic examination of stained preparations.

b. Other meats. Surface bacterial counts of picnic ham, sliced large bologna, and fresh beef and pork were made by swabbing a definite area of meat surface; counts were reported as the number of bacteria per square centimeter. The method was similar to that employed by Ayres et al. (1950), in sampling chicken. A cork borer was used to cut holes having an area of 2 sq. cm. in filter paper strips. The paper ring prepared in this way was sterilized, placed on the meat surface and a moistened cotton swab rolled over the enclosed area several times. The swab was transferred to a dilution bottle containing 99 ml. of sterile distilled water; the bottle was shaken 100 times and serial dilutions of the mixture were used in making pour plates. This method was also employed in determining contamination due to packaging materials.

The same plating media used for frankfurters were utilized in making counts of organisms growing on other meats. Incubation times and temperatures were the same as those indicated for plates in frankfurter studies.

IV. RESULTS AND DISCUSSION

A. Storage of Packaged Fresh Meats

1. Effect of packaging materials

Before undertaking studies concerning the influence of light, relative humidity, and gases on packaged meats, it was believed desirable to gain information about the relation of packaging films and foils to changes in refrigerated meats during storage.

a. Color changes. Permeabilities of materials to oxygen and moisture vapor appeared to be principal factors responsible for differences in color changes of packaged fresh meats. MSAT-60 cellophane was effective in preserving the red color of oxygenated beef for the first 3 days of storage at 4.4°C.; after 3 days, brown discolorations were observed. In the early phase of storage this film permitted conditions favorable for retention of the desired color. However, during prolonged storage, oxygen pressure was reduced; lowering of oxygen pressure promoted the formation of brown metmyoglobin. Fresh beef wrapped with other cellulosic materials such as LSAT cellophane, cellulose acetate, and cellulose acetate butyrate appeared darker red during the first few days of storage than did samples packaged with MSAT-60 cellophane; darkening continued on further holding. Meat packaged with waxed paper showed similar changes.

With Pliofilm FM-1 and with polystyrene Q-641, change in color occurred at about the same time as that observed when MSAT-80 cellophane was used as the wrapping material; discoloration of beef wrapped with the two former materials resembled that found when the cellophane was used. Meat packaged with Pliofilm FF-120 and with Pliofilm F-197 demonstrated visible reduction of myoglobin within 12 to 46 hours after packaging. Brown discoloration resulted soon thereafter. Samples wrapped with most Saran films, Cry-O-Rap, Flexvac 183A, Parafilm-M, Tyton Tite, polyethylene laminated to cellophane and Saran laminated to cellophane provided interesting illustrations of the course of color changes that commonly occur with fresh meats over a period of time. After 1 or 2 days in storage at 4.4°C., the central portion of the meat was purple in color, indicating that the myoglobin was in the reduced state. It was in this general area that the meat was in most intimate contact with the wrapping material and air space was at a minimum. The oxygen pressure was sufficiently low to permit the formation of reduced myoglobin from oxymyoglobin without further oxidation to metmyoglobin. Toward the outer edge of the meat, brown discoloration was observed as a narrow band extending over the entire meat surface and to a depth of 2 to 3 mm. In the region of the brown band, reduced myoglobin had undergone oxidation to metmyoglobin. The outermost edge, where oxygen pressure was high, remained bright red in color during the first few days of storage. By the end of the 12th day, all samples

packaged with the above materials showed an increase in brown discoloration. Aluminum foil, aluminum foil laminated to Pliofilm and 200 gauge Saran B-517 provided a reduced condition of the myoglobin with the purple color evidenced throughout storage periods of 12 or 16 days. On exposure to air, brightening occurred and the color resembled that of the meat immediately before it was packaged and stored.

At 7.2°and 10°C., discoloration proceeded more rapidly than it did at 4.4°C. Beef packaged with polystyrene Q-641 and stored at 7.2°C. demonstrated undesirable darkening of the red pigment after a holding period slightly less than 2 days, while at 4.4°C., darkening did not become noticeable until the third day of storage. When Pliofilm FM-1 was used for packaging fresh beef, brown discoloration became apparent approximately 1 day earlier at 7.2°C. than it did when the meat was held at 4.4°C. Color changes in beef wrapped with Saran laminated to cellophane, with polyethylene laminated to cellophane, and with 200 gauge Saran A-517 were more rapid at 10°C. than they were with storage at 4.4°C. The role of temperature in the oxidation of myoglobin has been described in the Review of Literature; in general, at higher temperatures metmyoglobin forms more rapidly than it does at lower temperatures. However, oxidation of myoglobin may not completely account for the increase in discoloration observed at the higher temperatures of storage. In all cases, samples held at 7.2°C. and at 10°C. lost more moisture than did beef packaged with the same kind of materials

and stored at 4.4°C. When polystyrene Q-641 and Pliofilm FM-1 were employed, desiccation may have contributed to discoloration. With the laminated materials and with Saran, differences in weight losses of meat kept at 4.4°C. and 10°C. were inappreciable and it is doubtful whether dehydration was of importance in influencing color changes. Unpackaged control samples stored in open petri dishes darkened very quickly and appeared dark red after 1 day at 4.4°, 7.2°, and 10°C. On prolonged storage, such meat became brown-black in color.

The predominant change in color of the lean of packaged pork loin was that of darkening of the light red pigment to a darker red or red-brown color. This type of discoloration was observed when pork loin was held at 4.4°C. after being wrapped with the following materials: ISAT cellophane, MSAT-80 cellophane, 200 gauge Saran A-517, Parafilm-M, Pliofilm FF-120, Cry-O-Rap, aluminum foil, and the laminate of aluminum foil and Pliofilm. No appreciable differences were detected in color of pork packaged with these materials and kept for 2 weeks, except in the case of meat wrapped with the foil-film laminate; darkening was very slight when the laminated wrapper was used. With all materials tested, the fat changed from white to yellow or yellow-brown at the end of approximately 2 weeks. The color of the lean of unpackaged control samples demonstrated progressive darkening from dark red after 1 day to dark red-brown at the end of 2 weeks at 4.4°C.

b. Weight losses. Average weight changes of beef and pork packaged with various materials are shown in Tables 3 and 4. Relative humidity

in refrigerators ranged from about 70 to 75 per cent. In several instances, a gain in weight was observed, due presumably to condensation of moisture on the outer surface of bags. At 4.4°C., all materials provided barriers to the transfer of moisture vapor when compared with unpackaged controls, but considerable variation in moisture vapor permeability was observed among materials. The effect of moisture loss on color of meat has been mentioned previously. Waxed paper, both types of cellophane, polystyrene Q-641, and Pliofilm FM-1 permitted greater weight losses than did other materials tested. In general, coated films, laminated materials and Saran films were the most effective barriers to transfer of moisture vapor.

At 7.2°C., beef packaged with Pliofilm FM-1 and with polystyrene Q-641 lost more moisture than did similar meat when wrapped with the same kind of materials and stored at 4.4°C. As given by Lange (1949, p.1483-1484), the vapor pressure of water in contact with air at 4.4°C. is 6.279 mm. Hg; at 7.2°C., the vapor pressure is 7.623 mm. Hg. Increased moisture loss at 7.2°C., may have been due to the higher vapor pressure than that obtaining at 4.4°C.

While Saran films allowed only negligible moisture losses after packaged beef was stored for 2 weeks at 10°C., very high weight losses were noted when beef was packaged with cellulose acetate or with cellulose acetate butyrate and held at 10°C. The latter materials have been characterized as being highly permeable to moisture vapor. Loss of nearly 17 per cent of the wet weight of meat wrapped with cellulose

Table 3. Average weight changes of fresh beef packaged with various materials*

Packaging material	Temp. (°C.)	Storage time (days) 2	5	8	10	12	14
None (unpackaged control)	4.4	-5.86	-16.82	-16.77		-25.97	
Waxed paper		-1.68	-6.32	-7.85		-8.10	
MSAT-80 cello.		-0.65	-2.08	-3.58		-6.83	
LSAT cello.		-0.62	-1.51	-4.33		-6.03	
Pliofilm FM-1		-0.74	-1.12	-2.36		-3.91	
Polystyrene Q-641		-0.39	-1.18	-2.07		-2.77	
Pliofilm F-197		-0.09	-1.23	-1.29		-1.45	
Cry-O-Rap		0.52	-0.13	-0.55		-1.18	
Saran B-517 (100 ga.)		0.00	0.00		-0.54		-0.76
Pliofilm FF-120		-0.11	-0.66	-0.71		-0.75	
Aluminum foil		0.02	-0.49	-0.22		-0.66	
Saran A-517 (200 ga.)		0.25	-0.14	-0.34		-0.60	
Tyton Tite		-0.07	-0.09	-0.16		-0.40	
Flexvac		0.00	-0.01	-0.05		-0.27	
Polyethylene-cello. laminate		0.00	0.00	-0.14		-0.23	
Saran A-517 DW		0.00	-0.04		-0.06		-0.16
Saran B-517 (200 ga.)		0.00	0.00		0.00	-0.10	
Saran Q-684 DW		0.00	0.00		0.00		-0.09
Parafilm-M		0.03	-0.04	-0.05		-0.08	
Saran-cello. laminate		0.00	0.00	-0.01		-0.01	
Alum. foil, Pliofilm laminate		0.25	0.22	0.14		0.01	
Polystyrene Q-641	7.2	-1.16	-4.52	-6.18			
Pliofilm FM-1		-0.63	-2.16	-2.60			
Wax-coated MAT cello. mold inhibitor		-0.02	-0.39	-0.64			-0.90
Wax-coated MAT cello. no mold inhibitor		-0.09	-0.19	-0.34			-0.35
Cellulose acetate	10.0	-16.95	-45.50	-51.40			
Cellulose acetate butyrate		-9.63	-25.50				
Saran A-517 (100 ga.)		0.00	0.00	0.00			-0.17
Saran 517 (100 ga.)		0.00	0.00	0.00			-0.13
Saran A-517 (200 ga.)		0.00	0.00	0.00			-0.09
Saran 517 (200 ga.)		0.00	0.00	0.00			-0.09

*Weight changes expressed as per cent initial wet weight

acetate and stored for 2 days was calculated to correspond to a muscle moisture content of approximately 58 per cent, or a relative humidity of 97.5 per cent at the surface (Scott, 1936). At the end of 5 days, beef packaged with cellulose acetate had a moisture content of about 30 per cent of the wet weight. Cellulose acetate butyrate allowed moisture losses to the extent that the water content of beef approached 50 per cent after 5 days at 10°C. One might expect that, owing to dehydration of the meat, bacterial growth on the surface of beef packaged with the cellulose ester films would be arrested after short periods of storage at 10°C. However, undesirable changes in color and decrease in tenderness due to desiccation would nullify any advantage to be gained from reduction of bacterial growth.

With pork loin (Table 4), the general trend of weight losses approximated that of fresh beef wrapped with the same type of materials. Moisture losses tended to be somewhat less with pork than with beef.

c. Storage life. (1) Comparison of spoilage criteria. Average keeping times of packaged fresh beef as determined by several methods are given in Table 5. Formation of H_2S was followed by off-odor and slime formation in that order. The production of H_2S was not considered as reliable an index of spoilage as was off-odor or slime formation because in many instances the gas apparently was lost from packages before any visible blackening of the lead acetate paper was observed. Blackening of the paper generally was more pronounced when materials having low rates of gas transmission were employed. Surface bacterial counts

Table 4. Average weight changes of fresh pork packaged with various materials*

Packaging material	Temp. (°C.)	Storage time (days) 2	4	8	10	14
None (unpackaged control	4.4	-5.11	-8.00	-16.57	-22.34	-26.07
LSAT cellophane		-0.35	-1.02	-3.02		-5.12
MSAT-80 cellophane		-0.40	-1.62	-2.02	-3.41	-4.46
Pliofilm FF-120		-0.02	0.00	-0.02		-0.45
Cry-O-Rap		0.11	0.14	0.00		-0.12
Aluminum foil		0.12	0.11	0.00		-0.08
Parafilm-M		0.05	0.13	-0.01		-0.06
Saran A-517 (200 ga.)		0.15	0.10	-0.04		-0.06
Alum. foil-Pliofilm laminate		0.50	0.10	0.04		0.00

*Weight changes expressed as per cent initial wet weight

ranged from 5.5×10^6 to 8×10^7 when off-odor became apparent, with an average count of 3×10^7 bacteria per square centimeter of surface. Slime was detected when the number of organisms was between 2×10^8 and 6×10^8 per square centimeter; the average value for slime formation was 4×10^8 bacteria. The interval between detection of off-odor and slime varied from 1 to 4 days and depended on differences in rates of growth of surface organisms when different materials were used. Slime was not evident on beef packaged with 200 gauge Saran B-517 after storage for 16 days at 4.4°C., or on meat wrapped with the foil-film laminate and held for 14 days at 4.4°C.

Analyses of the atmospheres of packages of beef wrapped with MSAT-80 cellophane or with Pliofilm FM-1 indicated that the production of

Table 5. Keeping times of packaged beef as determined by development of H_2S, off-odor, slime, production of CO_2, and rise in pH*

Packaging material	Temp. (°C.)	Keeping times (days)				
		H_2S	Off-odor	Slime	CO_2 production**	pH***
MSAT-80 cellophane	4.4	5	6.8	10.6	7	3.5
LSAT cellophane	4.4	-	5.0	8.0	-	-
Pliofilm FM-1	4.4	-	7.0	8.0	7	6.0
	7.2	5	6.0	8.0	-	3.5
Polystyrene	4.4	5	7.0	11.0	-	9.5
Q-641	7.2	5	6.0	8.0	-	3.5
Pliofilm FF-120	4.4	-	9.0	10.7	7	9.0
Saran-cello. laminate	4.4	11	12.0	16.0	-	13.3
	10.0	5	5.0	-	-	-
Saran A-517 (200 ga.)	4.4	6	11.2	12.5	-	4.5
Saran B-517 (200 ga.)	4.4	10	12.0	-	-	6.3
300 MAT cello. wax-coated	7.2	9	12.0	-	-	7.0
Alum. foil-Plio. laminate	4.4	-	12.7	-	-	-
Alu. foil	4.4	-	9.1	10.7	-	-

*Storage conditions:
Closed refrigerators
Relative humidity 70-75%

**Number of days in storage when an increase of 1% CO_2 was obtained

***Average time for increase after initial decline in pH

CO_2 at a level of about 1 per cent (0.8 to 1.0 per cent) occurred at about the same time that off-odor developed. With Pliofilm FF-120, off-odor was not detected until 2 days after the CO_2 concentration reached the 1 per cent level. Some variation is to be expected between the two indices of keeping time due to differences in the diffusion rates of CO_2 through the packaging films.

No definite relationship between keeping time and changes in pH of stored beef was established. The pH of beef was found to fall during the early phase of storage after which a rise and second decline occurred. Bate-Smith (1948) pointed out that when meat has an excess of glycogen after it attains its ultimate pH, the growth of microorganisms may not cause a rise in pH, because further breakdown of glycogen to lactic acid results as a consequence of production of base. After the glycogen reserve is exhausted, bacterial action causes an increase in pH. With these trends in mind, observations were made of the time at which a rise in pH took place after the initial decline. Since pH determinations were performed at intervals of 2 or 3 days, the actual day at which an increase occurred was not known. Values presented in Table 5 are averages representing the mid-point between the day for the lowest level of pH and day at which rise in pH was observed. In most instances, keeping time based on this method was shorter than that found when other indices were employed, although several exceptions were noted. The method of evaluating pH changes with respect to stor-

age life undoubtedly detracted from the use of pH as an index of keeping time. Also, the initial pH of fresh beef was found to vary considerably, ranging from 5.4 to 6.1. The mean pH of twenty-eight determinations was 5.8. Variation in initial pH conceivably may have resulted in differences in bacterial growth and consequent production of off-odor and slime.

As indicated by Ogilvy (1950), there are many difficulties associated with establishing an infallible index for determining when meat is spoiled. With self-service meats, off-odor and slime probably serve as adequate criteria for the consumer. In the laboratory, off-odor was usually employed to determine storage end-points for packaged meats. Surface slime generally followed off-odor by a few days.

(2) Keeping times with various materials. In preliminary experiments, it was found that the storage life of round steak varied when different packaging materials were used. Further trials were directed at comparing the effect of materials on keeping times of packaged beef; the results of these experiments are presented in Table 6. MSAT-80 cellophane was chosen as the standard for comparison, primarily because it has received widespread usage in packaging self-service meat items.

Of the materials tested, Saran films and laminated wrappers permitted the longest storage life of packaged beef kept at 4.4°C. The laminate of aluminum foil and Pliofilm was most effective in retarding spoilage; keeping time of beef packaged with the film-foil laminate was almost twice that of similar meat wrapped with MSAT-80 cellophane.

Table 6. Effect of packaging materials on keeping time of fresh beef*

Packaging material	No. of trials	Average keeping time (days)	Ratio of keeping time to keeping time with MSAT-80
MSAT-80 cellophane	10	6.8	1.00
Alum. foil-Pliofilm laminate	7	12.7	1.87
Saran-cellophane laminate	2	12.0	1.76
Saran B-517 (200 ga.)	2	12.0	1.76
Saran A-517 DW	2	12.0	1.76
Tyton Tite	1	12.0	1.76
Saran Q-684 DW	1	12.0	1.76
Saran A-517 (200 ga.)	5	11.2	1.65
Flexvac	3	10.5	1.54
Aluminum foil	4	9.1	1.34
Parafilm-M	5	9.0	1.32
Pliofilm FF-120	7	9.0	1.32
Cry-O-Rap	2	8.0	1.18
Pliofilm F-197	1	8.0	1.18
Pliofilm FM-1	4	7.0	1.03
Polystyrene Q-641	4	7.0	1.03
LSAT cellophane	2	5.0	0.74

*Storage conditions:
Temperature 4.4°C.
Relative humidity 70 to 75%
Keeping time determined by onset of off-odor

Keeping times of pork generally were similar to those of beef wrapped with the same type of material and stored at 4.4°C. Off-odor was detected on pork loin wrapped with aluminum foil, Pliofilm FF-120, and MSAT-80 cellophane after 9 days. With the laminate of aluminum foil and Pliofilm, spoilage was not apparent until the meat had been stored for 14 days.

(3) Growth curves. Figures 2, 3, and h show growth curves of bacteria on the surface of fresh beef packaged with several materials and stored at h.h°C. and at 10°C. In Figures 5 and 6, bacterial growth curves are given for fresh packaged pork loin held at h.h°C.

Examination of the linear portion of growth curves in Figure 2 indicates that the rate of reproduction of surface organisms varied with the type of packaging material employed. Of the four films, the most rapid growth was obtained with polystyrene Q-6h1 while the slope of the linear part of the curve for the foil-film laminate illustrates that the laminate retarded growth of surface organisms to a greater extent than did other materials. Also, the numbers of bacteria did not reach as high a level during the storage period as was noted when other materials were used. During the lag period, a decrease in bacterial count was observed when the foil-film laminate served as the wrapper, a feature in common with counts obtained from beef packaged with Pliofilm F-197 (Figure 3).

The curve for Pliofilm FM-1 (Figure 3) rather closely approximated growth curves for bacteria on beef wrapped with MSAT-80 cellophane or with polystyrene Q-6h1, indicating that the three materials were about equal in allowing proliferation of surface organisms. Table 6 shows that keeping times of meat packaged with these films were also similar. The trend of bacterial growth on beef packaged with Parafilm-M (Figure 2) was much like that for samples held in Pliofilm FF-120 (Figure 3).

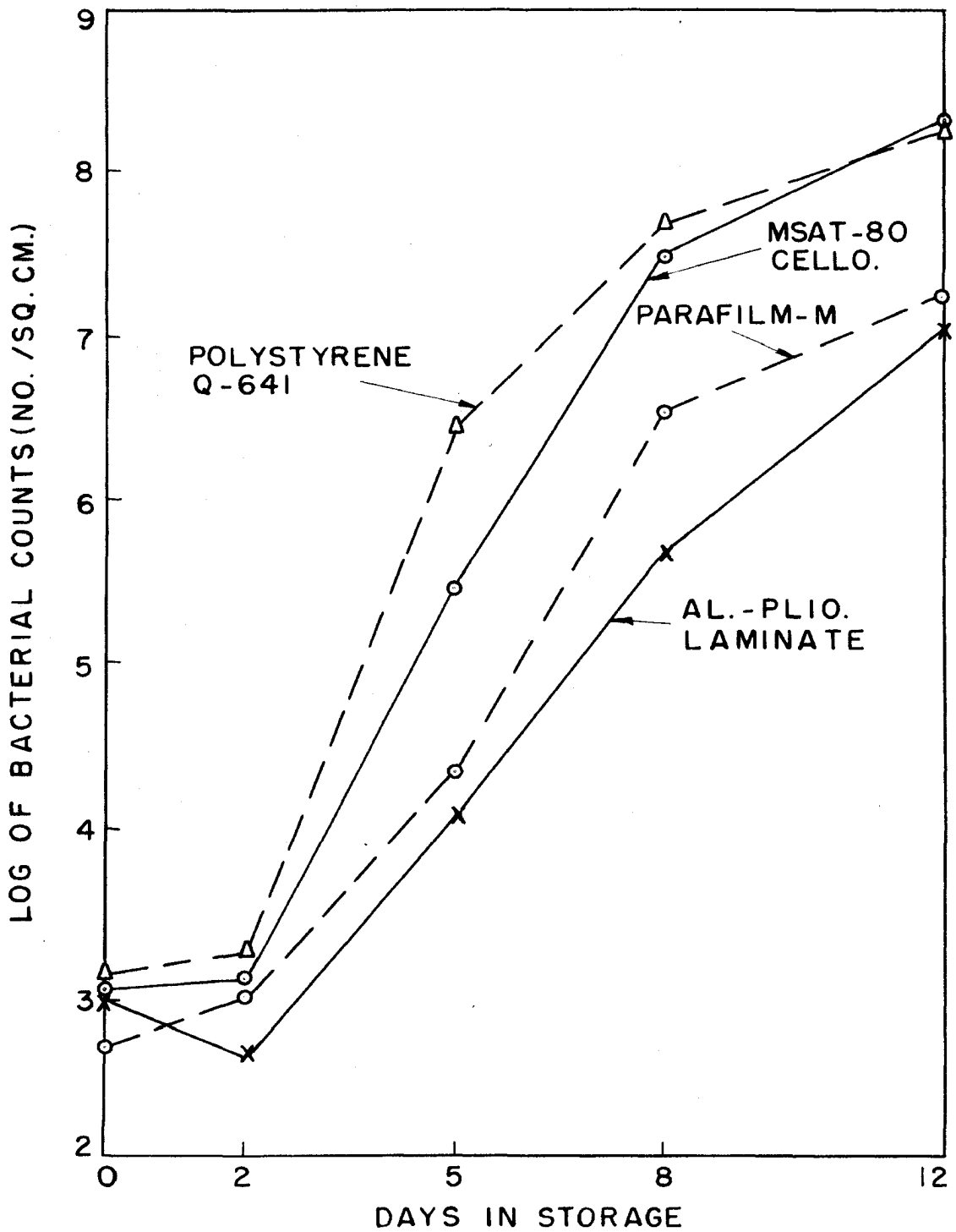


Figure 2. Effect of polystyrene Q-641, MSAT-80 cellophane, Parafilm-M, and aluminum foil laminated to Pliofilm on bacterial growth on fresh beef stored at 4.4°C.

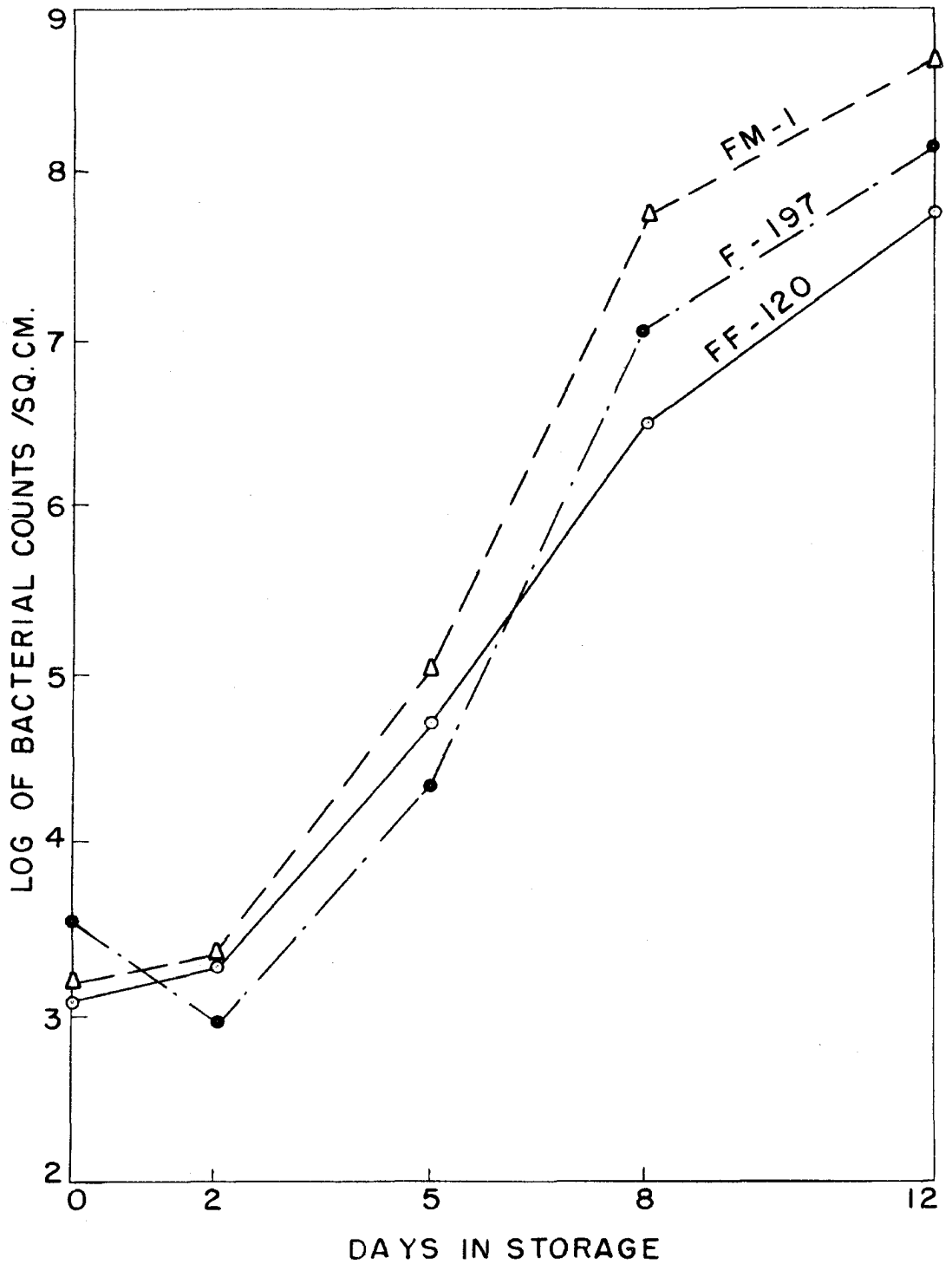


Figure 3. Effect of different types of Pliofilm on bacterial growth on fresh beef stored at 4.4°C.

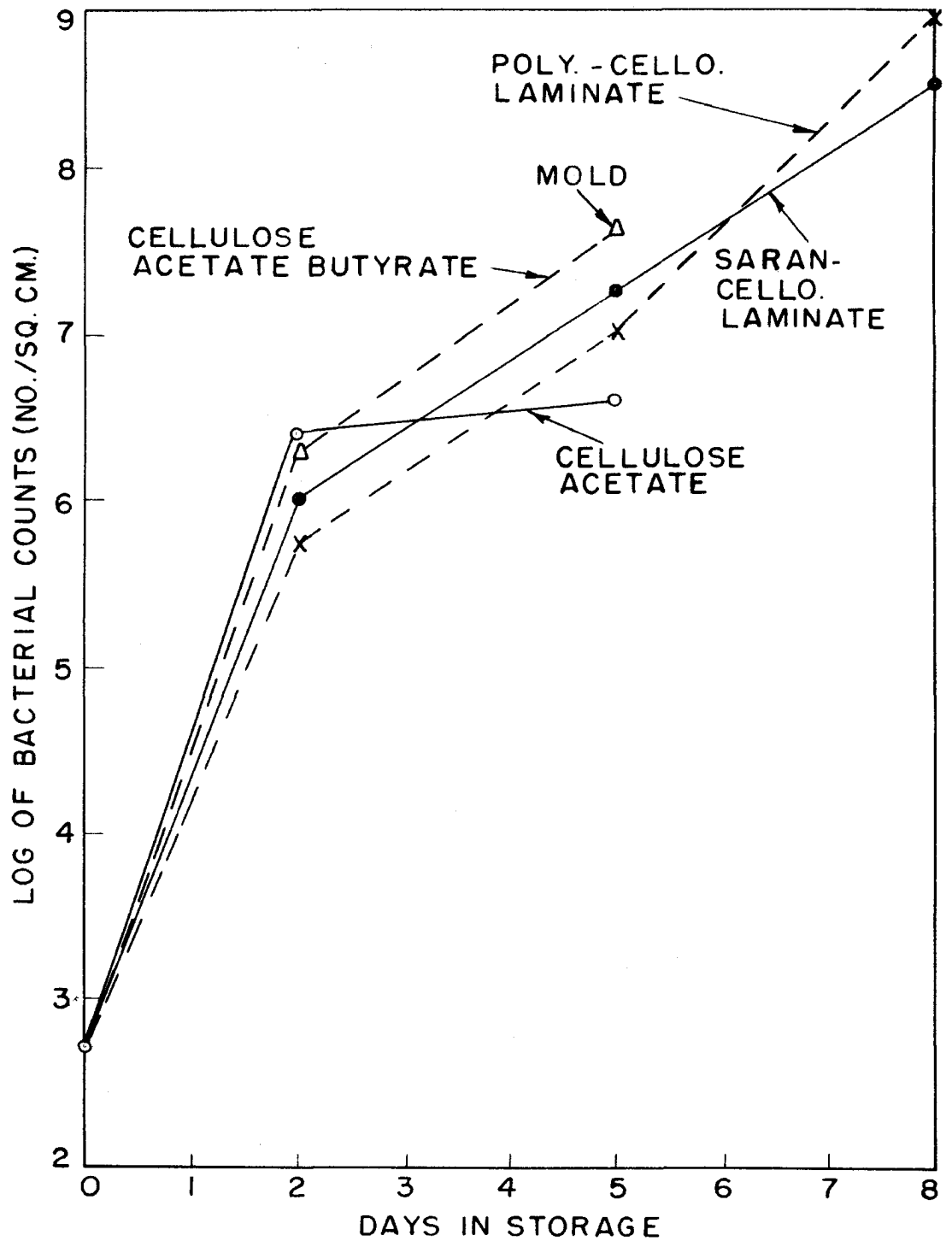


Figure 4. Effect of laminated films and cellulose ester films* on bacterial growth on fresh beef stored at 10°C.

*Limit of storage 8 days for beef wrapped with cellulose ester films

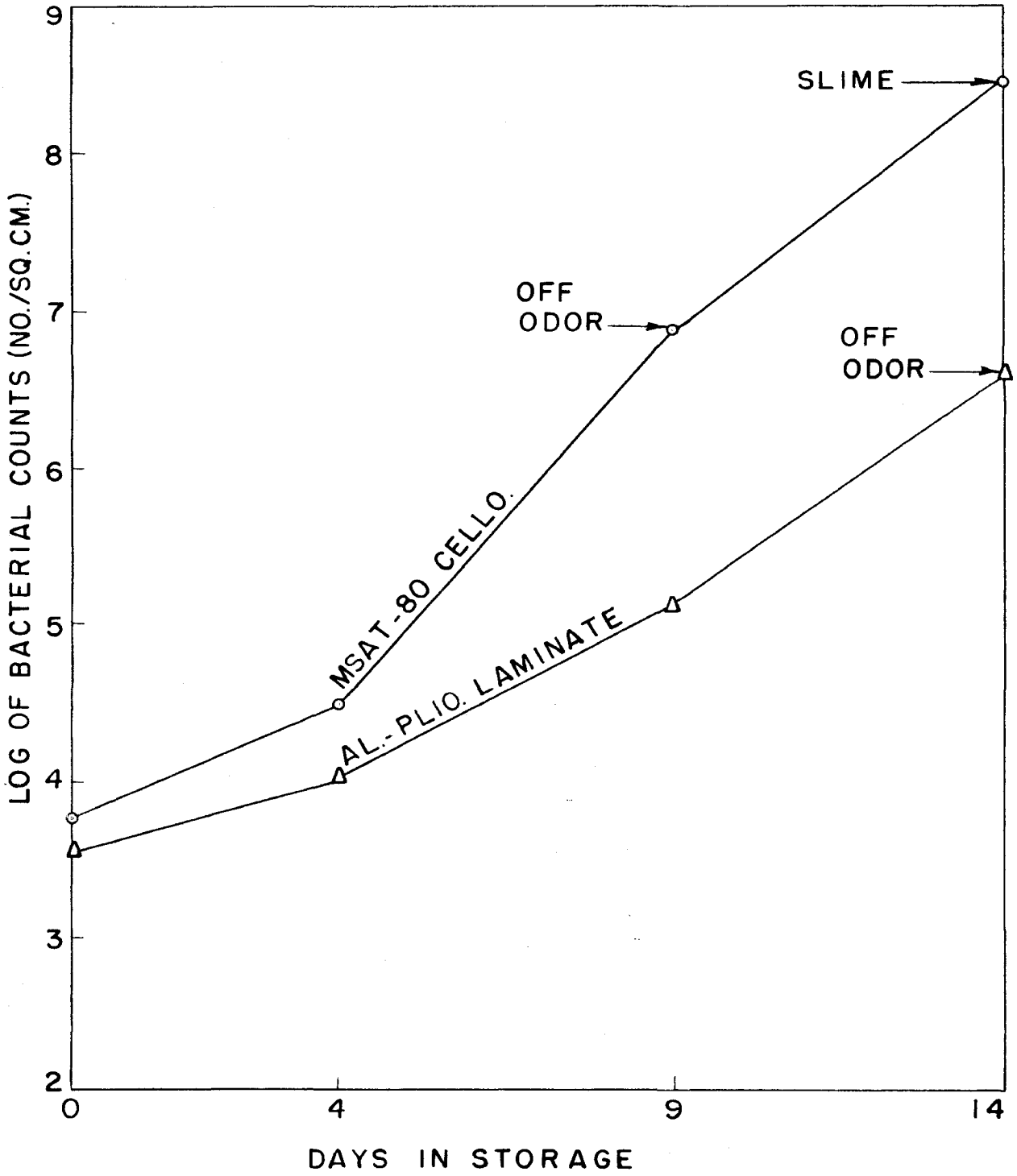


Figure 5. Effect of MSAT-80 cellophane and aluminum foil laminated to Pliofilm on bacterial growth on pork loin stored at 4.4°C.

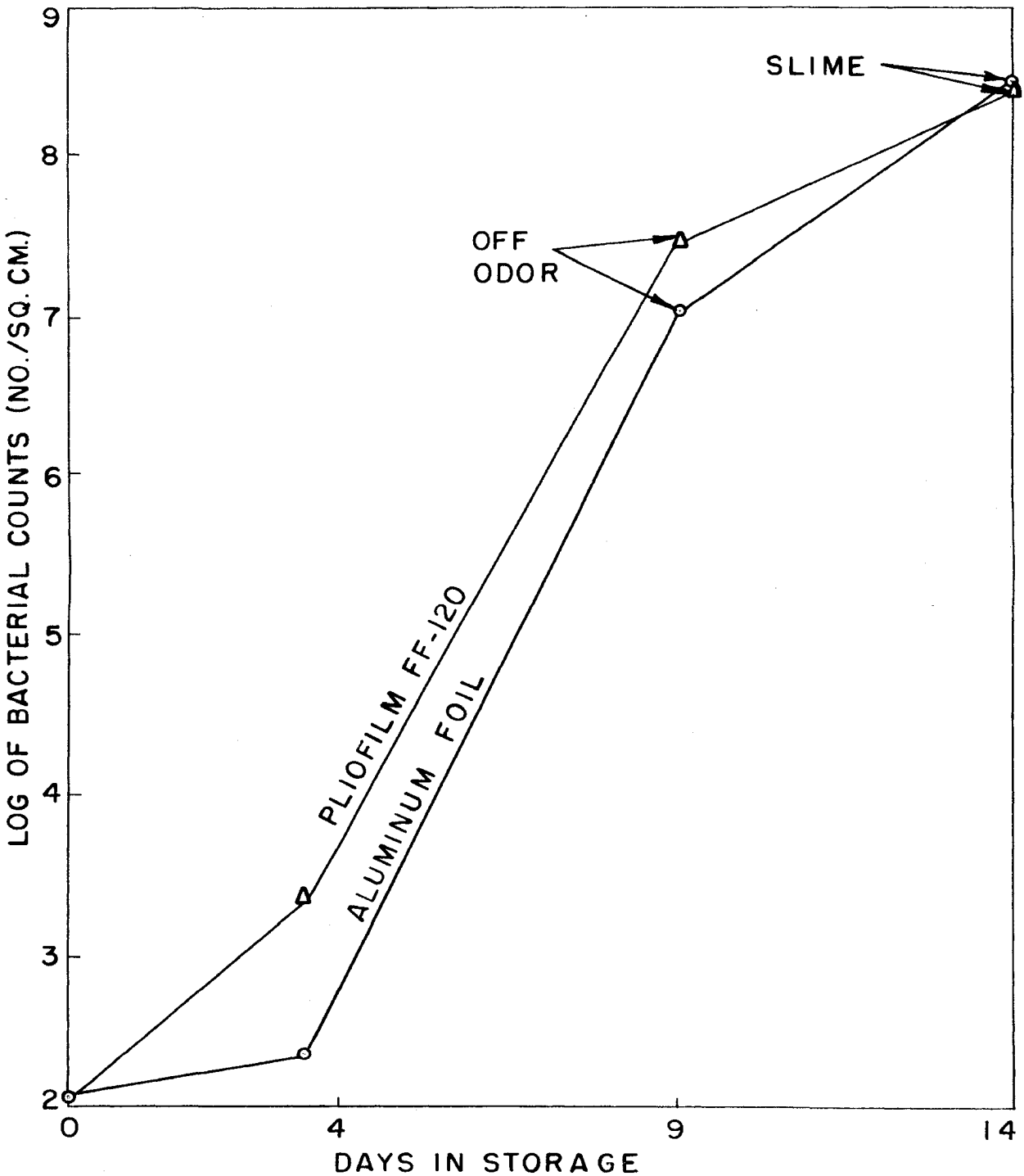


Figure 6. Effect of Pliofilm FF-120 and aluminum foil on bacterial growth on pork loin stored at 4.4°C.

Curves presented in Figure 4 demonstrate that the characteristic lag period was not observed for organisms growing on the surface of packaged beef stored at 10°C. All materials permitted rapid reproduction of surface bacteria. At the end of 5 days at 10°C., mold growth was observed on beef packaged with cellulose acetate butyrate. Although bacterial counts were high at the time mold appeared, it seems likely that due to the low moisture content of the meat, mold growth would be favored on further storage while development of bacteria would be retarded.

The reason for differences in keeping time of beef packaged with various materials becomes apparent when consideration is given to bacterial growth curves. Findings presented here are in agreement with reports given in the Review of Literature that off-odor and slime formation are manifestations of development of surface organisms.

Counts obtained from pork loin chops generally appeared to follow trends similar to those for beef packaged with the same type of material (Figures 5 and 6). Although initial bacterial loads were higher on pork packaged with MSAT-80 cellophane than was the case with other packaging materials, during the later phases of storage, pork wrapped with the cellophane had counts approximating those obtained when Pliofilm FF-120 and aluminum foil were employed. In comparison with other packaging films, then, MSAT-80 cellophane allowed less rapid proliferation of surface bacteria on pork than it did on beef. In previous experiments wherein initial levels of bacteria were the same, growth

curves secured from pork packaged with the three materials were similar. Each point on the curve given for MSAT-80 cellophane in Figure 5 represents the mean of counts made from three chops. The chops were originally cut from a single large section of loin. This trial was conducted as an additional check on the performance of MSAT-80 cellophane.

Similar to the results obtained with beef, the laminate of aluminum foil and Pliofilm was most effective in retarding growth of surface organisms on pork.

Growth curves of lipolytic and anaerobic bacteria found on the surface of fresh meats packaged with several materials are presented in Figures 7, 8, and 9. Counts of lipolytic bacteria as a rule followed trends similar to those of total counts. That is, those materials which permitted rapid growth of total surface organisms to high levels also allowed the greatest development of lipolytic bacteria. This was not the case with organisms growing anaerobically. Little difference was found in counts of anaerobic bacteria on beef packaged with MSAT-80 cellophane, Pliofilm FM-1, or the aluminum foil-Pliofilm laminate. Indeed, with fresh pork loin, the foil-film laminate allowed anaerobic organisms to reach higher levels than were found when the cellophane wrapper was used (Figure 9). In all cases, the numbers of anaerobic bacteria were low compared with total and lipolytic counts and it is doubtful whether the former were important as causative agents of surface spoilage of packaged meats.

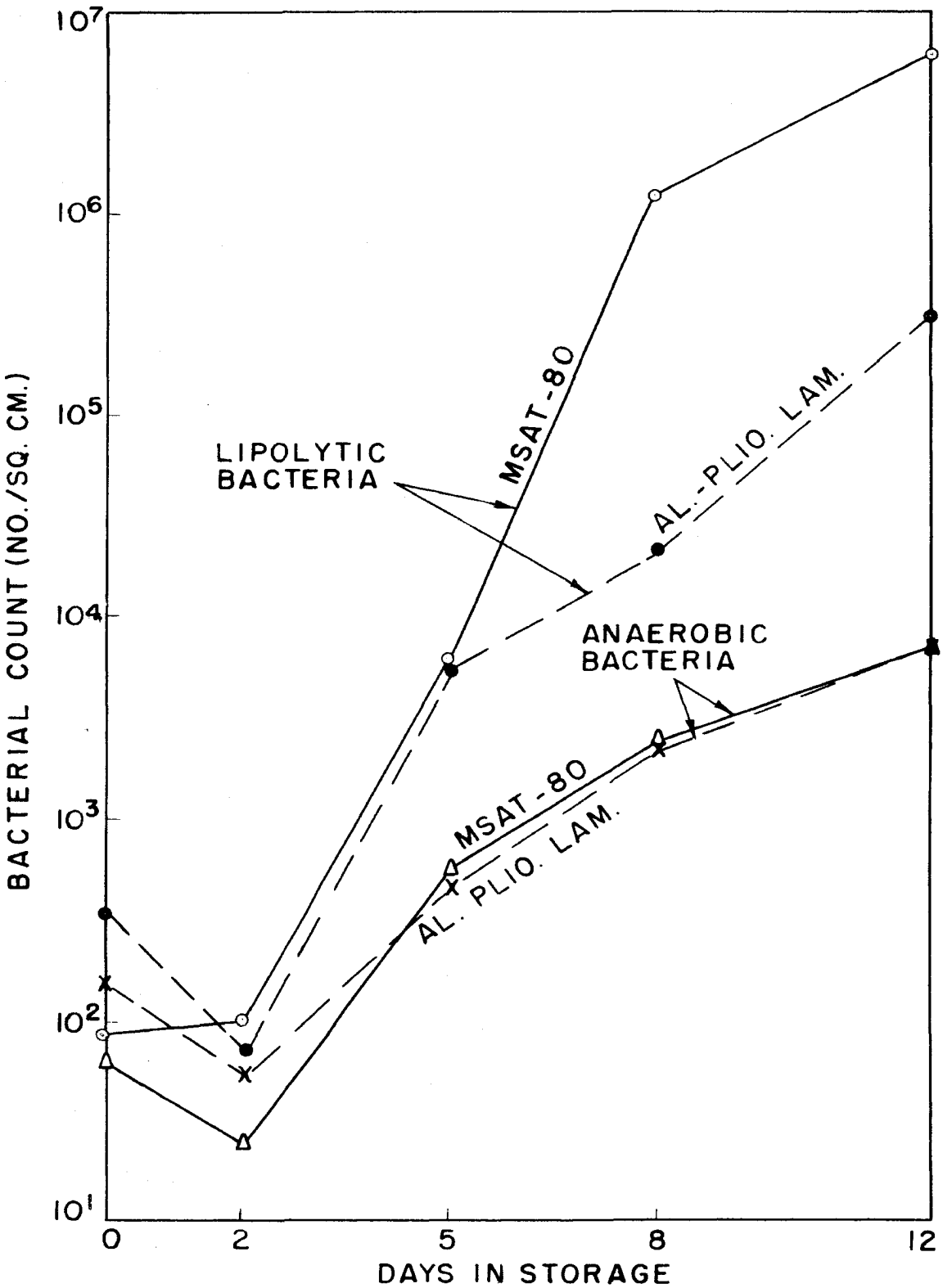


Figure 7. Effect of MSAT-80 cellophane and aluminum foil laminated to Pliofilm on growth of lipolytic and anaerobic bacteria on beef stored at 4.4°C.

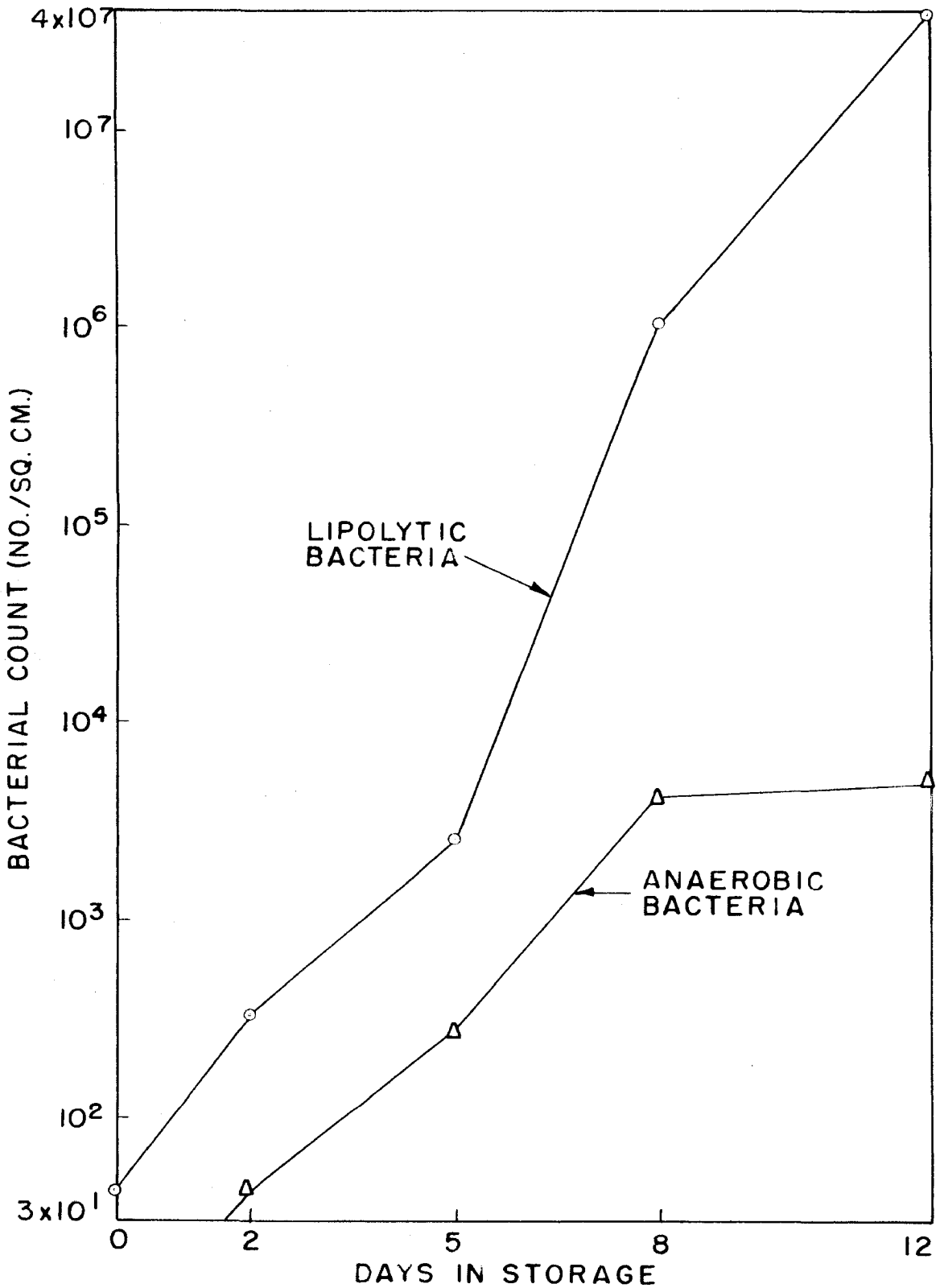


Figure 8. Effect of Pliofilm FM-1 on growth of lipolytic and anaerobic bacteria on beef stored at 4.4°C.

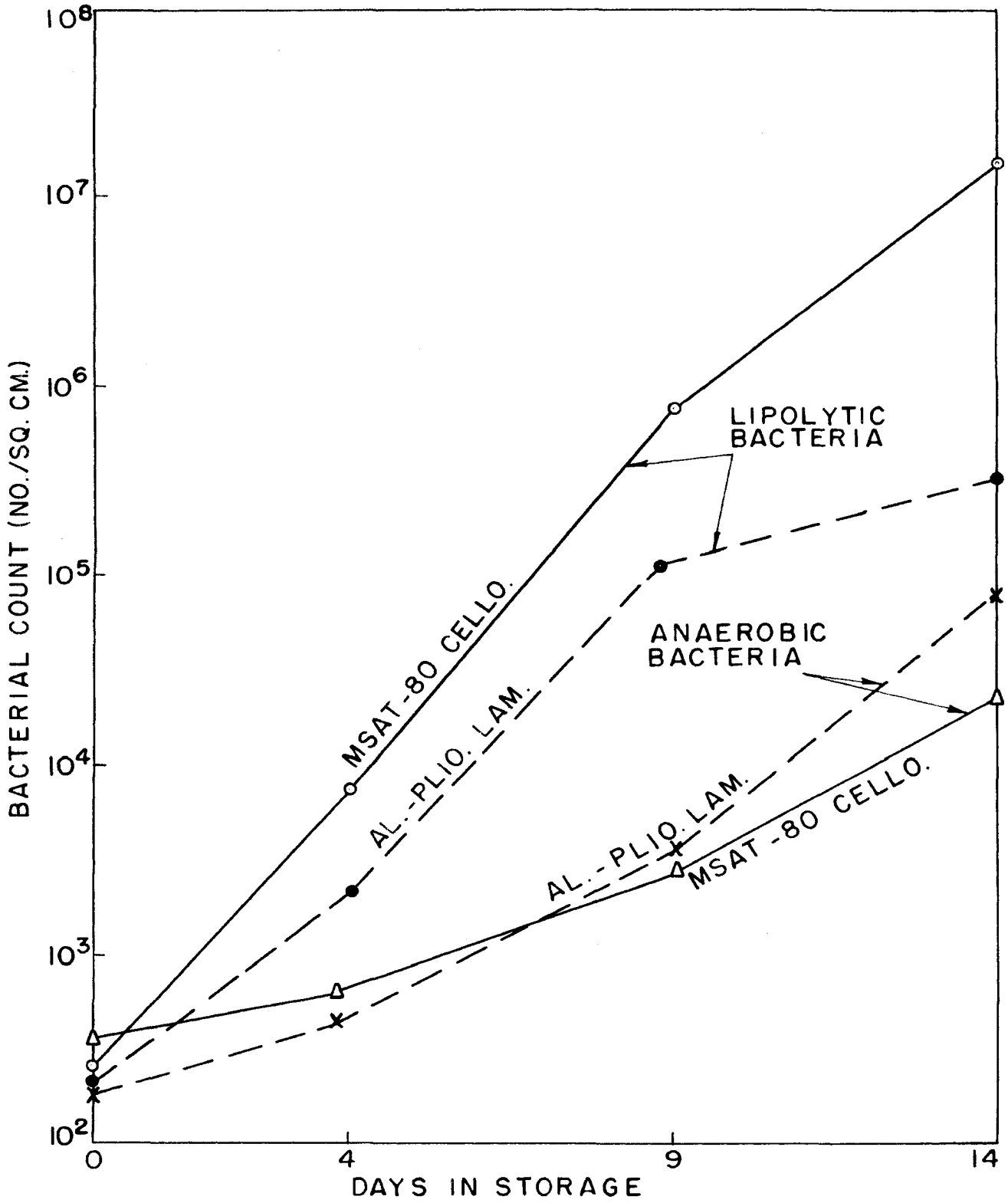


Figure 9. Effect of MSAT-80 cellophane and aluminum foil laminated to Pliofilm on growth of lipolytic and anaerobic bacteria on pork stored at 4.4°C.

The finding that numbers of lipolytic bacteria generally increased as total counts became greater suggested that lipolytic organisms increased in numbers proportionate to those of the total flora. For beef, lipolytic counts ranged from 1.5 to 7.5 per cent of total counts; the range for pork was about 4.5 to 13.5 per cent. During storage, quite large variations were observed in the per cent of lipolytic bacteria; the most frequently occurring values varied from 10 to 20 per cent of the total numbers. The per cent of lipolytic organisms was found to increase during the first week of storage, after which values tended to be erratic with no marked trends discernible. In general, however, the per cent of lipolytic organisms was lower after storage for 12 or 14 days than it was at the end of 2, 4, 5, 8, or 9 days. With pure cultures of lipolytic species of Pseudomonas, Goldman and Rayman (1952) found that the number of viable cells declined as titratable acidity increased and pH of the medium became lower. While numbers of lipolytic bacteria did not show a decrease during storage of beef or pork in the present work, the possibility existed that lowering of pH during the later phases of holding may have restricted complete development of these types.

(4) Effect of initial contamination. In order to effectively evaluate the influence of various films and foils on keeping quality of meats, consideration must be given to the extent of contamination of the product at the time it is packaged. Several investigators have reported that the storage life of meats held in the unpackaged state is markedly

affected by bacterial loads originally present on the surface; it was found here that the same condition prevailed with packaged meat items. Initial counts from fresh beef generally varied between 10^2 and 10^4 bacteria per square centimeter of meat surface; for pork somewhat lower levels were noted. Figure 10 gives keeping times plotted against logarithms of initial bacterial counts for fresh meats packaged with MSAT-80 cellophane and for unpackaged round steak stored at 50 per cent relative humidity. When the initial contamination was reduced, storage life was prolonged. Ogilvy and Ayres (1951a) concluded that a linear relationship existed between keeping time of chicken and initial loads of bacteria; this relationship was essentially substantiated for packaged beef and pork. Curves for the two types of meat packaged with the same material differ slightly in slope, but the variation in keeping time was only about 1 day with high or low initial counts.

Using the same methods as were employed for determining numbers of surface organisms on fresh meats, counts were made of the bacteria present on packaging films prior to wrapping the meat. These determinations were conducted at a number of intervals during the course of work with meats. It was found that the materials contributed very little to total bacterial loads; in most instances, bacterial counts were less than 10^1 per square centimeter of film surface.

(5) Influence of temperature. The effect of temperature on keeping time of fresh beef packaged with several films is illustrated in Figure 11. Reduction in temperature resulted in prolongation of

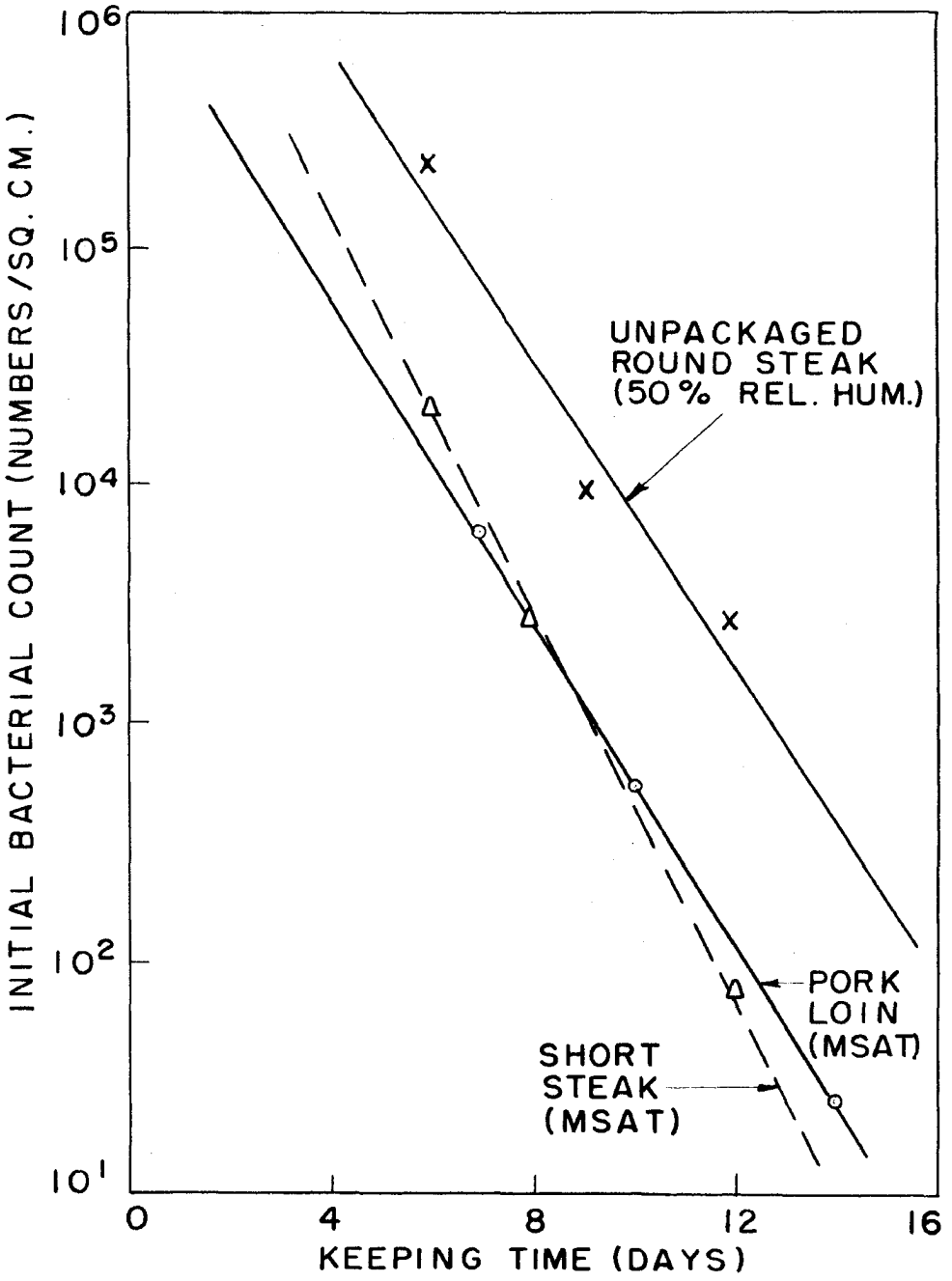
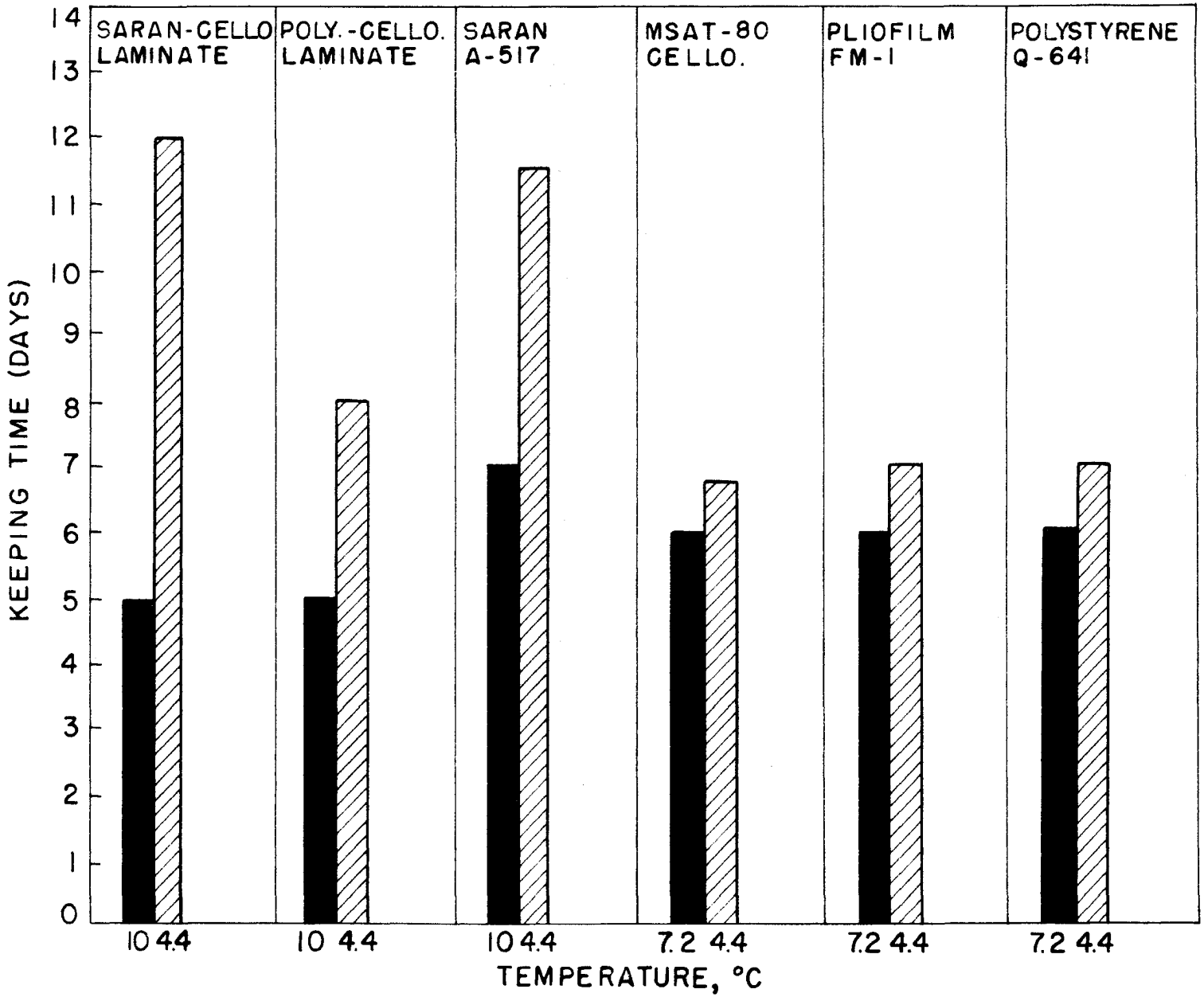


Figure 10. Influence of initial contamination on keeping time of beef and pork

Figure 11. Relation of packaging materials and temperature to keeping time of fresh beef


SARAN-CELLO LAMINATE
POLY.-CELLO. LAMINATE
SARAN A-517
MSAT-80 CELLO.
PLIOFILM FM-1
POLYSTYRENE Q-641
KEEPING TIME (DAYS)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
10 4.4
10 4.4
10 4.4
7.2 4.4
7.2 4.4
7.2 4.4
TEMPERATURE, °C

storage life. The amount of increase in keeping time varied with packaging materials employed; however, keeping time for beef packaged with MSAT-80 cellophane, polystyrene Q-641, and Pliofilm FM-1 was extended about equally when the storage temperature was lowered from 7.2°C. to 4.4°C. This suggests that the storage life of beef packaged with any of the three materials may be predicted for a given holding temperature provided that the keeping time at that temperature is known for beef wrapped with one of the packaging films.

As may be expected, a lowering of temperature from 10°C. to 4.4°C. resulted in even greater extension of storage life than was observed when the temperature was reduced from 7.2°C. to 4.4°C.

(6) Effect of film thickness. A property of packaging materials that appeared to exert an influence on keeping time of fresh beef was the thickness of films. For a given type of material, storage life of beef was greater with heavier gauge films. Growth curves for bacteria from meat wrapped with Saran films of 100 and 200 gauge thicknesses are shown in Figures 12 and 13. In general, development of surface organisms tended to be slower with the 200 gauge films in comparison with bacterial growth when 100 gauge materials were used. Although not shown in the graphs, counts obtained from meat wrapped with Saran A-517 were also lower with the 200 gauge film than they were with the 100 gauge material.

d. Discussion. As is the case with meats held in the unpackaged

state, storage life of packaged meats is conditioned by such factors as temperature of storage and bacteriological quality of the meat at the beginning of the holding period.

However, from consideration of the preceding findings, it is apparent that color changes, desiccation, and keeping time of packaged fresh meats depend to a great extent on the properties of the wrapping materials used. While a packaging material may be effective in retarding discoloration, it may not necessarily possess all of the properties requisite for preservation of meat. Weight losses of packaged meat indicated that the humidity of atmospheres within the packages varied with the materials employed. Those wraps that failed to prevent dehydration permitted lowered humidity conditions in the air space surrounding the meat. Desiccation did not proceed to the extent that levels of humidity inhibitory to bacterial growth were obtained until the meat was considered to be unacceptable by reason of discoloration and excessive dehydration.

Proliferation of surface microorganisms generally was most rapid with films that were ineffective as moisture barriers. These materials promoted aerobic conditions favorable for the growth of surface bacteria. Improvement of storage life resulting from packaging with relatively gas-impermeable films appeared to be due to the low rate of exchange of gases between the atmosphere of packages and the external atmosphere. Consequently, surface bacterial growth and moisture loss of meats

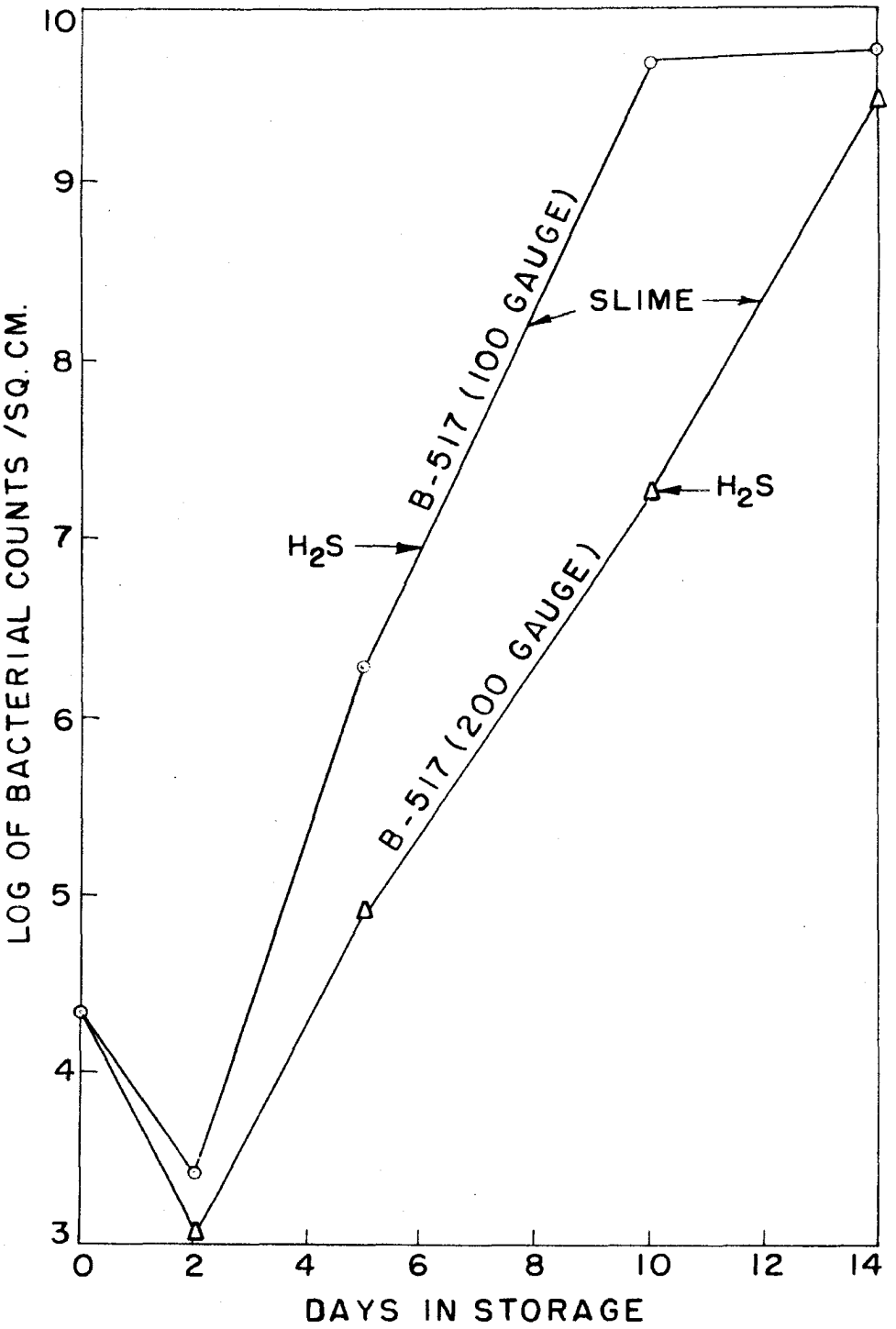


Figure 12. Effect of film thickness on bacterial growth on beef packaged with Saran B-517 and stored at 4.4°C.

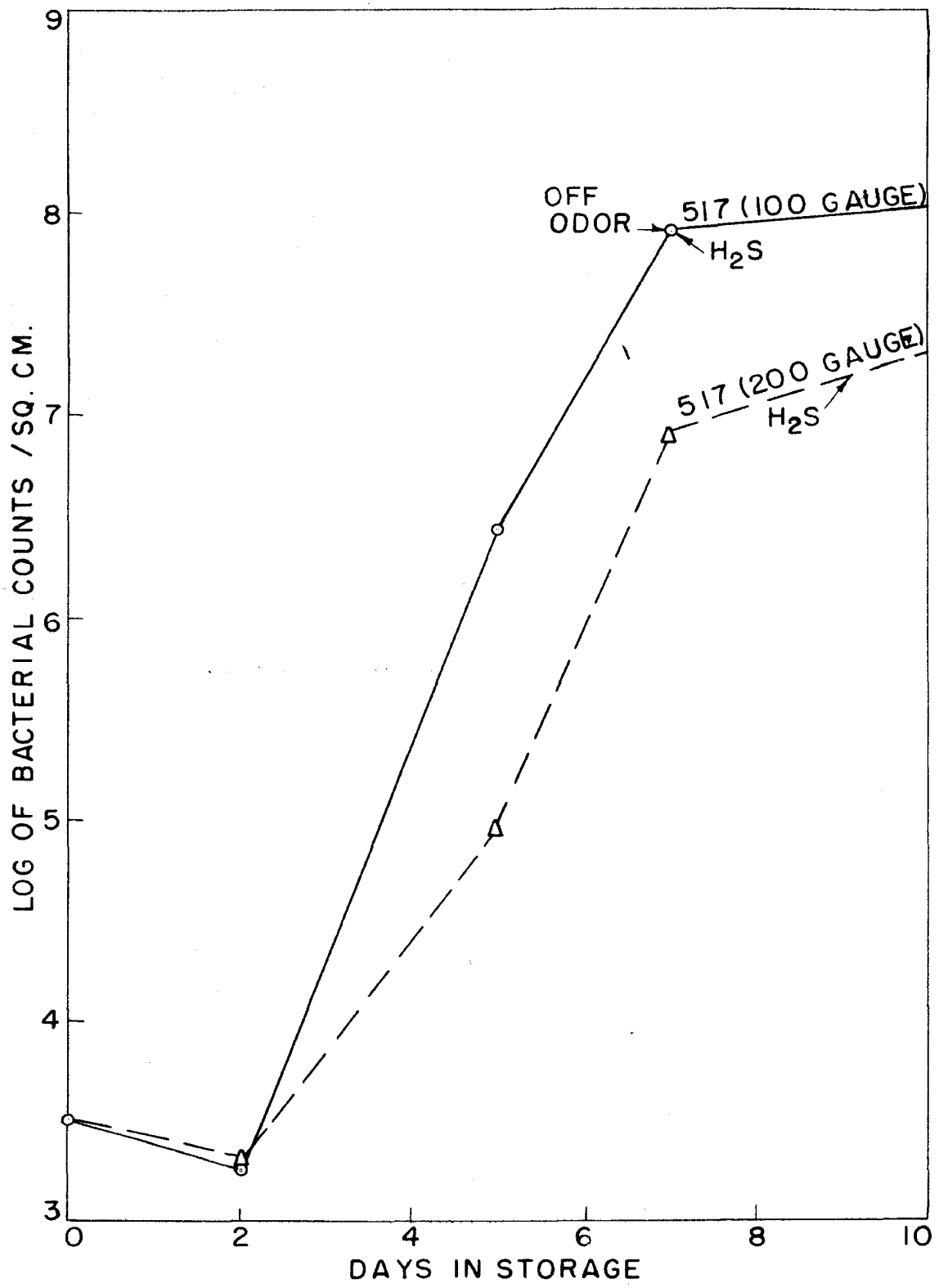


Figure 13. Effect of film thickness on bacterial growth on beef packaged with Saran 517 and stored at 10°C.

packaged with these materials proceeded at a markedly slower rate than that observed when fresh meat was wrapped with the more permeable cellulose derivatives, polystyrene, and Pliofilm. Further evidence for the relation between gas transfer and keeping time was gained by observations made with similar type films of different thicknesses; in all instances storage life was prolonged by the use of heavier gauge materials.

Reduction of myoglobin to the purple pigment was associated with the use of materials that permitted extended keeping time. While the purple color is not wholly desirable in displaying fresh meats, it is possible that the bright red color of the oxygenated pigment could be regained by opening the packages and displaying the meat unwrapped. When opaque materials are used, in order to permit visibility of package contents another alternative might be the use of a double wrap, the inner transparent material remaining after the outer opaque material is removed. The additional expenditure of time and materials by the use of these procedures might be worthwhile in view of the prolongation of storage life obtained. Such packaging procedures may be necessary with centralized packaging and later distribution to retail markets.

2. Effect of CO_2 on beef

a. Absorption of CO_2 by meat. Since the application of CO_2 to preservation of fresh meats is limited to concentrations no greater than 20 to 30 per cent because of the resultant discoloration when

higher levels are used, the question arises as to how effective these low concentrations may be in prolonging storage life of packaged meats. While 20 to 30 per cent CO_2 has been shown to exert a preservative influence on meats held in rigid gas-impermeable containers, extension of keeping time may not necessarily be as pronounced when meats are packaged with flexible gas-permeable materials. With packaged meats, free gas space is apt to be at a minimum, since the meat occupies a major part of the package volume. In this connection, solution of CO_2 in the meat with subsequent diffusion into the atmosphere of the package may play a role in increasing the effectiveness of the gas.

In Table 7, average values are given for CO_2 uptake of fresh beef stored in jars containing approximately 25 per cent CO_2. Individual figures are presented in Appendix Table 32.

Table 7. Absorption of CO_2 by fresh beef stored at 4.4°C.

Average initial CO_2 conc. (%)	Days in storage	Average weight of meat to volume of CO_2 (g./ml.)	Average CO_2 uptake* (ml./g.)
25.5	1	7.54	0.092
	2	8.39	0.069
	3	8.86	0.067
	4	8.89	0.058

*Calculated for 25°C.

As indicated in Table 7, the volume of gas taken up by the meat in an atmosphere containing 25 per cent CO_2 was considerably less than the solubility value of 1.56 for CO_2 in water at 4.4°C. (Lange, 1949, p.1265; corrected to 740 mm. pressure and 25°C.). According to Brooks and Moran (1934), a 150-lb. quarter of beef in equilibrium with 100 per cent CO_2 at 0°C. would absorb 70 liters of the gas. Calculated for 25°C., this figure would be 1.12 ml. CO_2 per gram of meat. Further, Brooks and Moran contended that such a quarter would provide a concentration of 25 to 30 per cent CO_2 in a gas-tight chamber having 105 cubic feet of total space per ton of meat. In other words, the ratio of meat to gas would have to be roughly 0.43 grams per ml. in order to attain a concentration of 25 per cent CO_2 in an atmosphere initially composed of air. As given by Brooks and Moran, the level of 25 per cent CO_2 would be realized only after the meat was allowed to come to equilibrium in an atmosphere originally composed completely of CO_2.

While the data presented in this study were not sufficiently extensive to warrant making general conclusions, indications are that with the relatively high ratios of meat to gas used, the amount of CO_2 in the atmosphere of packages would fall far short of the desired concentration of 25 per cent.

b. Discoloration. Beef stored in 25 per cent CO_2 at 4.4°C. generally remained red in color for 6 days, although progressive darkening of color was noted. Darkening may have been caused partly by desiccation. At the end of 6 days, the edges of samples appeared dark

red and the central portion of the meat was light brown. In later experiments, meat held in 25 per cent CO_2 for 2 days and later packaged in an atmosphere approaching 95 per cent CO_2 demonstrated brown discoloration within 2 days after packaging.

c. Storage life. (1) Keeping times and bacterial counts. The method adopted by Ogilvy and Ayres (1951a) of computing the ratio of storage life in CO_2 to that in air, and termed the "storage index", was employed in the present study in order to evaluate the effect of CO_2 on keeping time of packaged beef. Keeping times of fresh beef packaged with several materials in atmospheres of CO_2 and air are given in Table 8. The ratio of meat to gas ranged from 0.1 to 0.2 grams of meat per ml. of CO_2. Materials employed in these experiments were characterized as having high, low, and intermediate permeabilities to CO_2.

Table 8. Effect of packaging materials and CO_2 on storage life* of fresh beef at 4.4°C.

Packaging material	No. of trials	Average keeping time in air (days)	Average keeping time in CO_2 (days)	Average storage index
MSAT-80 cellophane	4	6.5	10.5	1.62
Pliofilm FM-1	3	6.8	10.0	1.47
Pliofilm FF-120	3	9.0	11.2	1.24
Alum. foil-Pliofilm laminate	2	12.3	15.0	1.22

*Limit of storage determined by onset of off-odor

The use of CO_2 resulted in an increase in storage life with all wrappers tested. In general, those materials that permitted greatest keeping time in air also allowed longest storage life in CO_2. Examination of the values for storage index with each material reveals that the relative effectiveness of CO_2 decreased as keeping time in air increased.

Bacterial growth curves constructed from counts obtained from beef packaged with materials listed in Table 8 are presented in Figures 14 through 17. In order to determine growth rates, counts were plotted in accordance with the method of least squares. With all materials, CO_2 tended to cause a greater decrease in numbers of surface organisms during the lag period than that observed when no CO_2 was added. Also, the slope of the linear portion of growth curves generally was decreased by addition of CO_2 to the atmosphere of packages, indicating that the rate of reproduction of bacteria was lower than that in an atmosphere of air. This effect was more marked with MSAT-80 cellophane and with Pliofilm FM-1 than it was with Pliofilm FF-120 and the laminate of aluminum foil and Pliofilm.

Table 9 gives generation times for bacteria growing on fresh beef in CO_2 and in the air. Counts used to calculate growth rates and the method of computation are given in Table 33 (Appendix). Generation times were longer with CO_2 than with air. Since keeping time depends to a considerable extent on the rate of reproduction of surface organisms it is not surprising to note that the increase in generation time

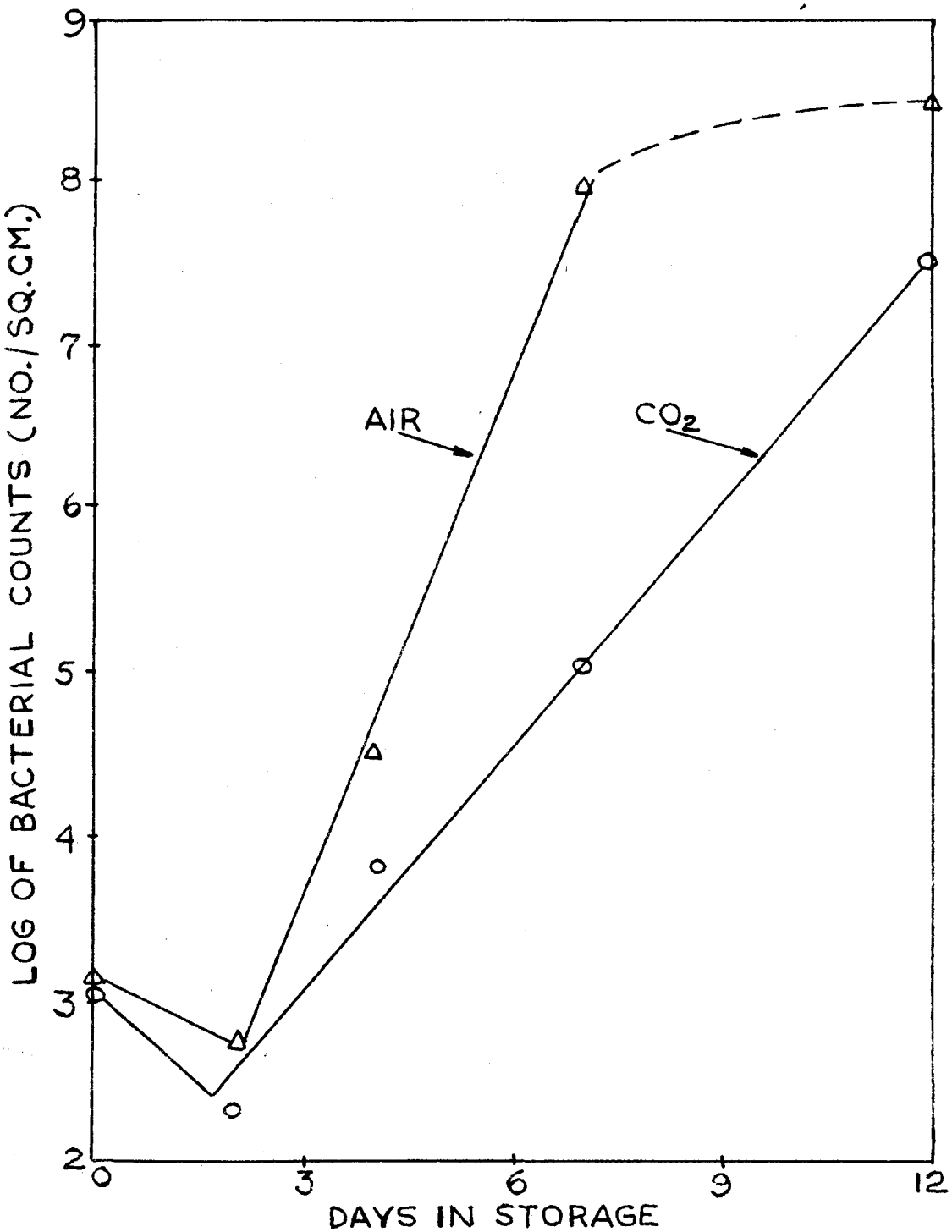


Figure 14. Growth curves of bacteria on beef packaged with MSAT-80 cellophane in CO_2 and air.

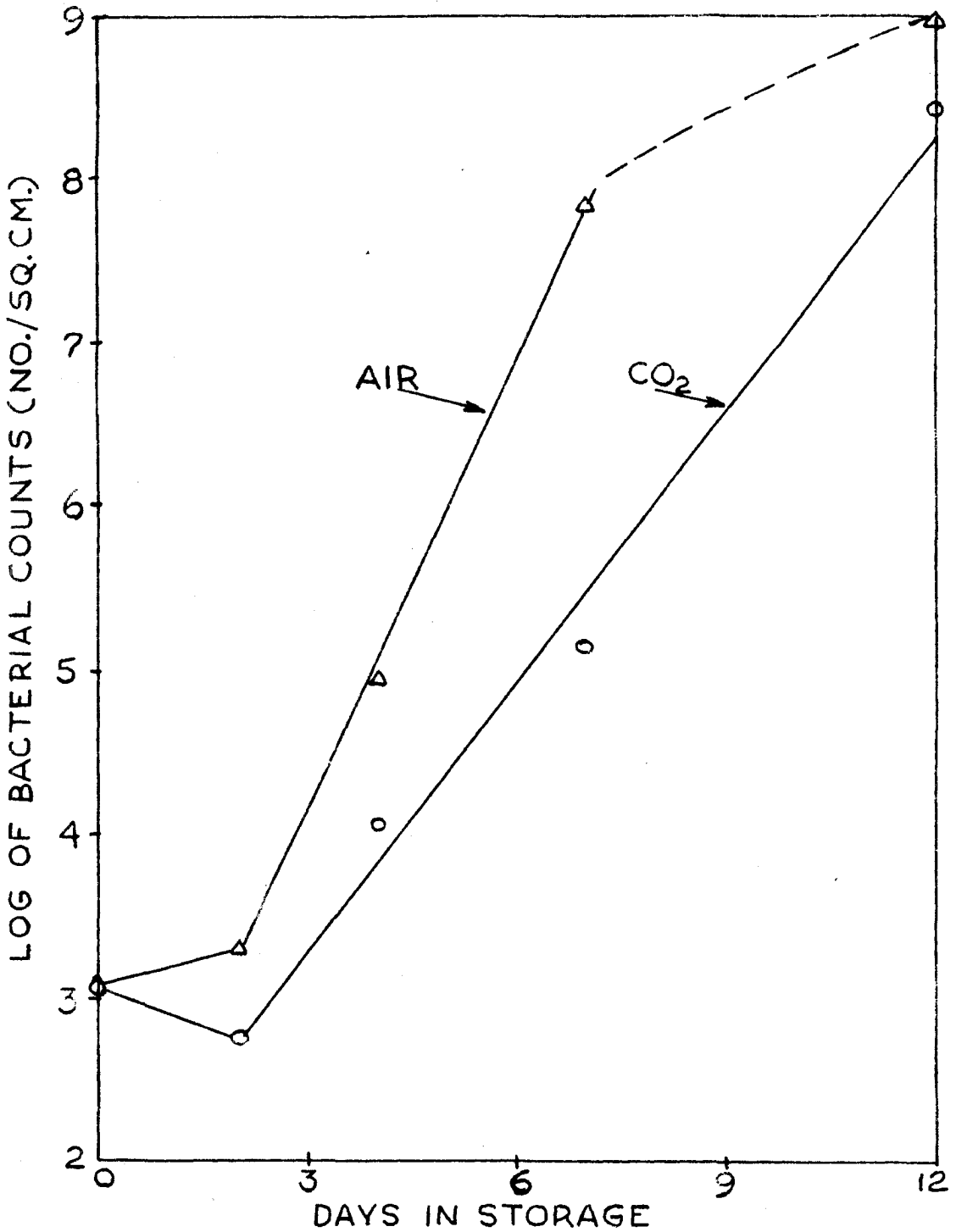


Figure 15. Growth curves of bacteria on beef packaged with Pliofilm FM-1 in CO_2 and air.

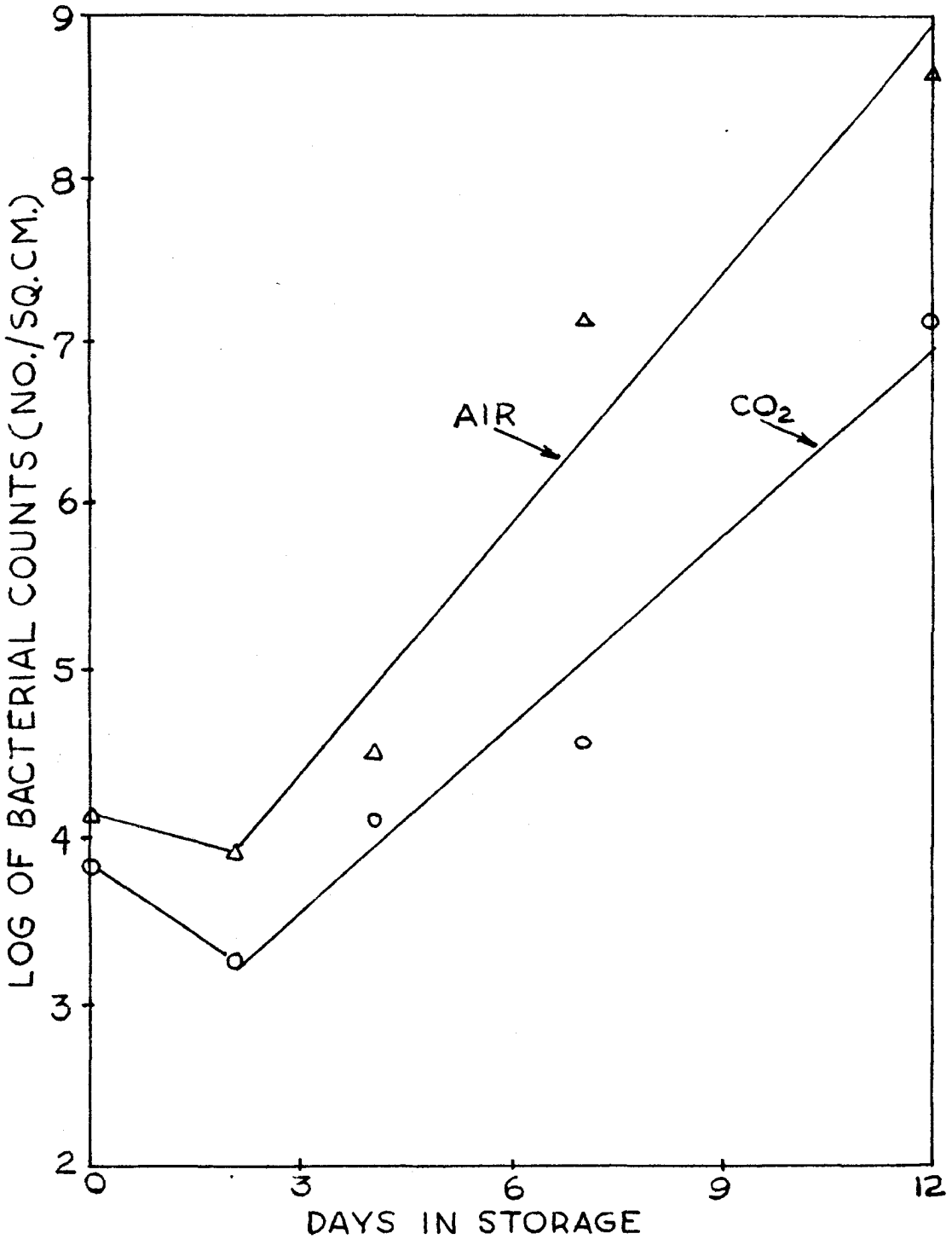


Figure 16. Growth curves of bacteria on beef packaged with Pliofilm FF-120 in CO_2 and air.

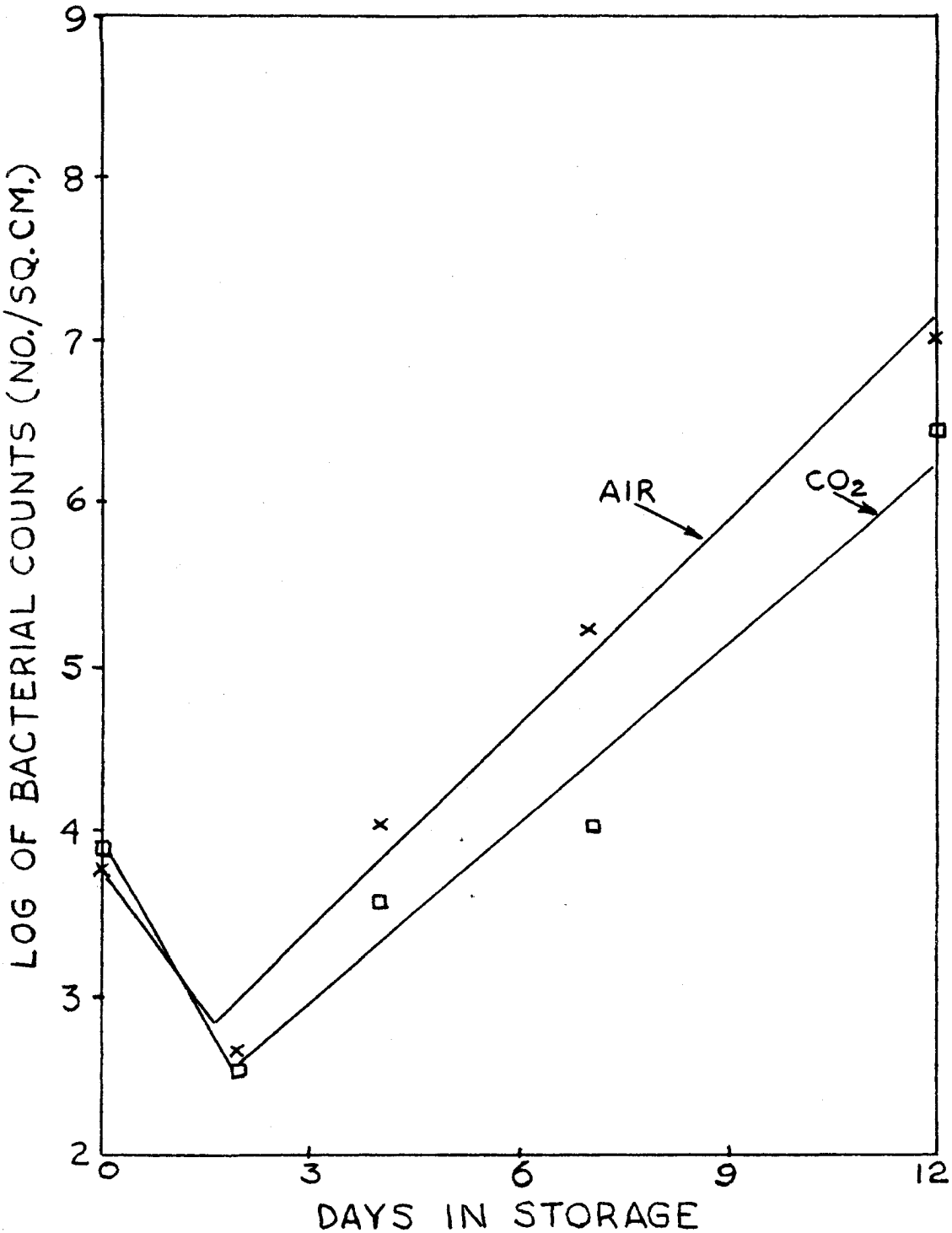


Figure 17. Growth curves of bacteria on beef packaged with aluminum foil laminated to Pliofilm; meat packaged in CO_2 and air

by the use of CO_2 followed the same order for packaging materials as did improvement in storage life.

Table 9. Generation times of bacteria on fresh beef packaged in CO_2 and in air and stored at 4.4°C.

Packaging material	Atmosphere	Average generation time (hours)	Ratio of generation time in CO_2 to generation time in air
MSAT-80 cellophane	Air	6.84	2.10
	CO_2	14.34	
Pliofilm FM-1	Air	7.96	2.08
	CO_2	16.52	
Pliofilm FF-120	Air	14.55	1.33
	CO_2	19.28	
Alum. foil-Pliofilm laminate	Air	16.89	1.15
	CO_2	19.49	

Forty colonies of predominant types were picked from plates prepared from round steak which had reached the slime stage. All colonies were circular, entire, glistening, convex, white, and opaque. Organisms were determined to be Gram-negative non-sporulating short rods; thirty-one of the forty exhibited polar flagella. No further studies were undertaken to identify these bacteria. The sketchy characterization presented here might at best be considered presumptive evidence only for placing the majority of the organisms in the genus Pseudomonas (Bergey's Manual of Determinative Bacteriology, 6th ed., 1948). Additional tests would be required before the identity of these bacteria could be established.

(2) Changes in gas concentration. During storage, residual concentrations of CO_2 in packages varied appreciably when different films were employed. An indication of the variation in gas permeability of the wrappers may be gained from examination of the data given in Table 10 for the materials used in the above studies on storage life.

Table 10. Changes in concentration of CO_2* in packages of fresh beef stored at 4.4°C.

Packaging material	Atmosphere	Days in storage				
		0	2	4	7	12
MSAT-80 cellophane	CO_2	23.2	94.1	10.7	3.2	3.9
	Air	0.0	0.0	0.0	1.0	2.8
Pliofilm FM-1	CO_2	24.4	92.8	16.3	3.0	3.9
	Air	0.0	0.0	0.0	0.8	1.5
Pliofilm FF-120	CO_2	25.7	94.2	27.6	13.1	4.8
	Air	0.0	0.0	0.1	1.5	3.9
Aluminum foil-Pliofilm laminate	CO_2	24.4	94.7	89.2	79.6	78.7
	Air	0.0	0.0	0.1	0.2	0.6

*Carbon dioxide concentration expressed as %
Samples held in 25% CO_2 for 2 days, then 95% CO_2 added to packages prior to sealing

With the first three materials shown in Table 10, reduction in CO_2 concentration took place rather rapidly and continued throughout storage until the level of the gas was considerably less than that at the time packages were gassed. On the other hand, loss of CO_2 was gradual when the foil-film laminate served as the wrapper; this material had the

greatest ability of the four to retain the gas within the package. In several instances, during the last week of storage, CO_2 treated meat packaged with the laminate was less firm than was similarly treated meat wrapped with other materials. The pH of the former samples was 0.1 to 0.3 lower than were values for meat packaged with either type of Pliofilm or with cellophane. Increase in concentration of CO_2 in control packages was considered to be due to action of microorganisms. Differences in loss of CO_2 undoubtedly influenced variation in keeping times of beef packaged with these materials.

d. Discussion. Ability of packaging materials to retain CO_2 within the atmosphere of the package seems to be a primary requirement for most successful application of the gas in preservation of self-service meats. However, improvement in keeping time of beef by the use of CO_2 was not limited to materials having low gas permeability. Storage life was prolonged even when films that provided poor barriers to gas transfer were employed. This suggests that initial exposure of meat to CO_2 before packaging was of value in extending keeping time. Presumably, as CO_2 passed through the wrappers into the outer atmosphere, a portion of the gas diffused from the meat into the atmosphere of the packages. This effect would assume greater importance if the quantity of meat relative to the amount of gas space were decreased.

Storage indices listed in Table 8 deserve special attention. Increase in storage life by the use of CO_2 was pronounced when the more

gas-permeable films were employed. This does not mean that keeping time was greater with these materials; on the contrary, with or without added CO_2, off-odor was detected earlier during storage than it was when less permeable materials were used. Apparently, the typical spoilage organisms which were inhibited by CO_2 were also retarded in development in the absence of added CO_2 when materials having low permeability to gases served as wrappers.

3. Influence of relative humidity

Early experiments concerning the relation of humidity to changes in fresh beef were carried out at relative humidities approximating those stated to exist in self-service meat display cases. Unpackaged cuts of beef were held at relative humidities in the neighborhood of 50 and 70 per cent. In later work, packaged beef was stored at relative humidities of 10 and 95 per cent. The latter levels were chosen in order to study the effects of extremes of humidity on changes in fresh meat packaged with various materials. It was believed that changes induced by variations in humidity would be more pronounced at low and high levels than at some intermediate relative humidity. Further, the use of the humidities specified was considered to be an expedient means for evaluating the general relationship of humidity to differences in performance of packaging films.

Materials employed in these studies included MSAT-80 cellophane,

Pliofilm FM-1, Saran laminated to cellophane, and polyethylene laminated to cellophane. The cellophane and Pliofilm types are more permeable to moisture vapor and gases than are the laminated films.

a. Color changes. Until storage progressed for 9 days and longer, little difference was observed in surface color of unpackaged beef stored at 50 and at 70 per cent relative humidity. At both humidities the meat appeared dark red after 3 days at 4.4°C.; increased darkening occurred during further storage. At the end of 9 days, beef held at 50 per cent relative humidity was dark maroon-brown in color; the edges of such samples were shrivelled and were darker than other portions. At 70 per cent relative humidity, the only color changes discernible were darkening of the red pigment and browning at the edges of the cuts of beef.

With storage at 10 per cent relative humidity, beef packaged with MSAT-80 cellophane and with Pliofilm FM-1 developed brown discoloration at the end of 3 days. Samples wrapped with the same kinds of materials but kept at 95 per cent relative humidity still remained red in color although they were darker than the bright red observed at the beginning of the storage period. At the higher humidity, brown discoloration was noted as storage continued. In the case of storage at 10 per cent relative humidity, the brown was less intense with Pliofilm FM-1 than it was with MSAT-80 cellophane: when the cellophane was used the surface color of packaged beef approached black. In addition, the meat wrapped with the latter film appeared to have a leathery consistency

after prolonged holding at 10 per cent relative humidity.

The color of beef packaged with the laminate of Saran and cellophane became purple within a few hours after packaging and remained in this condition throughout storage at both 95 and 10 per cent relative humidity. Upon exposure to air, the samples appeared bright red, similar in color to that of the meat just prior to packaging. With polyethylene laminated to cellophane, similar changes were noted when storage was carried out at 10 per cent relative humidity. However, at 95 per cent relative humidity, beef wrapped with this material showed a gradual reduction of myoglobin to the purple pigment with subsequent oxidation to brown metmyoglobin. After 9 and 12 days, the color of such beef was best described as reddish brown.

b. Desiccation. The role of humidity in bringing about the color changes described above may be explained on the basis of moisture losses of beef wrapped with materials having different degrees of permeability to moisture vapor. In Table 11, figures are given for weight losses of meat stored at the test humidities. Each value is the mean for three samples.

Table 11. Effect of relative humidity and packaging materials on weight losses* of fresh beef stored at 4.4°C.

Packaging material	Relative humidity (%)	Days in storage			
		3	6	9	12
MSAT-80 cellophane	95	0.36	1.36	1.78	2.89
	10	5.17	12.10	16.83	27.60
Pliofilm FM-1	95	0.19	0.43	0.96	1.15
	10	2.00	4.11	6.81	8.77
Saran-cellophane laminate	95	0.00	0.00	0.10	0.26
	10	0.19	0.55	0.68	2.29
Polyethylene-cellophane laminate	95	0.00	0.00	0.00	0.00
	10	0.00	0.04	1.24	1.56
None (unpackaged)	70	3.84	9.45	12.55	16.88
	50	9.11	13.75	22.30	28.25

*Weight losses expressed as % initial wet weight

It should be understood that humidities at the surface of cuts of beef were not as low as the values indicated in Table 11. In his studies on the effect of humidity on growth of microorganisms, Scott (1936) stated that the relative humidity at the surface of meat would be higher than that of the surrounding atmosphere because of diffusion of moisture from the interior of the meat to the surface. These conditions apparently prevailed in the work presented here, since samples were not allowed to reach an equilibrium moisture content at the specified humidities prior to storage. In these trials, limits of storage were determined by development of off-odor and slime or by severe de-

hydration; these end points were observed before the meat attained equilibrium with its external atmosphere.

Data given by Scott (1936) and Kefford (1948) for muscle moisture contents of beef at various humidities were employed in order to estimate humidities obtaining at the surface of packaged and unpackaged samples. Moisture contents were calculated from observed weight losses; these data together with relative humidities are presented in Table 12.

Table 12. Muscle moisture contents and relative humidities at surface of round steak stored at 4.4°C.

Packaging material	Relative humidity of external atmosphere (%)	Days in storage	Approximate moisture content*	Relative humidity at meat surface (%)
MSAT-80 cellophane	95	3	75.1	99.3
		12	72.6	99.1
	10	3	70.3	99.0
		12	48.0	96.0
Pliofilm FM-1	95	3	75.3	99.3
		12	74.4	99.2
	10	3	73.5	99.1
		12	66.7	98.5
Saran-cellophane laminate	95	3	75.5	99.3
		12	75.2	99.3
	10	3	75.3	99.3
		12	73.2	99.1
Polyethylene-cello. laminate	95	3	75.5	99.3
		12	75.5	99.3
	10	3	75.1	99.3
		12	73.9	99.2
None (unpackaged)	70	12	58.0	97.5
	50	12	47.2	95.8

*Moisture content expressed as % wet weight

It may be noted that even with storage at 10 per cent relative humidity moisture vapor at the surface of beef did not fall below about 96 per cent after 12 days; in most instances, with packaged samples, the humidity remained above 98 per cent. Dehydration was severe when moisture contents decreased to 47 or 48 per cent of the wet weight, as was the case when meat was packaged with MSAT-80 cellophane and stored at 10 per cent relative humidity or held unpackaged at 50 per cent relative humidity. When the laminated films were employed as wrappers, desiccation of beef was negligible.

c. Bacterial growth. Growth curves of bacteria found on packaged beef held at external relative humidities of 10 and 95 per cent are given in Figures 18, 19, and 20. Figure 18 indicates that with MSAT-80 cellophane bacterial counts were about one hundred times greater at 9 or 12 days with storage at 95 per cent relative humidity than at 10 per cent relative humidity. The lag period appeared to be extended by the use of the low humidity. At the end of 3 days, moisture content of beef held at 10 per cent relative humidity was about 70 per cent of the wet weight, corresponding to a surface humidity of 99 per cent (Table 12). Scott (1936) found that the lag period for Pseudomonas was increased by 5 days when the relative humidity at the surface of meat was lowered from 99.3 to 99.0 per cent. In the present work, lowering of the moisture content of cellophane-wrapped beef may have resulted in an increased lag period for surface flora. Off-odor was

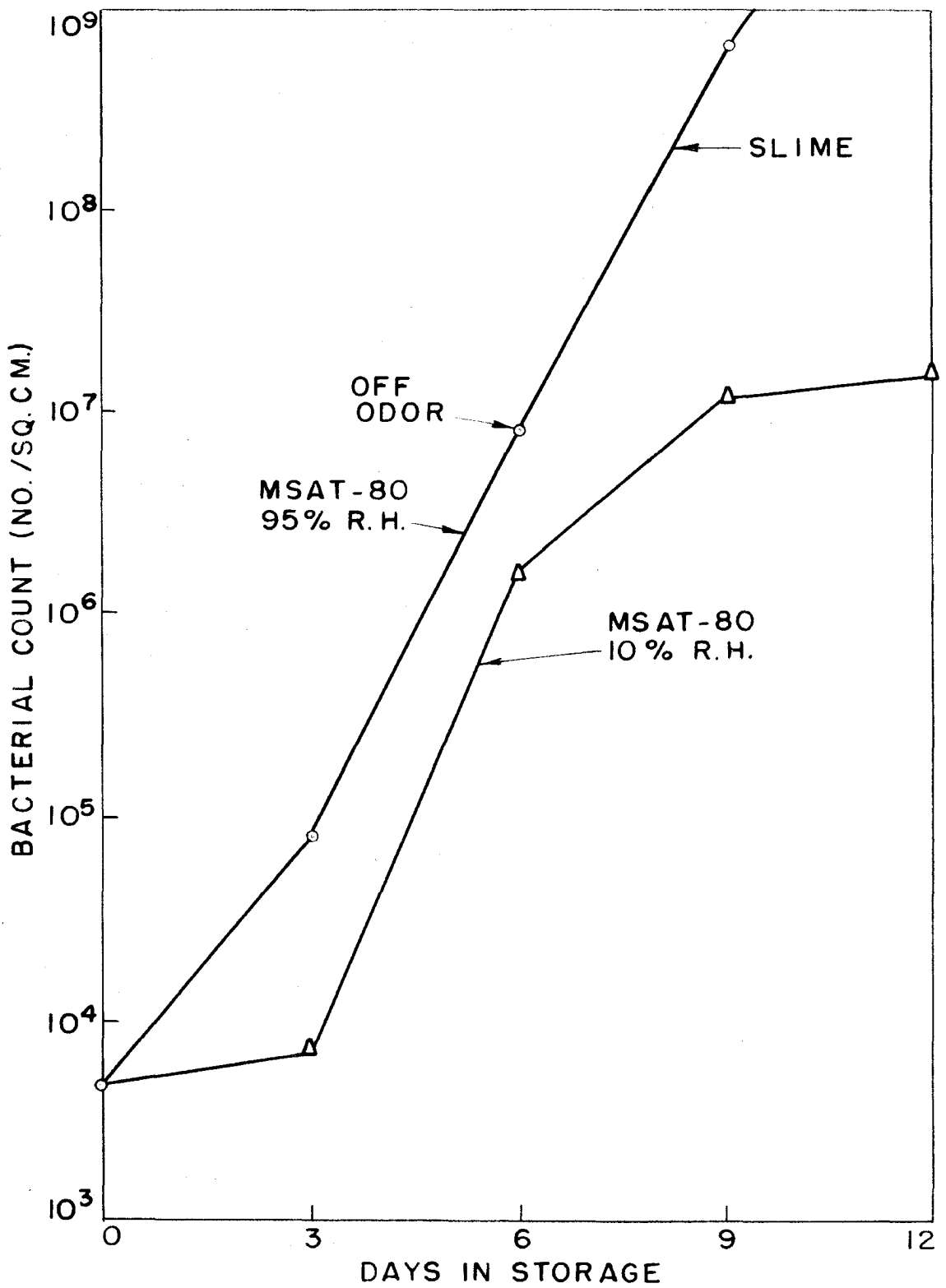


Figure 18. Effect of relative humidity on growth curves of bacteria on beef packaged with MSAT-80 cellophane and stored at 4.4°C.

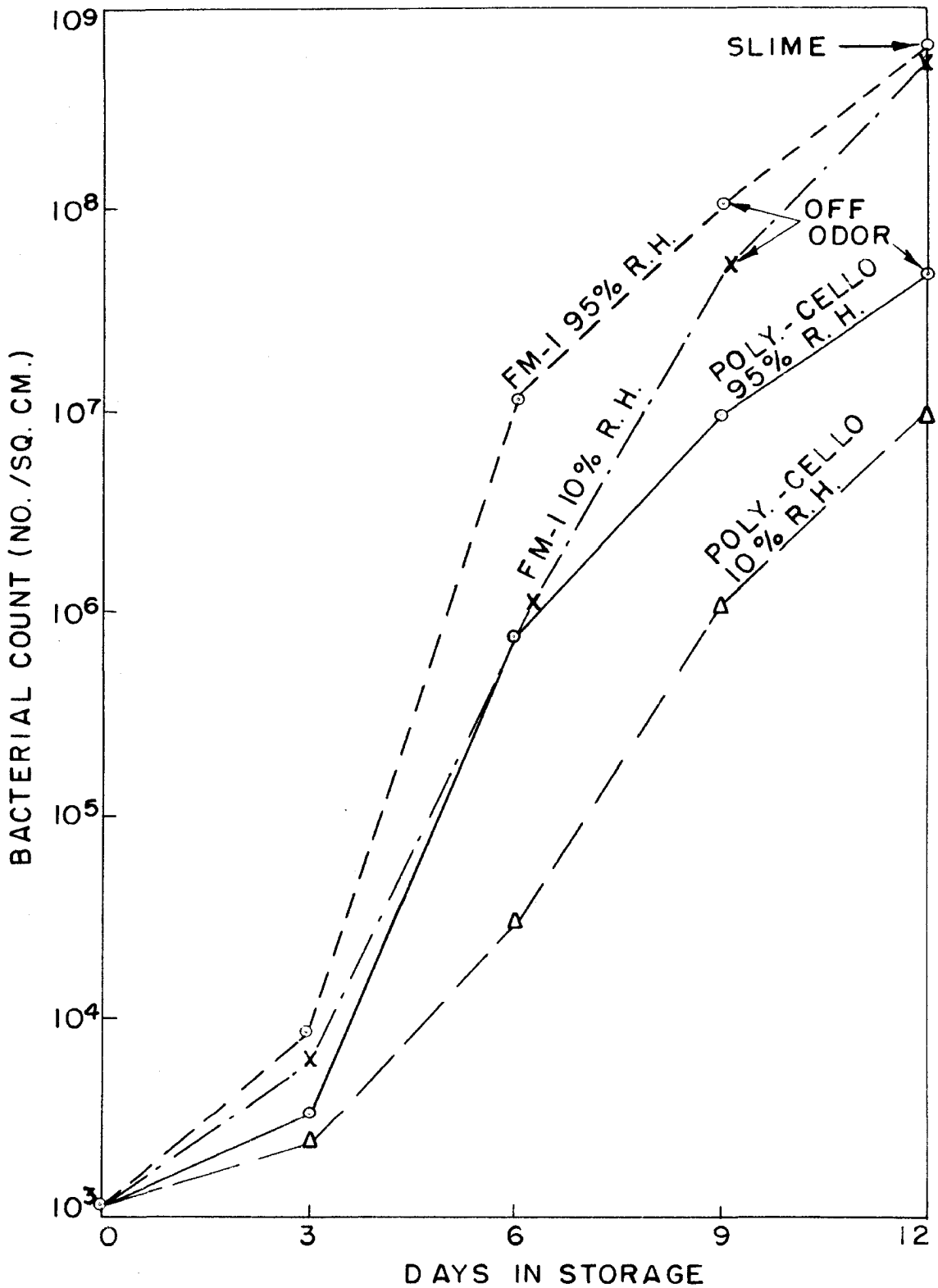


Figure 19. Effect of relative humidity on growth curves of bacteria on beef packaged with Pliofilm FM-1 and polyethylene laminated to cellophane and stored at 4.4°C.

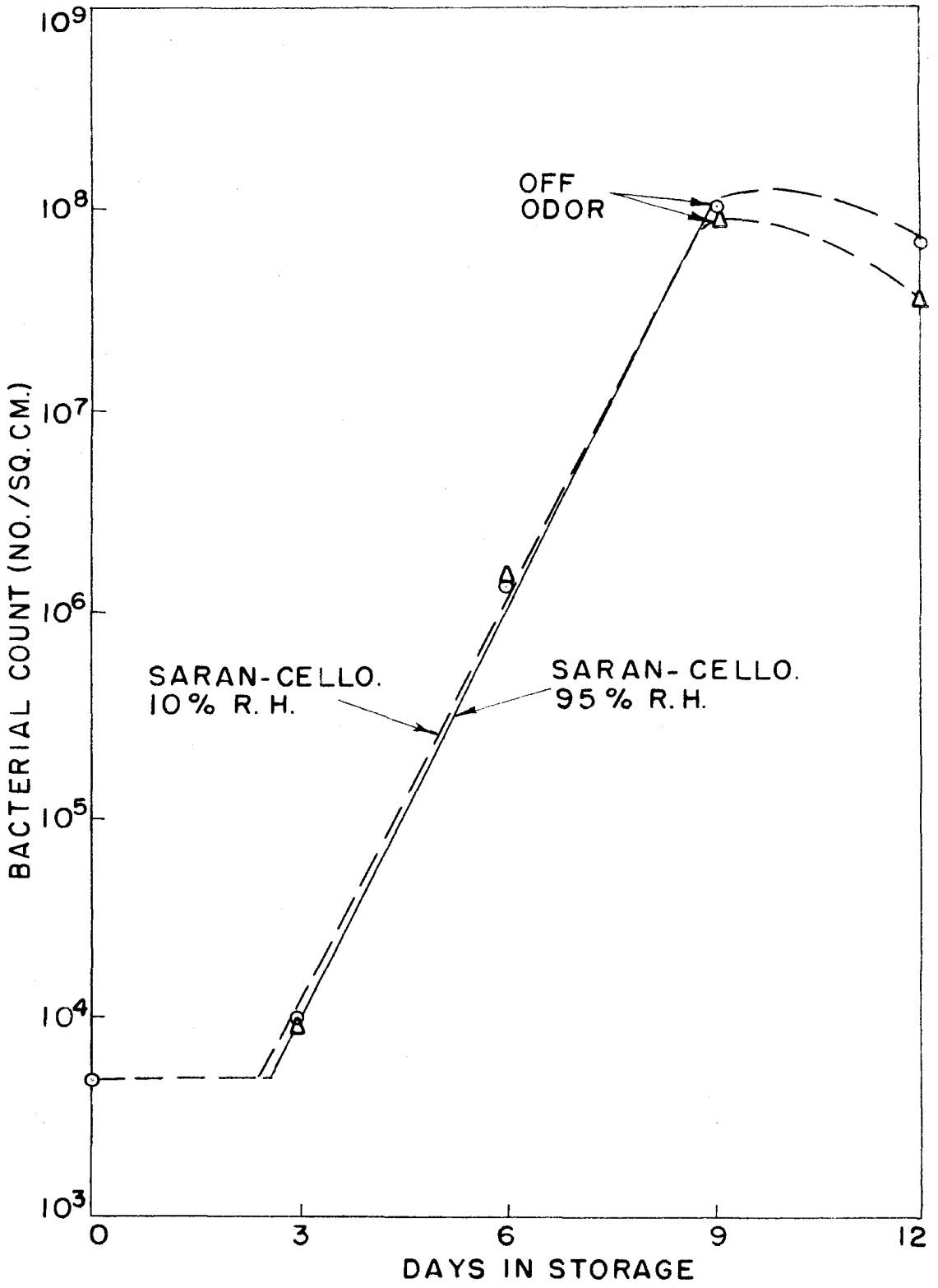


Figure 20. Effect of relative humidity on growth curves of bacteria on beef packaged with Saran laminated to cellophane and stored at 4.4°C.

not detected on meat held at 10 per cent relative humidity at any interval during storage. However, at the end of 6 days, dehydration had advanced to the point where samples were considered to have reached the limit of storage.

Although bacterial growth appeared to proceed more slowly on meat wrapped with Pliofilm FM-1 (Figure 19) and kept at 10 per cent relative humidity than it did on samples packaged with the same material but held at 95 per cent relative humidity, off-odor was observed at the same time with both humidities of storage. Unfortunately, the meat was not examined for spoilage at any time other than the days on which it was sampled. As indicated previously, on the average, off-odor was noted when surface counts reached 3×10^7 bacteria per square centimeter. Assuming that a similar relationship existed in these experiments, the lower humidity would have provided a longer storage life than that with storage at 95 per cent relative humidity.

After meat was held for 9 and for 12 days, bacterial numbers were appreciably lower at either humidity when polyethylene laminated to cellophane was used than when Pliofilm FM-1 served as the wrapping material. In view of the fact that moisture contents of beef packaged with the laminated material were higher than those of similar meat wrapped with Pliofilm FM-1, desiccation evidently was not responsible for the lower counts obtained when the laminate was utilized. Growth of surface organisms appeared to be conditioned to a greater extent

by the packaging materials than by the humidity of the atmosphere outside the packages.

The keeping time of round steak packaged with Saran laminated to cellophane (Figure 20) was longer than that of beef having the same initial count but wrapped with MSAT-80 cellophane (Figure 18). Bacterial growth was independent of the outside relative humidity when meat was packaged with the laminate. Generation times were approximately equal at both storage humidities; at 95 per cent relative humidity the generation time of surface flora was 10.6 hours and at 10 per cent relative humidity the value was 10.9 hours.

d. Discussion. The humidity of storage had little effect on keeping time, changes in color, or desiccation of beef packaged with Saran laminated to cellophane. All three of the specified criteria of keeping quality are related and it seems reasonable to believe that any condition affecting one index of quality also influences one or both of the others. Variations in relative humidity within the range 10 to 95 per cent then might not be expected to influence the performance of the Saran-cellophane laminate with respect to the above quality factors. An altogether different set of conditions existed when MSAT-80 cellophane was employed as the packaging material. Bacterial counts were higher at 95 per cent relative humidity than they were at 10 per cent relative humidity, and the rate of growth of surface microorganisms was greater at the higher humidity of storage.

Meat held at the lower humidity suffered extreme desiccation and exhibited undesirable darkening of color before microbial growth was appreciably retarded. Changes effected by relative humidity were not as great with Pliofilm FM-1 as they were with the cellophane, but little advantage was gained by reducing humidity when the former material served as the wrapper for beef.

A packaging material that prevented development of bacteria on the surface of meat at reduced humidities without allowing any deleterious changes would probably be considered ideal for practical application. While no such material was found in this limited study, the laminate of polyethylene and cellophane most nearly satisfied these criteria when the relative humidity external to the packages was 10 per cent. Differences between growth curves at 10 and 95 per cent relative humidity with polyethylene laminated to cellophane cannot be readily explained. It does not seem reasonable to assume that the almost negligible loss of moisture which occurred at the lower humidity was the cause of decreased numbers of surface organisms in comparison with those found at 95 per cent relative humidity. It was stated earlier that the myoglobin of beef packaged with the polyethylene-cellophane laminate was maintained in the reduced condition when the humidity of storage was 10 per cent; this condition did not prevail at the higher humidity. This observation raises the possibility that the lower humidity favored movement of dissolved oxygen from the meat through the material to the atmosphere outside the packages. That the meat lost moisture with

storage at 10 per cent relative humidity is known from observed weight losses; loss of oxygen from the packages would be enhanced by solution of the gas in the water vapor transmitted through the walls of the packages. Under these circumstances, conditions prevailing within packages held at 10 per cent relative humidity would be less aerobic than those existing with storage at 95 per cent relative humidity. Consequently, surface bacterial growth would tend to be less profuse on meat kept at the lower humidity of storage than on similar samples stored in an atmosphere of 95 per cent relative humidity. As indicated in a previous section, those materials that promoted reduction of myoglobin generally permitted extended storage life; a similar situation may have occurred when beef was packaged with the polyethlene-cellophane laminate and held at 10 per cent relative humidity.

Although benefit was derived from the use of 10 per cent relative humidity and the polyethylene-cellophane wrapper, from the practical point of view a problem would arise with regard to maintenance of low humidities in open display cases in retail stores.

4. Relation of light to changes in beef

a. Factors affecting color and reflectance. (1) Variation in product. Measurements were made ofthe spectral reflectance of fifteen slices of freshly cut round steak in order to determine the variation among samples before storage. Cuts were exposed to air for about 20 minutes; at the end of that time the color was bright red. Mean

reflectance values are indicated by the solid line curve in Figure 21; curves for confidence interval estimates (P=0.05) are shown by broken lines in the same graph. Data from which these curves were constructed may be found in Appendix Table 34.

The greatest variation in reflectance of different cuts of beef from the same section of the quarter was found in the region between 650 and 800 mμ, or in approximately the red portion of the spectrum. It should be mentioned that visible color changes would also be expected to occur largely in the red region. In later experiments wherein reflectance curves were used as objective measurements of differences in color of treated samples, account was taken of the variation in reflectance of untreated meat.

(2) Time of exposure. In evaluating factors responsible for discoloration, it was rather difficult to separate the effects of time of exposure per se from those of type of light. Discoloration of packaged beef exposed to Soft White fluorescent light generally followed the usual pattern over a period of time; namely, reduction of myoglobin to the purple pigment followed by oxidation to brown metmyoglobin. With all types of light tested, no fading was observed in fresh meat given an exposure of 6720 footcandle-hours. When materials having relatively high transparency to ultraviolet light were employed as wrappers, display under this type of light was characterized by progressive darkening of the meat pigment. These changes are described in greater detail in later sections concerning the effects of type of light and light intensity.

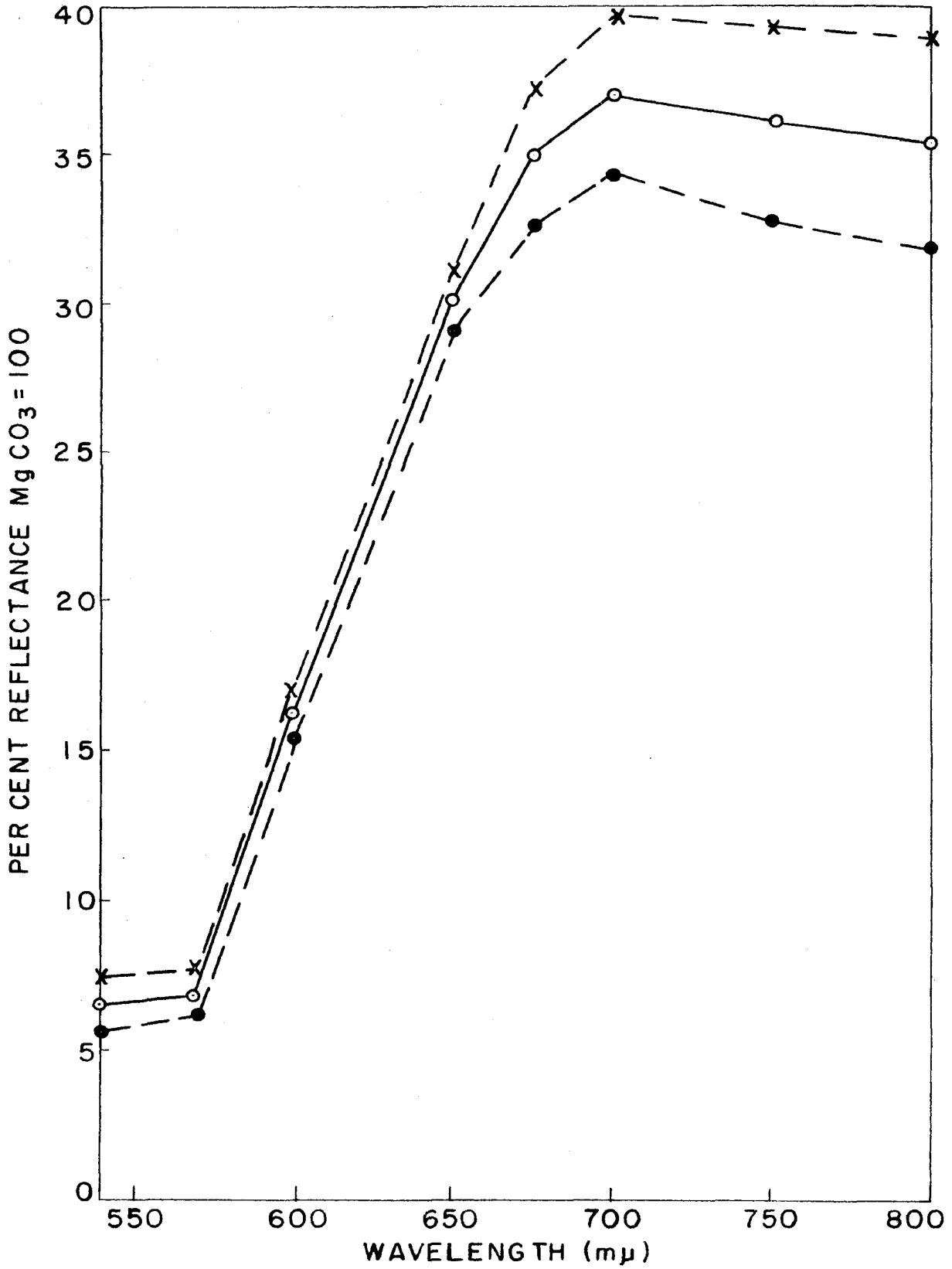


Figure 21. Mean spectral reflectance of fresh red beef

(3) Transmission of light by materials. Degree of transparency of materials to light of different wavelengths is an important consideration in studying the action of radiant energy on pigments of packaged fresh meats. Curves for transmission of light by several materials used for wrapping fresh beef are shown in Figures 22 and 23. MSAT-80 cellophane (Figure 22) transmitted considerably more radiation in the ultraviolet and visible regions of the spectrum than did Visten-C; Pliofilm FF-120 was intermediate to these films in transparency. The curve for Pliofilm FM-1 (Figure 23) approximated that for Pliofilm FF-120 at wavelengths below 500 mμ, but the former type of Pliofilm was more transparent to higher wavelengths in the visible light range than was Pliofilm FF-120. Both Pliofilm FM-1 and polystyrene Q-641 allowed passage of a high proportion of light of wavelengths greater than 500 mμ. In addition, the latter film and MSAT-80 cellophane permitted transmission of appreciable amounts of ultraviolet radiation.

(4) Type of light. In preliminary experiments it was found that little difference existed between changes in color of packaged beef exposed to White fluorescent or Soft White fluorescent light having equal intensities. Again, discoloration was similar to that of meat wrapped with the same materials but held in the dark and examined at the same time intervals.

Germicidal ultraviolet light has been advocated as a means of controlling microbial growth on the surface of meats (Food Ind., 1938; James, 1936; Ewell, 1941), but the use of this type of illuminant has

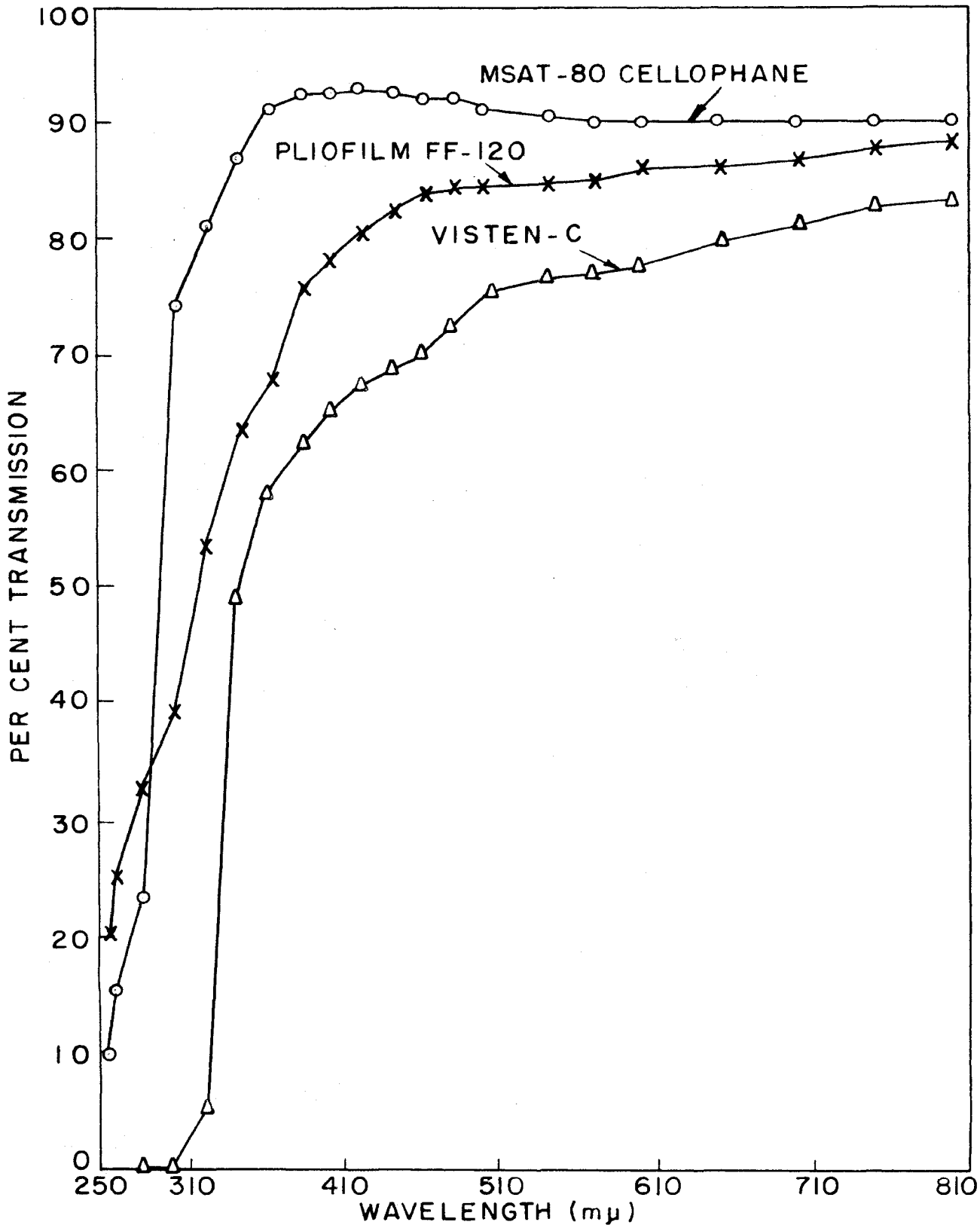


Figure 22. Transmission of light by MSAT-80 cellophane, Pliofilm FF-120, and Visten-C

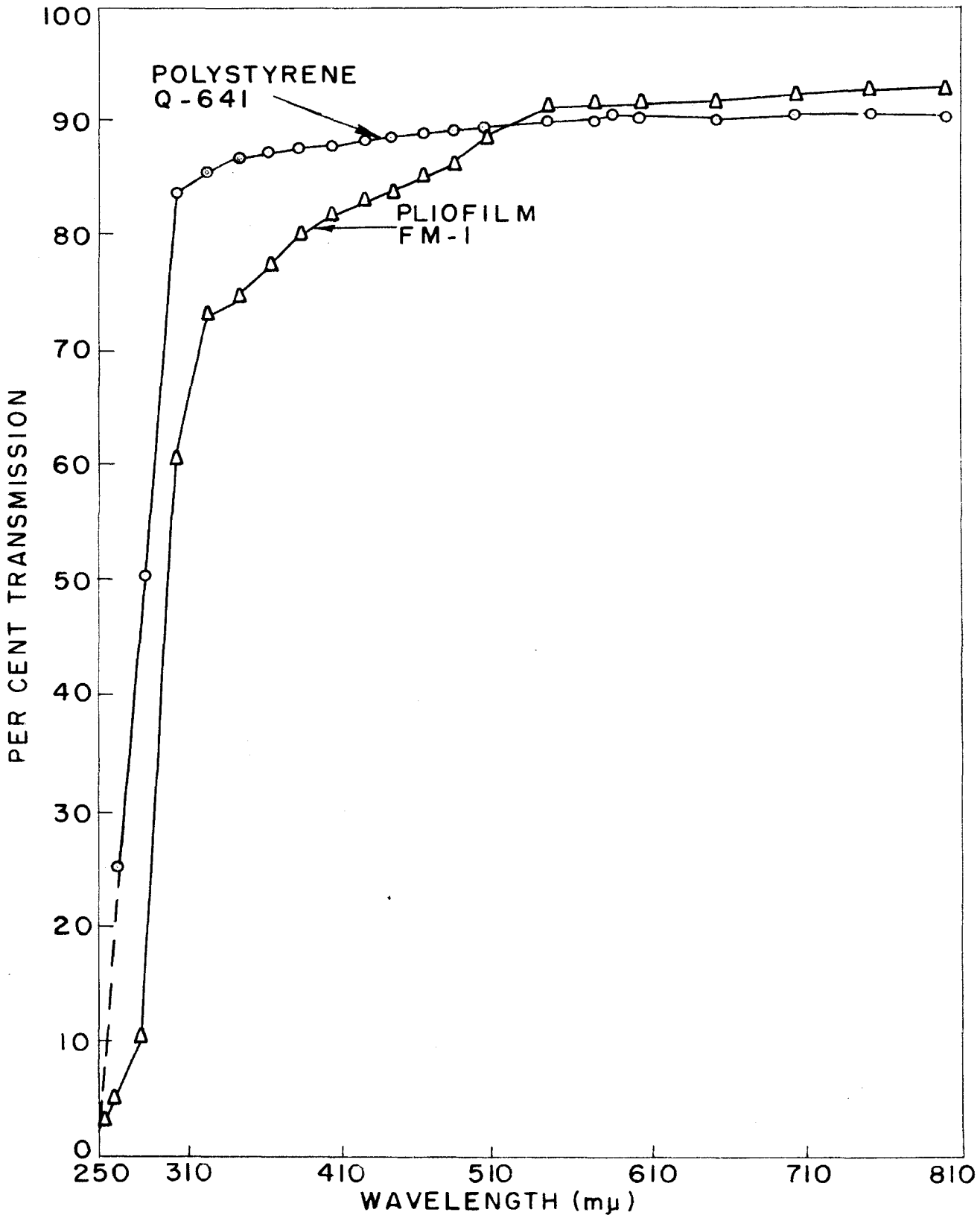


Figure 23. Transmission of light by polystyrene Q-641 and Pliofilm FM-1

also been criticized because of its harmful effects on color (Lea and Lea, 1937; Oser, 1946). It was believed desirable to compare changes in color of packaged beef exposed to germicidal ultraviolet radiations with color changes in similar meat stored under a commonly used display case illuminant such as Soft White fluorescent light.

In these trials, the intensity of ultraviolet radiation as measured with the light meter was 7 to 10 footcandles at the surface of samples, while the Soft White fluorescent lamp provided an intensity of 30 to 40 footcandles. It was recognized that the glass guard on the light meter may have absorbed a portion of the ultraviolet light and thereby reduced the intensity incident to the photocell. Also, the packaging materials employed in these experiments differed in their ability to transmit light in the ultraviolet and visible portions of the spectrum.

Curves for spectral reflectance of beef packaged with MSAT-80 cellophane and with Visten-C are shown in Figures 24 and 25. The color of samples at the time spectrophotometric measurements were made is also indicated. In Figure 22, it is demonstrated that the cellophane transmitted a much greater amount of light in both the ultraviolet and visible regions of the spectrum than did Visten-C; the relation between transparency of materials and color changes in meat is brought out by Figures 22, 24, and 25.

Cellophane-wrapped beef (Figure 24) exposed to ultraviolet light exhibited more drastic changes in color and reflectance than those noted when similarly packaged beef was held under the Soft White fluores-

cent light. With display under the ultraviolet lamp, reflectance decreased rapidly during storage for 2 days. The color of samples was dark brown in contrast to the dull red of meat exposed to Soft White fluorescent light. As storage progressed, reflectance values for meat subjected to ultraviolet radiation showed a more gradual decline; darkening of the surface continued until samples appeared brown-black in color. On the other hand, beef stored under fluorescent light demonstrated color changes similar to those previously observed for samples kept in the dark. A slight increase in reflectance occurred at the time when the myoglobin became reduced and the meat was a reddish purple color. Subsequent oxidation to metmyoglobin and increase in desiccation resulted in lower reflectance and brown discoloration. With respect to the time for appearance of dark brown discoloration, shelf life of beef exposed to fluorescent light was extended by 10 days in comparison with that for meat irradiated with germicidal ultraviolet light. Values for reflectance at 650 mμ for beef stored under ultraviolet light were significantly lower at the 1 per cent level than those of meat exposed to Soft White fluorescent light. Data obtained from experiments described here (Run RS-30) and from a subsequent trial (Run RS-32) were used in making the "t" test for significance (Appendix Table 35). Readings made at 650 mμ were selected as representative of reflectance in the red region of the spectrum.

Beef wrapped with Visten-C (Figure 25) was not affected by the two types of light in the same manner as was meat packaged with MSAT-80

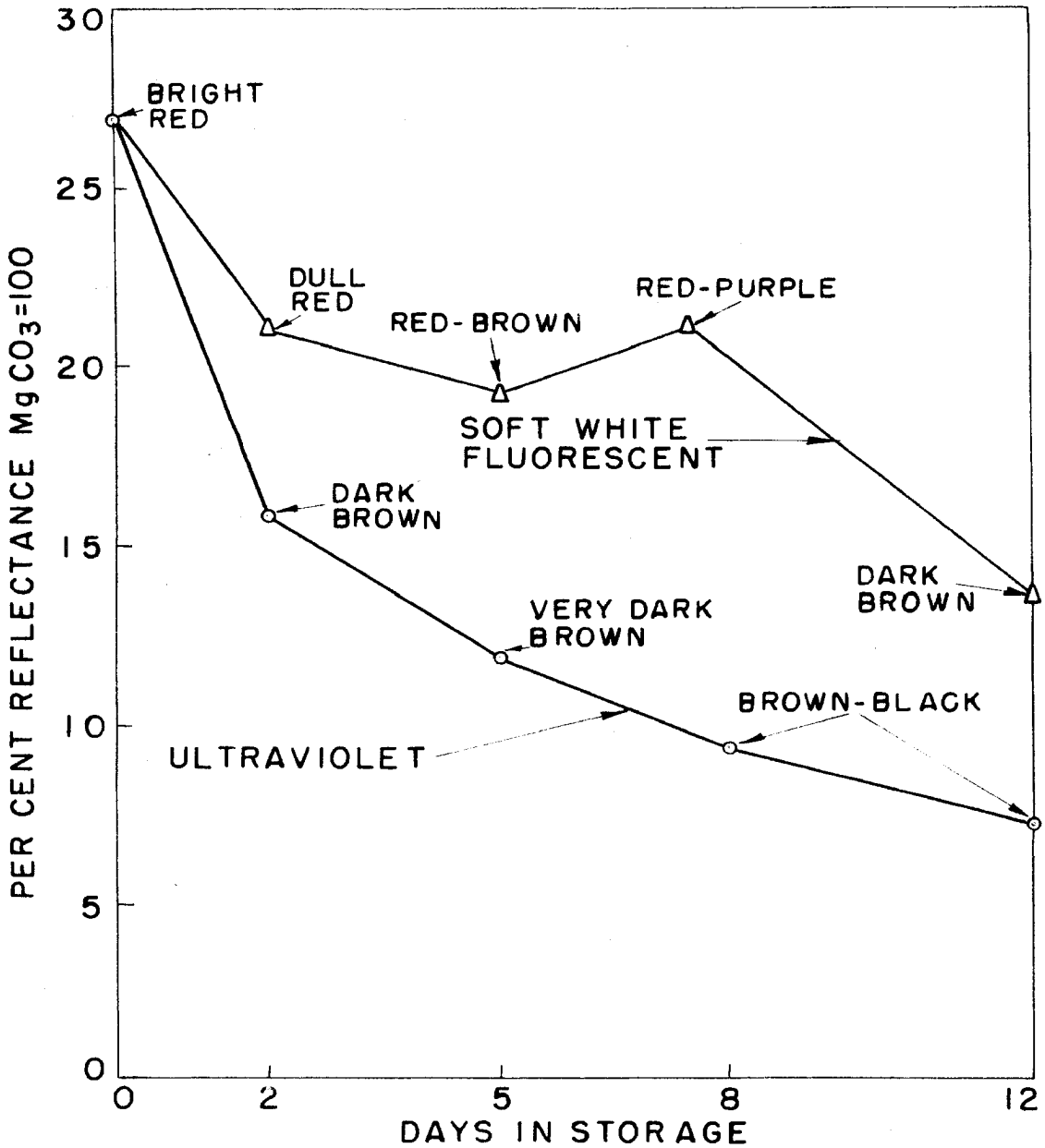


Figure 24. Reflectance and color of beef packaged with MSAT-80 cellophane and exposed to germicidal ultraviolet and Soft White fluorescent light*

*Each point is of reflectance for two samples measured at 540, 570, 580, 600, 635, 675, 700, 750, and 800 mμ.

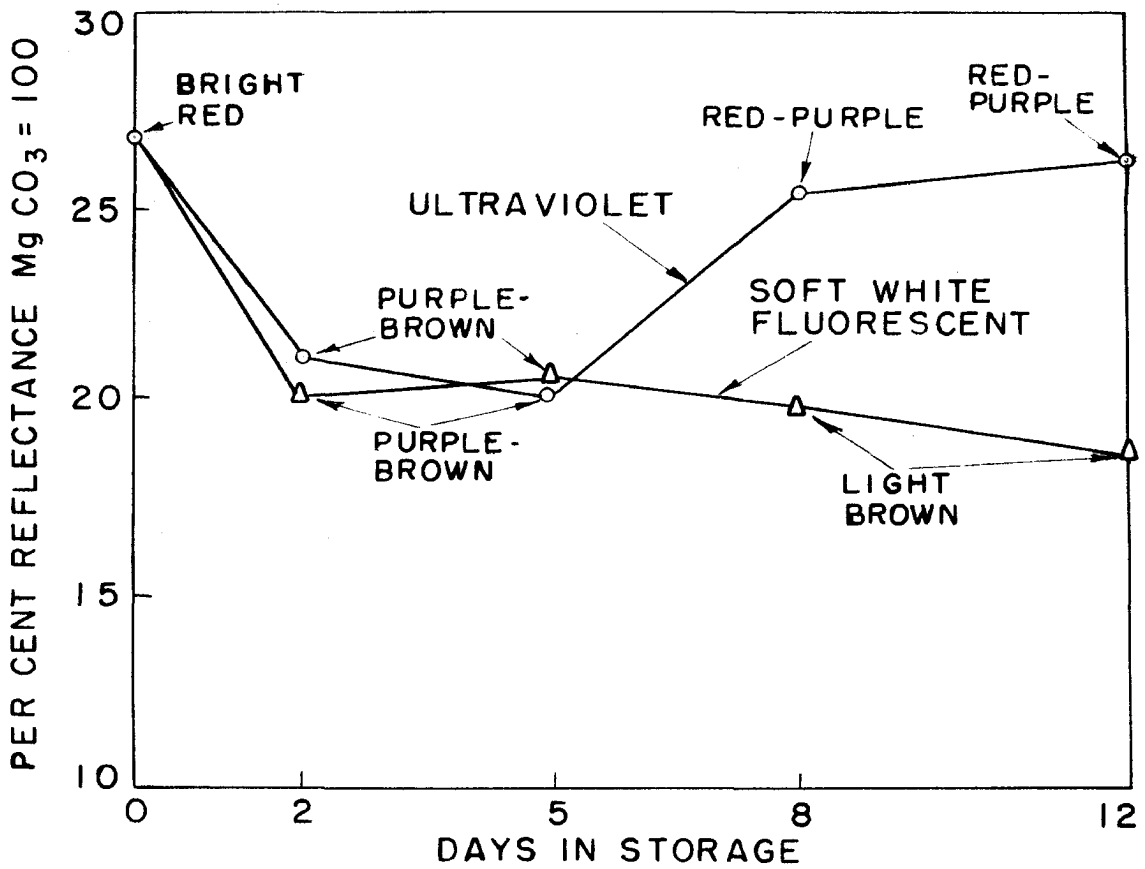


Figure 25. Reflectance and color of beef packaged with Visten-C and exposed to germicidal ultraviolet and Soft White fluorescent light*

*Each point is mean of reflectance for two samples measured at 540, 570, 580, 600, 635, 650, 675, 700, 750, and 800 mμ.

cellophane. From examination of the two curves in Figure 25, it appears that Soft White fluorescent light produced greater discoloration than did germicidal ultraviolet light. With exposure to either type of illuminant for 2 and 5 days, the meat pigment was converted to a partly oxidized, partly reduced condition. Evidently the oxygen pressure in packages was such that metmyoglobin was formed at a rate similar to that for production of reduced myoglobin. On further display, samples exposed to fluorescent light showed continued oxidation to metmyoglobin. Conversely, the myoglobin of beef held under ultraviolet light became reduced further as storage time increased. As was the case when MSAT-80 cellophane was used as the wrapper, meat packaged with Visten-C demonstrated an increase in reflectance upon reduction of myoglobin. From these observations, it seemed that the intensity of ultraviolet radiation on the surface of beef packaged with Visten-C was not sufficiently great to cause as marked discoloration as that which occurred when the meat was wrapped with the more transparent cellophane. This might suggest further that the use of Soft White fluorescent light resulted in greater discoloration of meat packaged with Visten-C than that observed when ultraviolet light was employed because the light intensity of the fluorescent light was higher. However, cellophane also transmitted more Soft White fluorescent light than it did ultraviolet light, but both types of light passed through the cellophane to a greater degree than was observed when Visten-C was tested. If the above reasoning is to be valid,

some minimum intensity of ultraviolet radiation is necessary for production of discoloration. These experiments were not designed to study the relation of light intensity provided by various illuminants to color changes in packaged meats. Rather, the object of the work here was to compare the general effect of ultraviolet radiation with that of visible light when materials having appreciable differences in transparency were utilized. Conditions of display as closely approximated those prevailing in retail stores as was possible; this required that both types of lamps be mounted at the same distance from the samples. Variation in light intensity of the two lamps was not under consideration.

(5) Desiccation. During the course of work with different kinds of illuminants, it was found that meat packaged with MSAT-80 cellophane and exposed to ultraviolet light suffered appreciable dehydration. The color of samples treated in the above manner resembled that of beef which was stored at low relative humidity without application of ultraviolet radiation. Beef held unpackaged showed rapid discoloration; weight losses of unpackaged meat exposed to germicidal ultraviolet and Soft White fluorescent lights are given in Table 13.

Table 13. Mean weight losses* of unpackaged beef exposed to different types of light

Type of light	Days in storage			
	2	5	8	12
	Per cent weight loss			
Ultraviolet	35.3	55.9	65.7	67.0
Soft White	33.4	59.5	63.0	66.7

*Weight losses expressed as % initial wet weight; mean of two samples
Relative humidity 40 to 75%
Temperature 2.5°C. ±1°

Losses in weight of packaged samples irradiated with the two types of light are presented in Table 14.

Table 14. Mean weight losses* of packaged irradiated beef

Packaging material	Type of light	Days in storage			
		2	5	8	12
		Per cent weight loss			
MSAT-80 cellophane	Ultraviolet	3.80	12.7	27.8	39.3
	Soft White	3.05	9.04	12.8	19.6
Visten-C	Ultraviolet	0.18	0.48	0.85	1.51
	Soft White	0.21	0.49	0.80	1.24

*Weight losses expressed as % initial wet weight; mean of two samples
Relative humidity 40 to 75%
Temperature 2.5°C. ± 1°

It appears that desiccation played an important role in causing discoloration of cellophane-wrapped beef exposed to ultraviolet radiation. Moisture content of samples stored for 12 days was calculated to be about 36 per cent of the wet weight, which provided a relative humidity of somewhat less than 95 per cent at the meat surface. It has been stated (Duggar, 1936, Vol. 1, p.310, 319) that affinity of proteins for water is decreased as a result of denaturation by ultraviolet radiation. This may partly explain the excessive desiccation of cellophane-wrapped beef exposed to germicidal ultraviolet light. Moisture losses were less severe with display under Soft White fluorescent light than with exposure to ultraviolet light.

The type of light had little effect on weight losses of meat wrapped with the less transparent, less moisture-permeable Visten-C film. When slices of beef were stored with no barrier to transfer

of moisture vapor, losses in weight were approximately equal regardless of the kind of illuminant employed. With both lights, dehydration of beef proceeded to the point where the meat was almost completely free of water of hydration. Reflectance curves for unpackaged beef are shown in Figure 26. Ultraviolet light exerted an influence above that of desiccation in the early phases of storage; at the end of 2 days the color of ultraviolet-irradiated samples was considerably darker than that of meat held under fluorescent light. Accordingly, reflectance values were markedly lower during the first 2 days of storage than were readings obtained after exposure to fluorescent light. End points for color changes apparently were attained at about the same time with both types of lamps; samples gave similar reflectance values and had about the same color after display for 5 days. Little change was noted on further storage. Darkening due to dehydration may have masked any brown discoloration resulting from further oxidation of myoglobin on prolonged exposure to ultraviolet radiation.

The above findings indicated that values for spectral reflectance followed a definite pattern in relation to visual changes in color over a period of time. Reflectance decreased as desiccation of beef progressed.

(6) Light intensity. When fresh beef was packaged with polyethylene laminated to cellophane, inappreciable differences in color

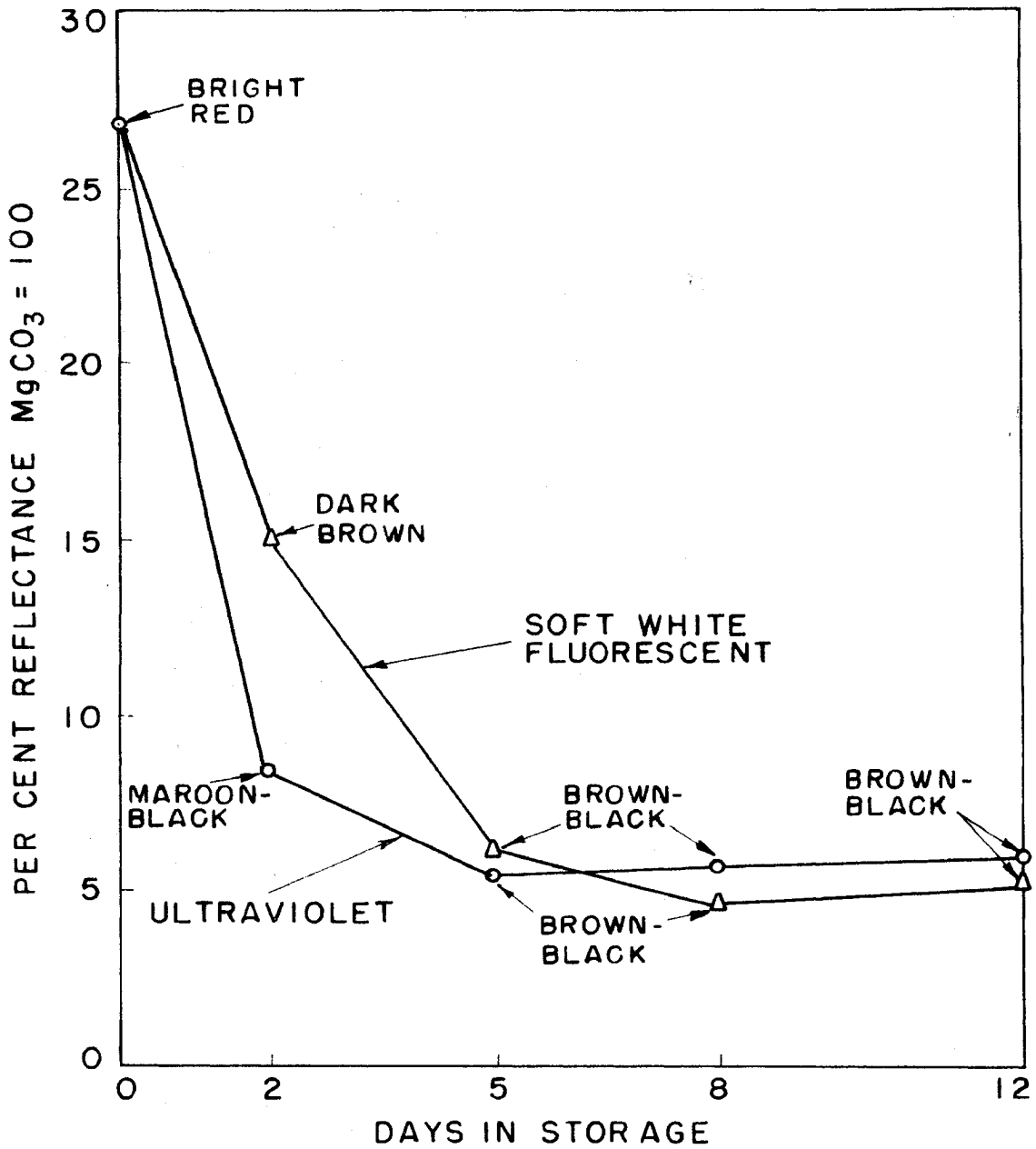


Figure 26. Reflectance* and color of unpackaged beef exposed to germicidal ultraviolet and Soft White fluorescent lights.

*Each point is mean of reflectance for two samples measured at 540, 570, 580, 600, 635, 675, 700, 750, and 800 mμ

and reflectance were evidenced by meat held in the dark or exposed to 70 footcandles of Soft White fluorescent light. Figure 27 shows reflectance curves for samples displayed under light and for those stored in the dark. Values for spectral reflectance at each of the wavelengths indicated are the means of determinations made at 2, 4, and 12 days. Beef stored in the absence of light gave slightly higher reflectance values than those obtained from similar meat exposed to light. By inspection, however, the variation between the two curves is no more than that which was found before storage (Figure 21). It may be noted that in either the presence or absence of light, reflectance of stored meat was shifted to values lower than those demonstrated by freshly cut bright red beef. During the storage period of 12 days, samples held in the dark or exposed to light exhibited color changes in accordance with the previously described sequence. Reduction of myoglobin occurred within 2 days and by the end of the 12th day the beef appeared purple-brown or light brown in color.

Further studies were conducted in order to evaluate the influence of high and low intensities of light on discoloration of packaged beef. MSAT-80 cellophane was considered to be well-adapted to determinations of this type because of its high transparency and popular usage in retail marketing. As indicated in Figure 28, reflectance of cellophane-wrapped beef stored at a light intensity of 150 footcandles differed little from that of similarly packaged meat exposed to 50 footcandles of Soft White fluorescent light. Changes in color also were in good

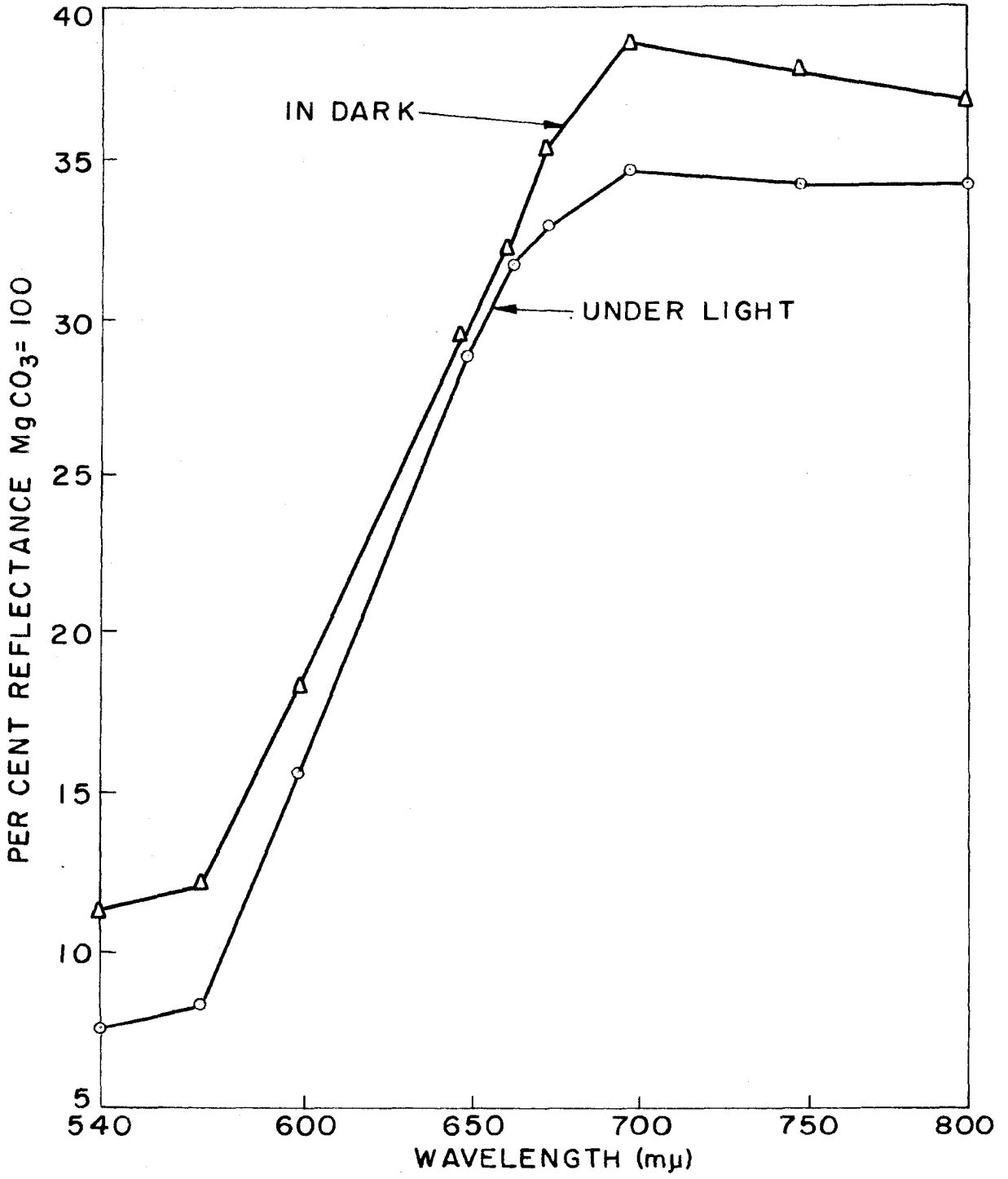


Figure 27. Effect of light intensity on reflectance* of beef packaged with polyethylene laminated to cellophane

*Light intensity 70 footcandles of Soft White fluorescent light

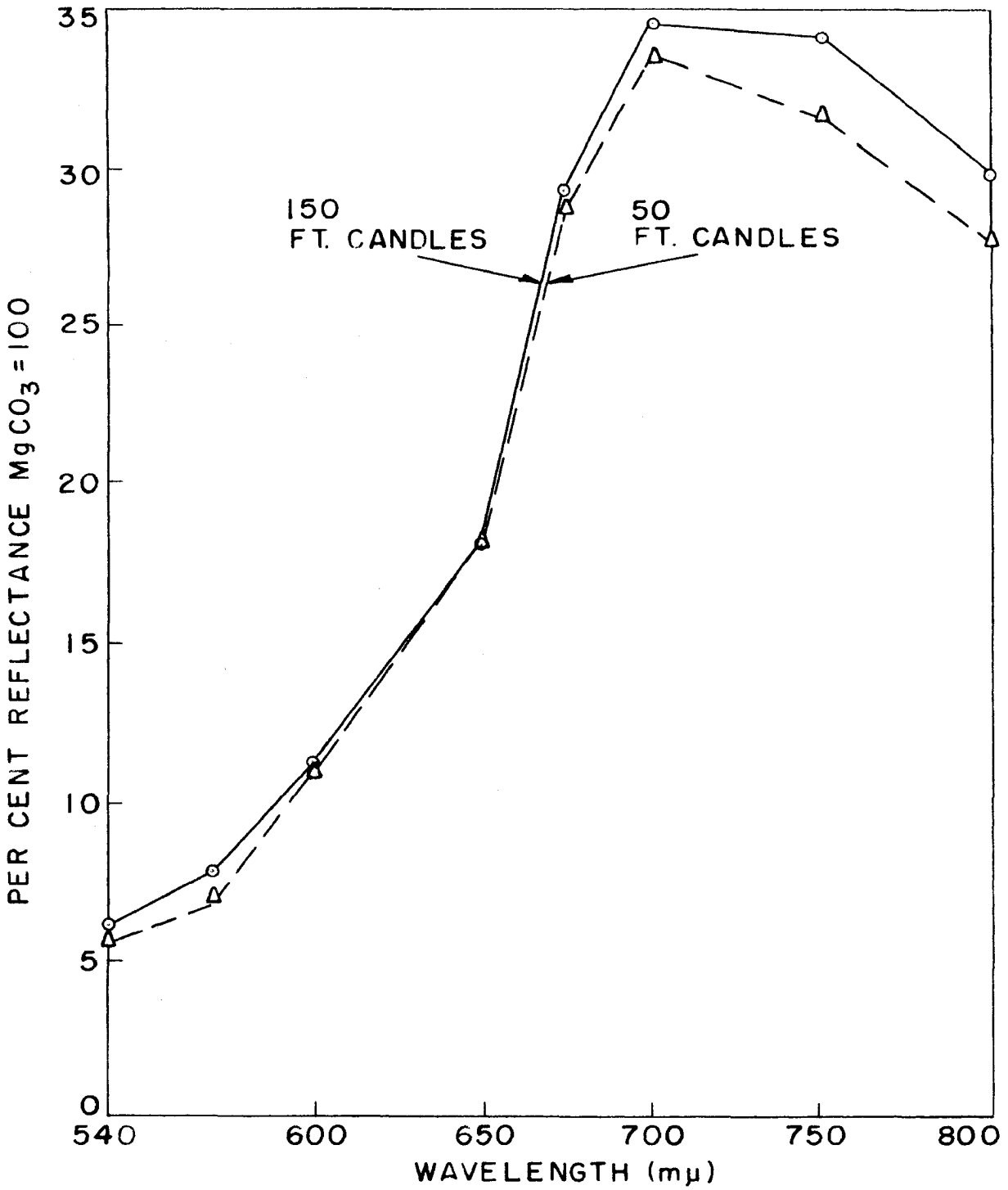


Figure 28. Effect of light intensity on reflectance* of beef packaged with MSAT-80 cellophane

*Each point is the mean of reflectance for four samples measured at 2, 5, 8, and 12 days

agreement and corresponded to those observed when beef packaged with MSAT-80 cellophane was stored in the dark.

(7) Deterioration of materials. Inasmuch as extreme desiccation of cellophane-packaged beef occurred on exposure to ultraviolet light, additional experiments were performed in order to explore possible causes of this observation. Tests to determine if the moisture vapor permeability of the cellophane was increased by treatment with ultraviolet radiation were considered as a feasible approach to this problem.

Cellophane bags containing no meat were stored in the display case for 20 days under ultraviolet light; similar bags were exposed to Soft White fluorescent light for the same length of time. After this preliminary exposure, slices of beef were packaged in the usual manner and displayed under the lights along with controls wrapped with freshly cut cellophane. Weight losses of samples are given in Table 15.

On the basis of these data, it seems reasonable to assume that transmission of moisture vapor was increased by exposure of cellophane to ultraviolet light. Weight losses were exceptionally high when preexposed cellophane was used. The per cent increase in loss over that observed with cellophane not previously irradiated decreased as storage time progressed. With preliminary exposure to Soft White fluorescent light, the permeability of the wrapping material also increased but the magnitude of this change did not approach that produced by ultraviolet light. Cellophane treated with ultraviolet radiation for 20 days appeared wrinkled within 2 days after it was used for packaging beef. In addition,

such cellophane lost much of the flexibility characteristic of the film which was held under Soft White fluorescent light.

Table 15. Mean weight losses* of irradiated beef packaged with MSAT-80 cellophane pre-exposed to light and with freshly cut cellophane

Cellophane treatment	Type of light	Days in storage			
		2	5	8	12
Exposed to light for 20 days	Ultraviolet	21.6	36.7	48.3	59.6
None (unexposed)		2.56	6.22	15.3	35.9
Exposed to light for 20 days	Soft White	3.50	8.44	15.6	19.2
None (unexposed)		2.05	5.36	9.02	12.8

*Weight losses expressed as % initial wet weight; mean of two samples
Relative humidity 50 to 75%
Temperature 2.5°C. ± 1°

The severe dehydration exhibited by beef wrapped with pre-irradiated cellophane and stored under ultraviolet light was reflected by marked discoloration. Samples darkened rapidly during the early phases of storage and continued to show darkening throughout the period of display. Preliminary irradiation of cellophane resulted in extraordinary decrease in reflectance of packaged meat (Figure 29).

In a later section, it will be shown that the germicidal power of ultraviolet light was increased when cellophane previously exposed to the light was used for wrapping beef. This suggests that the effect

of the radiation was to enlarge the pore size or to cause other opening of the structure of the material. Under these conditions, moisture vapor permeability and transmission of light would be increased.

b. Effect of type of light on bacterial growth. The action of germicidal ultraviolet radiation in reducing surface bacterial growth on packaged beef was dependent upon ability of materials to transmit light. Bacterial growth curves obtained from meat wrapped with the same materials employed in studies on color are given in Figure 30.

When MSAT-80 cellophane served as the wrapper, growth of surface bacteria on packaged beef was greatly retarded on exposure of packages to ultraviolet light. In the later phases of storage, further inhibition of bacterial development may have been due in part to desiccation. Also, increase in transparency of the film to ultraviolet light probably resulted on prolonged exposure. With Visten-C, which was shown to be less transparent than cellophane to light of short wavelengths, little difference was observed in counts made from samples held under either ultraviolet or fluorescent lights. However, during the lag phase, numbers of organisms decreased more markedly on exposure to germicidal radiation than they did with display under Soft White fluorescent light. At the end of the eighth day in storage, off-odor was detected on beef wrapped with Visten-C and held under either type of light.

Ultraviolet-irradiation of cellophane prior to packaging resulted in a great reduction in development of surface flora on beef stored under germicidal light (Figure 31). Pre-exposure of cellophane to

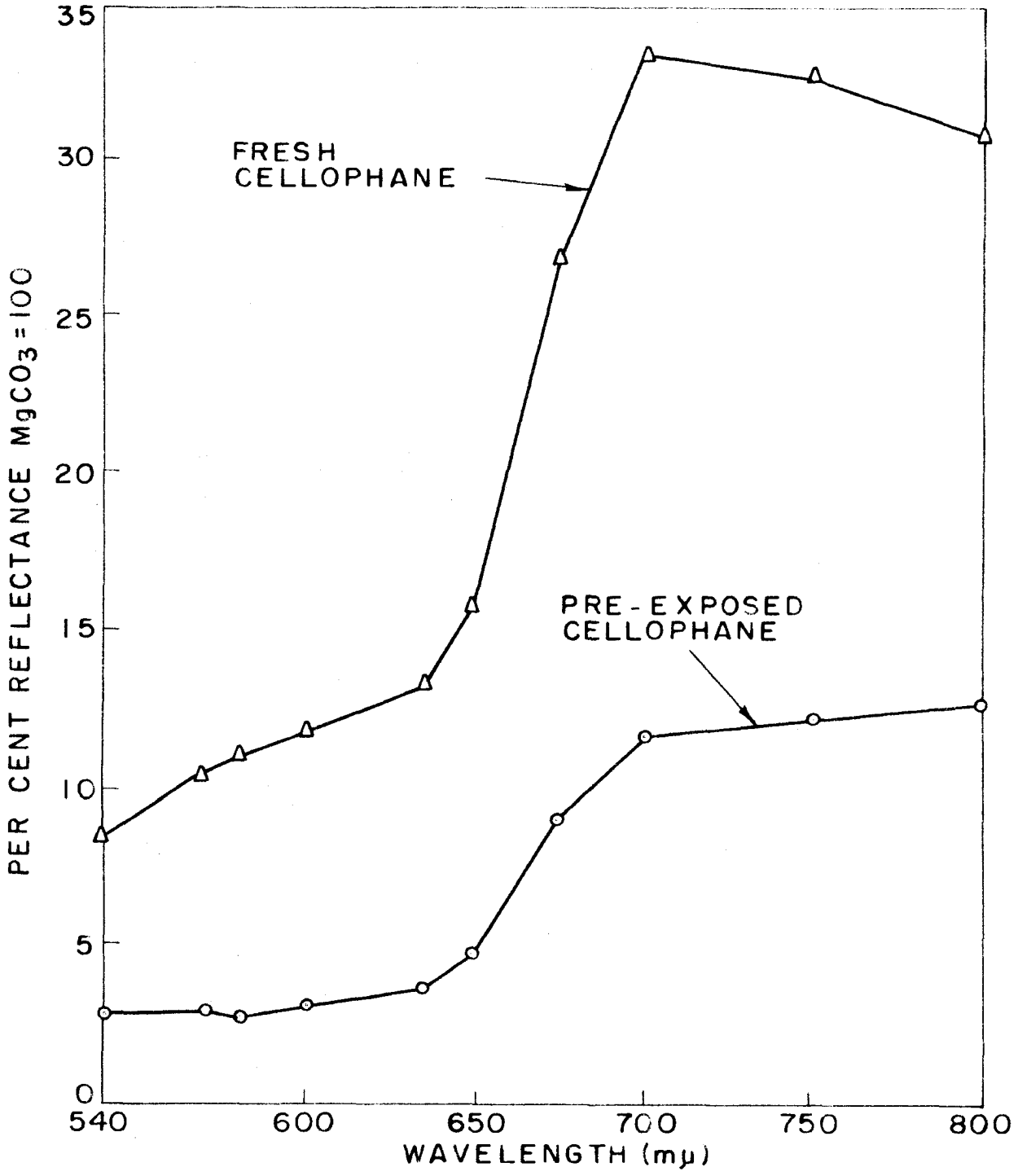


Figure 29. Effect of preliminary exposure of cellophane to ultraviolet radiation on reflectance* of packaged beef

*Each point is the mean of reflectance for two samples measured at 2, 5, and 8 days

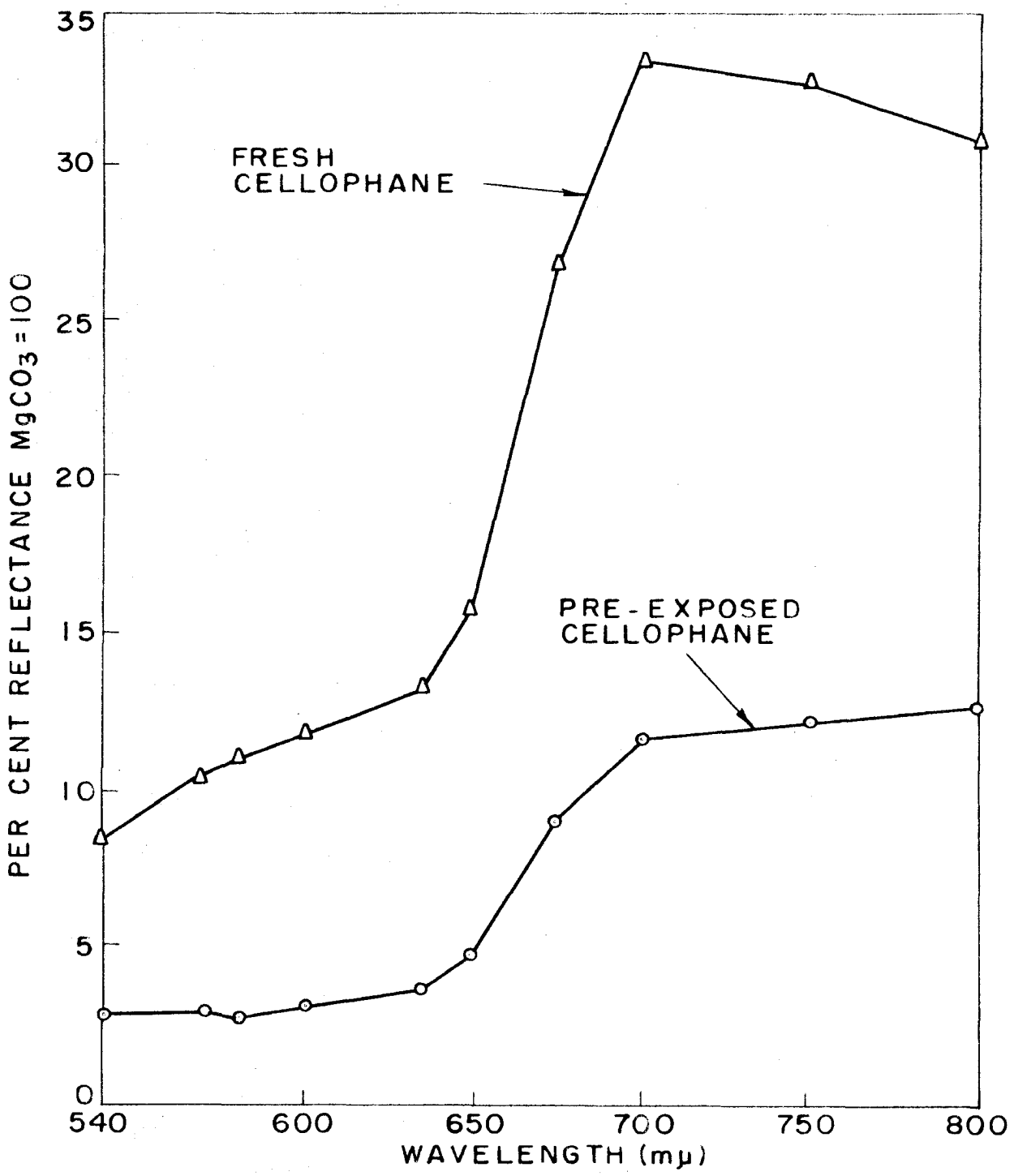


Figure 29. Effect of preliminary exposure of cellophane to ultraviolet radiation on reflectance* of packaged beef

*Each point is the mean of reflectance for two samples measured at 2, 5, and 8 days

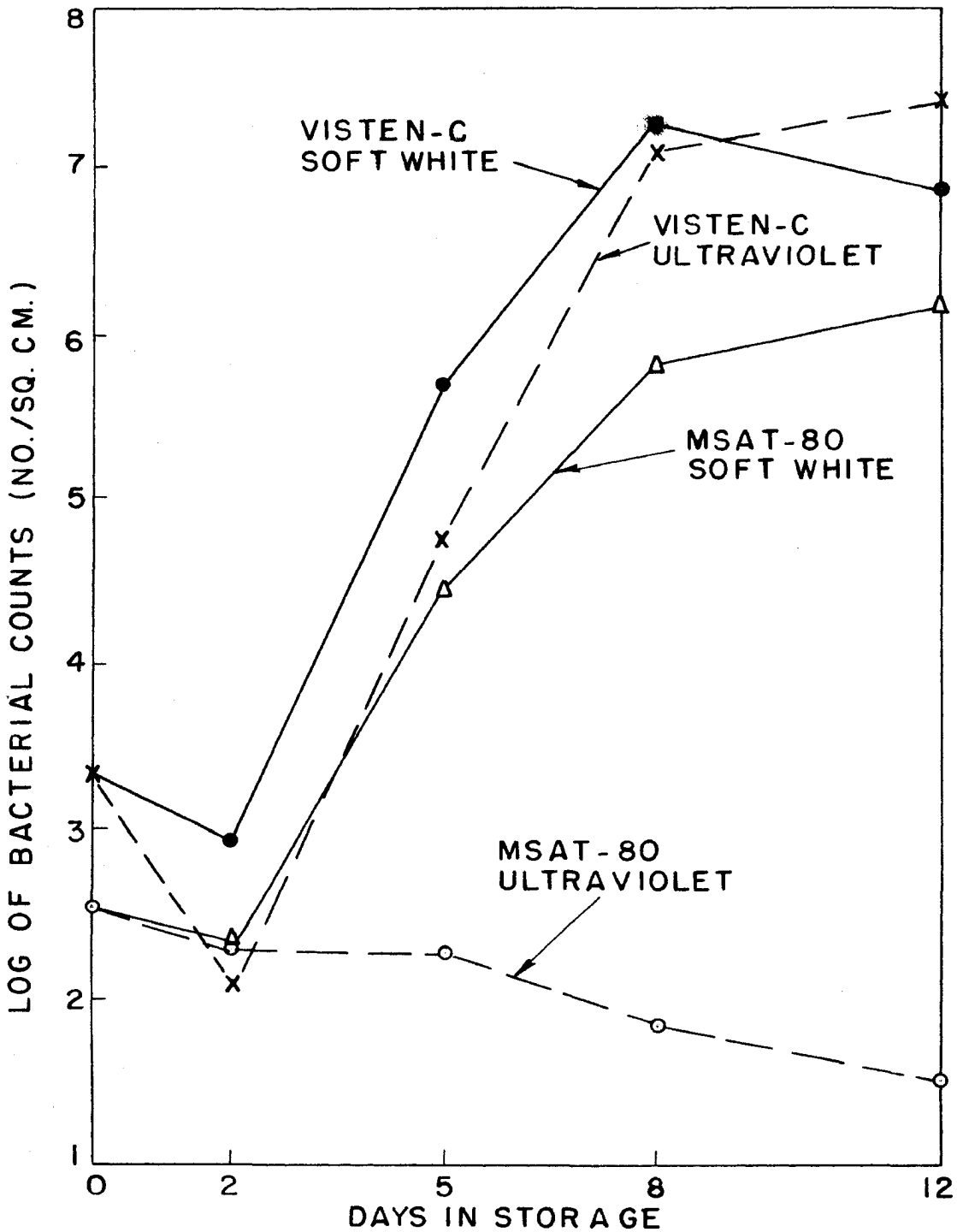


Figure 30. Relation of packaging materials and type of light to bacterial growth on beef

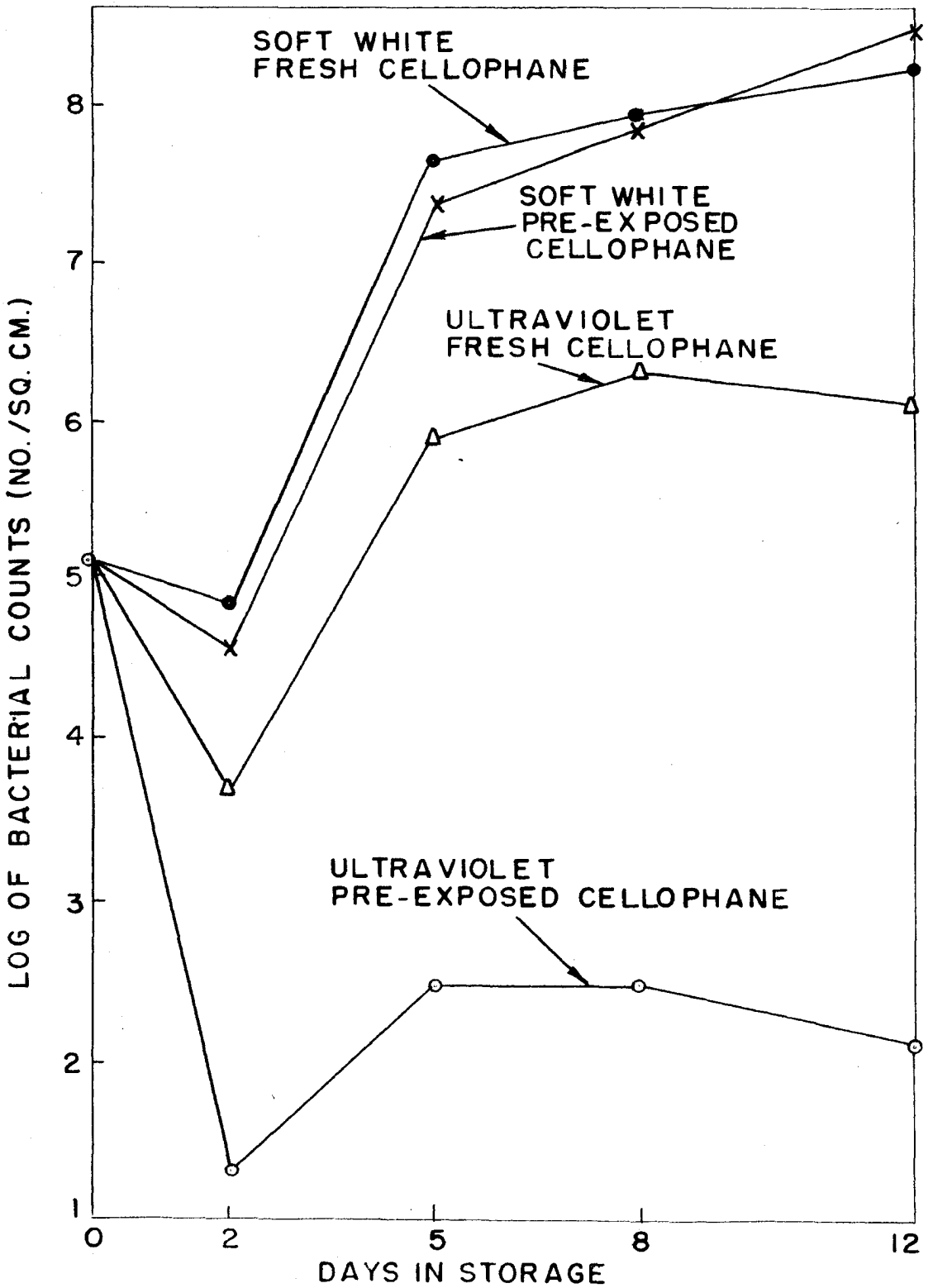


Figure 31. Relation of previous irradiation of cellophane and type of light to bacterial growth on beef

Soft White fluorescent light did not appreciably affect bacterial growth on meat wrapped with material receiving this treatment and held under the fluorescent lamp.

When initial counts were rather high, ultraviolet radiation appeared to be most effective in causing reduction of bacterial numbers during the lag period. This is illustrated in Figures 30 and 31 for counts obtained from cellophane-wrapped beef. Figure 30 also shows this effect when the average initial count and the count made at 2 days from meat wrapped with Visten-C is compared with those from samples packaged with MSAT-80 cellophane. With display under either type of light, lower counts during the lag phase generally were associated with fewer organisms during later periods of bacterial growth. The relation between initial loads and inhibition of surface organisms on beef exposed to ultraviolet light was similar to that observed by Ogilvy and Ayres (1951a) for chicken treated with CO_2.

Presumably, the intensity of ultraviolet radiation on the surface of meat wrapped with previously irradiated cellophane was greater than that which existed when freshly cut cellophane was used. The higher intensity of the germicidal radiation was probably responsible for more marked retardation of bacterial development than that found when untreated cellophane was employed. Carrying this one step further, decrease in counts during prolonged storage may have been due to increase in germicidal light intensity. The action of ultraviolet radiation in reducing bacterial numbers during the lag period was consider-

ably enhanced by preliminary irradiation of the packaging film (Figure 31).

Variation in resistance of surface organisms to ultraviolet radiation may account for the recovery manifested by increase in numbers of bacteria on beef immediately after the lag phase elapsed. Rentschler et al. (1941) found that, at low intensities of ultraviolet radiation, the rate of reproduction of resistant organisms was more rapid than was that of destruction. Evidently, when the initial contamination of beef was great, more of the resistant types were present than those occurring when the initial count was low.

c. Influence of light intensity on bacterial growth. Studies on the effect of light intensity were limited to observations made from packaged beef exposed to 150 and 50 footcandles of Soft White fluorescent light. Very little difference was found in bacterial numbers on meat held at these intensities for periods as long as 21 days. Counts are given in Table 16.

Surface bacterial counts were somewhat erratic and no definite trend in development of organisms was observed. Freezing temperatures were approached during storage and under these conditions the numbers of bacteria remained relatively low. Similar to its influence on color and reflectance, intensity of fluorescent light apparently was unimportant in affecting bacterial growth on packaged beef.

d. Discussion. It has been recognized for some time that ultraviolet radiations exert a germicidal effect and that these radiations also cause oxidation of myoglobin of fresh meats. However, no work

has been reported on the action of germicidal ultraviolet light in causing desiccation of fresh meats. In the present study, it was found that irradiation of packaged beef with this type of light produced marked dehydration of the meat. Further, loss of moisture resulting from irradiation appeared to be responsible for much of the darkening of the meat pigment and for retardation of surface bacterial growth.

Although only two materials were tested using germicidal ultraviolet light, results indicate that benefits derived from reduction in bacterial counts are nullified by accompanying darkening of color and desiccation. The extent to which these changes occur depends in large measure on the properties of packaging films. Light transmission, permeability to moisture vapor and gases, and stability to ultraviolet radiation are characteristics of materials that affect discoloration and development of surface organisms on beef exposed to germicidal ultraviolet radiations. In view of the findings given here, application of ultraviolet light in self-service display cases does not appear to be practical for retailing fresh meats.

Further attention should be given to factors responsible for color changes in fresh meats displayed under different types of light. While reduction and oxidation of myoglobin and drying may occur simultaneously, the importance of these changes in producing discoloration varies considerably. At the end of 12 days, cellophane-packaged beef displayed under Soft White fluorescent light demonstrated a weight loss of almost 20 per cent of the initial wet weight; the

Table 16. Logarithmic averages of bacterial counts from packaged beef exposed to 150 and 50 footcandles of Soft White fluorescent light*

Packaging material	Light intensity (foot-candles)	No. of sam-plings	Days in storage					
			0	2	5	8	12	21
			Logarithmic averages of counts					
MSAT-80	150	8	2.813	3.171	3.119	3.052	2.861	3.372
cellophane	50	8	2.813	2.902	2.825	3.363	3.245	2.336
Pliofilm	150	2	2.989	3.833	4.295	4.114	4.057	-
FF-120	50	2	2.989	4.041	3.699	3.484	3.431	-
Polystyrene	150	2	2.989	4.382	3.728	3.695	3.477	-
	50	2	2.989	4.369	3.947	3.544	3.816	-

*Storage conditions:
Victor refrigerator with top open
Temperature 1.5°C. ± 1°
Relative humidity 50 to 65%

color was dark brown, and the reflectance value was 13.5. It is significant that ultraviolet irradiation caused a similar decrease in reflectance after only 2 days, at which time the weight loss was approximately 3 per cent. Also, the color of beef subjected to ultraviolet light for 2 days very closely resembled that of meat held under Soft White fluorescent light for 12 days. From this it may be concluded that the ultraviolet light effected rapid oxidation of myoglobin to produce marked discoloration early in the storage period, whereas similar discoloration with display under fluorescent light required a longer time and resulted from a combination of desiccation and oxidation of the pigment.

A point of interest brought out by this work concerns the relation of light intensity to the degree of discoloration resulting from the use of different packaging films. Examination of Figures 21, 27, and 28 reveals that MSAT-80 cellophane permitted discoloration to reach a more advanced state than that noted when the laminate of polyethylene and cellophane was employed. Apparently, the properties of materials exerted a greater influence on color than did various intensities of light. Changes in color of beef wrapped with cellophane undoubtedly were affected by desiccation; such meat lost considerably more moisture than was observed when the laminated film served as the wrapper. In consideration of these findings, it seems that for a single type of illuminant, intensity of visible light is relatively unimportant in influencing the course of discoloration of packaged fresh meat. It was not determined whether this relation holds for all kinds of visible light. From the practical aspect, display of fresh meat in self-service markets could be carried out using high intensities of Soft White fluorescent light without the occurrence of undue discoloration. As pointed out in a previous section, some doubt exists relative to the effect of light intensity when Visten-C was used for wrapping beef exposed to Soft White fluorescent light and to ultraviolet light. In this instance, the possibility should not be ruled out that factors other than intensity of illumination were responsible for improved color retention with display under ultraviolet light.

Reports in the literature (Pracejus, 1949; Ramsbottom et al., 1951) have mentioned that fresh meats are very resistant to "fading" on exposure to light. The term should not imply a bleaching of color, since in all the work presented here, discoloration of fresh meat was evidenced by darkening of the pigment. Decrease in intensity of color was never observed in fresh meats displayed under light.

B. Storage of Packaged Cured Meats

1. Effect of gas and vacuum packaging

a. Absorption of CO_2 by meat. Work dealing with CO_2 uptake of cured meats was confined to frankfurters. In this trial, the ratio of meat to gas averaged 0.42 grams per ml. Carbon dioxide uptake is given in Table 17.

Table 17. Absorption of CO_2 by frankfurters stored at 4.4°C.

CO_2 conc. (%) in packages	Days in storage	CO_2 uptake* (ml./g.)
95.0	0	-
94.6	2 (pkgs. gassed)	-
82.6	4	0.340
81.8	7	0.543
64.4	14	0.524
38.6	21	0.505

*Calculated for 25°C.

In a later section, it will be shown that absorbed CO_2 caused an increase in storage life of frankfurters initially packaged in a high concentration of the gas and later stored in air.

b. Color changes. (1) Time for color fading. The term "fading" as used with reference to color of cured meats denotes a decrease in intensity of color. Oxidation of nitric oxide myoglobin or of nitric oxide myochromogen results in a faded pink, gray, or brown appearance. A number of tests were carried out in order to compare the time for fading of picnic ham packaged in air, in nitrogen, in CO_2, and under vacuum. The meat was exposed to various intensities of Soft White fluorescent light. Saran-coated cellophane was employed in all trials with picnic ham. This material was found to be well-suited for gas and vacuum packaging and also has a fair degree of transparency. Table 18 presents data for time of fading and exposure of picnic ham treated as described above. The influence of nitrogen in increasing the time and exposure required for fading is shown in Table 19.

Retardation of color fading in picnic ham by packaging in an atmosphere of nitrogen appeared to be proportional to light intensity. In general, the use of nitrogen resulted in greater protection of color at higher light intensities than was observed when the meat was displayed at lower intensities. However, values given in Table 18 indicate that, in the presence of either air or nitrogen, discoloration occurred earlier at high light intensities than it did when low intensities were used. When display was carried out at 30 footcandles

Table 18. Light intensity and time for visible fading of picnic ham packaged with Saran-coated cellophane and displayed under Soft White fluorescent light*

Atmosphere	Light intensity (footcandles)	Average time for fading (hours)	Exposure (footcandle-hours)
Air	150	12	1800
	120	12	1440
	70	30	2100
	50	48	2400
	40	48	1920
	30	60	1800
	25	96	2400
	10	144	1440
	0 (in dark)	504	-
Nitrogen	150	36	5400
	70	78	5460
	50	90	4500
	30	90	2700
	25	120	3000
	10	156	1560
	0 (in dark)	504	-
CO_2	30	90	2700
	0 (in dark)	504	-
Vacuum	30	90	2700

*Storage conditions:
Victor refrigerator with top open
Temperature 1.5°C. ± 1°
Relative humidity 50 to 65%

Table 19. Effect of nitrogen in prolonging color retention in picnic ham exposed to Soft White fluorescent light

Light intensity (footcandles)	Increase in time for fading over that in air (hours)	Increase in exposure for fading over that in air (footcandle-hours)
150	24	3600
70	48	3300
50	42	2100
30	30	900
25	24	600
10	12	120

of Soft White fluorescent light, meat packaged in nitrogen, in CO_2, or under vacuum, retained its color for equal lengths of time; in all cases the time before fading occurred was longer than it was when picnic ham was packaged in air. With frankfurters, no discoloration was observed when CO_2 was used and the packaged meat was stored in the dark for 28 days at 4.4°C.

As stated previously, the ratio of reflectance at 650 mμ to that at 570 mμ decreases as fading of cured meats progresses. In tests wherein color changes of packaged bologna were evaluated, reflectance ratios and scores given by judges were employed as measures of discoloration. Scores for controls were established using the reflectance ratios of these samples in accordance with procedures described in Methods. Values for reflectance ratios and judges' scores for color of sliced bologna packaged in air and under vacuum appear in Table 20.

Table 20. Effect of vacuum packaging on fading of sliced bologna displayed under Soft White fluorescent light*

Days in storage	Mean** of judges' scores		Mean** of reflectance ratios (650/570 mμ)	
	Air	Vacuum	Air	Vacuum
0	7.8	7.8	2.18	2.18
1	4.5	4.9	1.59	1.59
2	3.8	3.9	1.48	1.54
3	4.0	4.0	1.42	1.49
6	3.3	3.3	1.43	1.46

*Storage conditions:
Light intensity 45 to 60 footcandles
Packaging material Saran laminated to cellophane
Percival open-type display case
Temperature 2.5° C. ± 1°
Relative humidity 40 to 75%

**Mean of values for two samples

Vacuum packaging did not appreciably retard fading of sliced bologna packaged with Saran laminated to cellophane and exposed to fluorescent light. It should be noted that, in this study, an initial vacuum of about 28.5 inches of Hg was obtained in the packages; this value was less than the initial vacuum of 29.0 inches of Hg specified by Winans (1950) as requisite for maintenance of color of cured meats. At the end of 6 days, the vacuum decreased to approximately 27.0 inches of Hg, a level greater than the minimum of 24.0 inches of Hg cited by Winans for stored packages. From measurements of spectral reflectance and from scores given by the judges, the rate of fading was found to be greatest during the first day of exposure to light regardless of whether

packages were evacuated or sealed under atmospheric pressure. Similar to the results obtained with Saran laminated to cellophane, when LSAT cellophane served as the packaging material, differences in color and reflectance of bologna held under vacuum and in air were only negligible.

(2) Degree of discoloration. The use of nitrogen, CO_2, or evacuation of air allowed fading of picnic ham and bologna upon exposure of the meat to light, but the extent of this discoloration was not as great as that which occurred when samples were wrapped in air. Fading of color in air generally passed through stages of faded pink to gray and faded brown rather rapidly. With atmospheres of CO_2 or nitrogen, or with vacuum packaging, discoloration was not as pronounced until storage time had progressed further. Evacuation or replacement of air with nitrogen or CO_2 usually resulted in a faded pink appearance of of the meat at the time when samples packaged in air exhibited a faded brown color.

c. Storage life. (1) Keeping times and bacterial counts. When Pliofilm FF-120 was used as the packaging material, frankfurters treated with CO_2 and stored at 7.2°C. had a storage life approximately 1.5 times that of control samples packaged in air.(Table 21). Bacterial counts are listed in Table 22. Incipient spoilage was indicated by mold growth, which occurred on control frankfurters after 16 days in storage, but was not observed on treated samples until the 26th day. An interesting feature noted was that the concentration of CO_2 in control packages was approximately equal to that of the gassed packages at the

time control frankfurters showed growth of mold. This raised the possibility that the CO_2 had been lost from the treated packages, and that lengthened storage life of gas-packaged samples was due to diffusion of CO_2 from the frankfurters to the atmosphere of the packages.

Frankfurters packaged with aluminum foil laminated to Pliofilm and subjected to an initial CO_2 concentration of about 95 per cent in the packages did not give evidence of mold until 19 days after samples were unwrapped and held in air; the keeping time for these frankfurters was 40 days. Meat wrapped with the same material and stored in air continuously showed visible slime after 21 days. Storage life at 1.5°C. was prolonged by 19 days as a result of initial packaging in a high concentration of CO_2.

Additional experiments on the action of CO_2 in extending storage life of packaged frankfurters were conducted using Flexvac and the laminate of aluminum foil and Pliofilm. Table 21 shows keeping times and storage indices obtained when these materials and Pliofilm FF-120 were employed. In these tests, the ratio of meat to gas averaged 0.27 grams per ml.

Bacterial growth curves (Figure 32) illustrate that the preliminary gas exposure of frankfurters before packages were sealed resulted in a delay in germination of bacteria as compared with control samples not subjected to such treatment. In the case of both packaging materials, the rates of growth of surface microorganisms were greater with meat packaged in air than with samples wrapped in an atmosphere of CO_2.

Table 21. Effect of CO_2 on storage life of packaged frankfurters

Packaging material	Storage temp. (°C.)	Keeping time in air (days)	Keeping time in CO_2 (days)	Storage index
Pliofilm FF-120	7.2	16*	26*	1.6
Flexvac	4.4	9**	13**	1.4
Aluminum foil-Pliofilm laminate	4.4	9**	13**	1.4

*End point determined by appearance of mold

**End point determined by slime formation

Table 22. Logarithmic averages* of bacterial counts from frankfurters packaged with Pliofilm FF-120 and stored at 7.2°C.

Atmosphere	Days in storage					
	1	3	7	12	17	22
	Logarithmic averages of counts					
CO_2	2.792	5.000	4.633	4.763	5.832	7.845
Air	2.968	5.204	5.204	6.255	8.079	10.623

*Log averages of numbers of bacteria per frankfurter for two samples
Log average initial count 2.919

Control samples were considered to be spoiled after 9 days in storage, at which time a watery slime was observed. Gas-treated frankfurters did not show sliming until the 13th day.

In preliminary experiments, it was found that keeping time of packaged picnic ham was prolonged by treatment with CO_2 or by evacu-

ation of air. Keeping time in an atmosphere of nitrogen differed little from that in air. Further work was undertaken along these lines and results are summarized in Table 23.

Table 23. Effect of gas and vacuum packaging on storage life of picnic ham*

Atmosphere	Keeping time** (days)	Storage index
Air	14	-
Nitrogen	14	1.0
Vacuum	28	2.0
CO_2	28	2.0

*Storage conditions:
Victor refrigerator with top open
Light intensity 30 footcandles Soft White fluorescent light
Temperature 1.5°C. ± 1°
Relative humidity 50 to 65%

**Keeping time determined by onset of off-odor

Growth curves of surface bacteria are presented in Figure 33. Off-odor on picnic ham was associated with counts ranging from 4×10^6 to 1.0×10^7 bacteria per square centimeter of surface. Development of surface flora was less profuse on meat packaged under vacuum or in CO_2 than it was when the atmosphere of packages consisted of air or nitrogen. The use of CO_2 or vacuum packaging resulted in extension of the lag period. Growth of bacteria on packaged picnic ham held in nitrogen approximated that found when the meat was stored in air. With vacuum packaging, counts were similar to those obtained from meat packaged in a high concentration of CO_2. These findings do not support

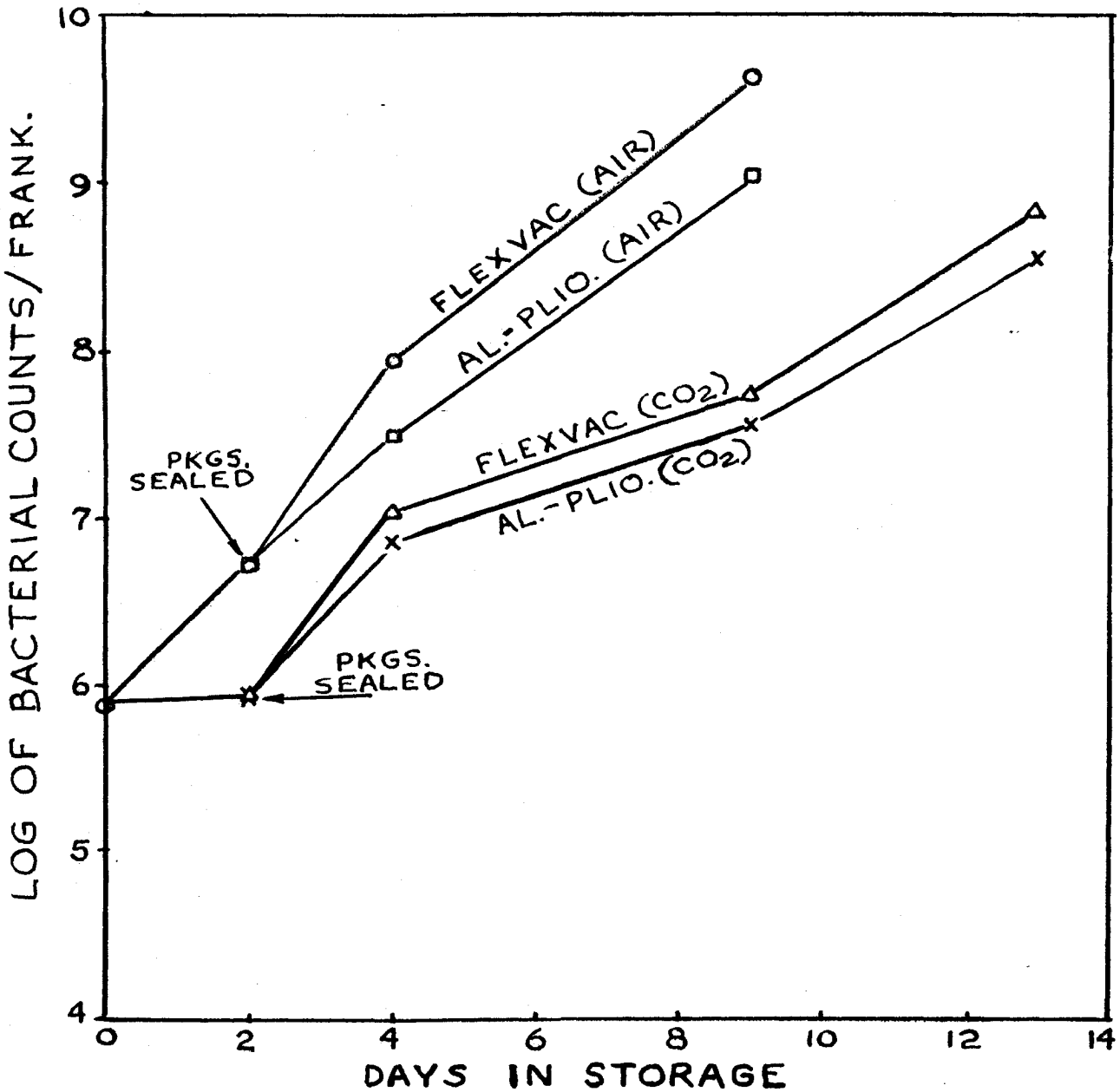


Figure 32. Relation of packaging materials and CO_2 to bacterial growth* on frankfurters stored at 4.4°C.

*Surface bacterial counts of frankfurters having approximately a 90 sq. cm. surface area and weighing about 50 g.

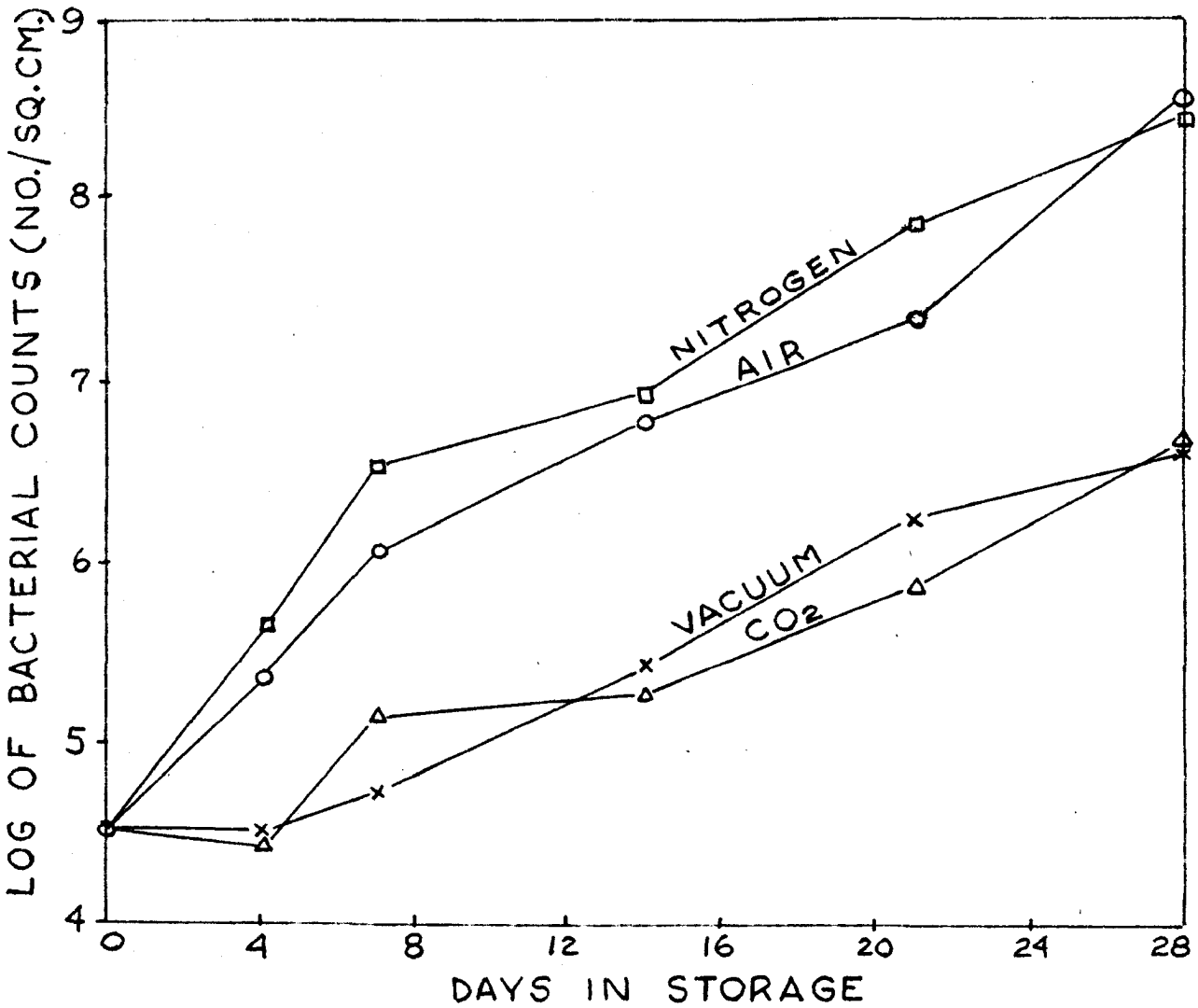


Figure 33. Effect of gas and vacuum-packaging on bacterial growth on picnic ham

the view of Garnatz (1950) that vacuum packaging resulted in conditions favorable for bacterial growth on cured meats.

(2) Changes in gas concentration. Decrease in levels of gas originally added in high concentrations or loss of vacuum occurred during storage of packaged cured meats (Appendix Tables 36 through 39). In view of the fact that bacterial counts were lower on control frankfurters packaged with aluminum foil laminated to Pliofilm and the levels of gas were higher than with Flexvac (Appendix Table 37), the former material was probably less permeable to CO_2 than was Flexvac. Both laminated materials were effective in retaining CO_2 within packages of frankfurters. Oxygen concentration increased during storage of picnic ham packaged in CO_2 or in nitrogen (Appendix Table 38). In general, the level of CO_2 became greater as holding time of control samples wrapped in air progressed; this was believed due to the action of microorganisms.

d. Discussion. Color protection of cured meats packaged with an inert gas such as nitrogen, or with CO_2, appeared to depend on retention of the gas within packages. Similarly, when vacuum packaging was employed, as the vacuum decreased during storage, fading took place on exposure of bologna or picnic ham to light. Although a material considered to be an excellent barrier to oxygen transfer was used in tests with sliced bologna, incomplete evacuation of air did not succesafully maintain the original color of the meat. Fading with display under fluorescent light was attributed to the action of the small amount of

residual oxygen in the package after the evacuation process.

For retarding fading of cured meats in self-service refrigerated display cases, benefits derived from nitrogen packaging might be greater if the light intensity were high than if low intensities were used. It should be emphasized, however, that in this work discoloration was more rapid at high light intensities than it was at low intensities regardless of gas treatment.

A finding of interest related to the degree of discoloration observed with gas or vacuum packaging in comparison with that noted when cured meats were wrapped in air. From the point of view of consumer acceptance, it would seem that the faded pink color of gas treated or vacuum packaged meat would be more desirable than the faded brown exhibited by untreated meat. Of course, fading in any form lowers the acceptability of cured meats, and whether the above assumption is justifiable could be determined only by an adequate testing program.

It is not clear why vacuum packaging resulted in inhibition of bacterial growth on picnic ham. If lowering of oxygen tension was responsible for decreased growth of surface microorganisms, one might expect that nitrogen would produce similar effects. Larson et al. (1918) found that nitrogen was inert toward bacteria; the gas produced no killing nor did it cause any morphological changes in the cells tested. These findings and the work reported here are in conflict with the conclusion of Callow (1932) that pure nitrogen was almost as effective as CO_2 in retarding spoilage of cured meat. As indicated

previously, oxygen content increased during storage of packages in which atmospheres were initially composed of high levels of nitrogen; oxygen uptake may have been sufficiently great that surface bacterial growth was not retarded.

While evacuation of air resulted in inhibition of development of surface organisms to an extent similar to that produced by the use of CO_2, CO_2 may prolong storage life of meat even after the gas has been lost from packages. Therefore, packaging in CO_2 atmospheres may be presumed to have a more beneficial effect than that provided by vacuum packaging in the event that packages lose their gas-tight characteristics.

2. Effect of relative humidity

a. Color changes. Sliced bologna was packaged with LSAT cellophane and with Visten-C and stored at 10 and 95 per cent relative humidity for 12 days at 4.4°C. Bologna wrapped with cellophane and held at the high humidity developed a slightly faded pink color as storage time increased. With storage at 10 per cent relative humidity, the color changed from bright pink to dark red or red-brown during the holding period. When Visten-C was utilized as the wrapper, little difference was noted in color of samples stored at either 10 or 95 per cent relative humidity. The meat appeared faded pink in color after 12 days in storage, but this discoloration was inappreciable.

In a long term experiment, sliced bologna was wrapped with LSAT cellophane and with Saran laminated to cellophane and held for 39 days

at the two humidities previously specified. Temperature of storage was 4.4°C. Cellophane-packaged meat held at 10 per cent relative humidity was severely dehydrated at the end of the storage period and presented a dark brown appearance. On the other hand, bologna wrapped with the laminated film was only slightly discolored after the same holding period at the low humidity. At 95 per cent relative humidity, meat wrapped with cellophane became dark red while bologna packaged with the laminate appeared faded pink in color.

b. Desiccation. Excessive moisture losses were observed when sliced bologna was packaged with LSAT cellophane and held at 10 per cent relative humidity. Dehydration was not as severe with Visten-C or with Saran laminated to cellophane when storage was carried out at the low humidity. At 95 per cent relative humidity, moisture content of packaged bologna was retained to a considerable degree with all materials tested. Table 24 gives data for weight losses of sliced packaged bologna stored at 10 and 95 per cent relative humidity for 12 days.

Storage of cellophane-wrapped bologna at 10 per cent relative humidity for 39 days resulted in weight loss approaching 50 per cent of the initial wet weight, while at 95 per cent relative humidity, about 3 per cent of the initial weight of the meat was lost. When bologna was packaged with Saran laminated to cellophane, weight losses were less than 5 per cent after 39 days' storage at either humidity. The relation of packaging materials to relative humidity in packages and consequent effects on color of sliced bologna will be discussed further in a later section.

Table 24. Effect of relative humidity and packaging materials on weight losses* of sliced bologna stored at 4.4°C.

Packaging material	Relative humidity (%)	Days in storage 3	6	9	12
		Per cent weight loss			
LSAT cellophane	10	17.1	25.6	37.7	42.7
	95	0.00	0.00	0.23	0.16
Visten-C	10	0.67	1.54	2.27	3.03
	95	0.00	0.09	0.04	0.00

*Weight losses expressed as % initial wet weight; mean of two samples

c. Bacterial growth. At the end of 39 days, the numbers of bacteria per square centimeter of surface of bologna wrapped with LSAT cellophane were about one thousand times greater when storage was carried out at 95 per cent relative humidity than when 10 per cent relative humidity was employed. With Saran laminated to cellophane, little difference was observed with regard to bacterial counts at either high or low relative humidity. A thin, watery slime was detected on meat packaged with the laminate and bacterial counts were somewhat greater than 1×10^8 cells per square centimeter at the end of the holding period. Slime was also apparent on meat wrapped with LSAT cellophane and held at 95 per cent relative humidity for 39 days; counts were approximately 8×10^8 bacteria per square centimeter.

Surface bacterial counts obtained from bologna packaged with Visten-C and with LSAT cellophane are shown in Table 25.

Table 25. Effect of relative humidity and packaging materials on bacterial counts* from sliced bologna stored at 4.4°C.

Packaging material	Relative humidity of atmosphere (%)	Days in storage				
		0	3	6	9	12
		Logarithmic averages of counts				
LSAT cellophane	10	3.177	1.500	2.072	1.738	2.575
	95	3.177	1.984	2.458	3.200	5.270
Visten-C	10	3.491	4.018	4.272	4.498	5.956
	95	3.491	3.317	4.339	5.978	6.147

*Logarithmic averages of counts obtained from four samples

Lowering of relative humidity to 10 per cent resulted in marked decrease in numbers of organisms on meat wrapped with the cellophane. Bacterial counts were lower during the lag period when the meat was held at the low humidity than when storage was conducted at 95 per cent relative humidity. Differences in development of surface organisms were negligible at either humidity when Visten-C was used to package bologna; little or no decrease in numbers of bacteria was found on initial storage at 10 and 95 per cent relative humidity. Neither off-odor or slime was detected on sliced bologna after storage after 12 days at 4.4°C.

d. Discussion. It is unfortunate that adequate control of microbial growth on packaged meats by reduction of humidity may not be achieved without occurrence of undue discoloration and desiccation. Although moisture content of cured meats is less than that of fresh meats, drying of bologna stored at low humidity was objectionable and no practical advantage was gained from the use of such humidity. Materials that

retarded desiccation of sliced bologna were of no value in inhibiting bacterial growth; however, such materials did diminish the extent of discoloration. The performance of Saran laminated to cellophane when used as a wrapper for cured meat paralleled that found when the material was employed to package fresh meat. In either case, extremes of relative humidity had little influence on discoloration, desiccation, or bacterial growth. The interdependence of these three criteria of keeping quality was brought out in studies with packaged fresh meats; apparently the same relation holds for packaged cured meats.

3. Relation of light to changes in cured meats

a. Factors affecting color and reflectance. (1) Variation in product. Since the color of cured meats is standardized to some extent by the processes employed in manufacture (i.e., curing, cooking) less variation is to be expected in color and reflectance than that found with fresh meats. Variation in spectral reflectance of large bologna was determined from measurements made of eighteen samples (Appendix Table 40). Curves for mean reflectance (solid line) and for confidence interval estimates (broken line) are given in Figure 34. The reflectance properties of fresh slices of bologna purchased on different days did not vary to an appreciable extent, indicating that only slight differences existed in color of samples at the initiation of each experiment.

(2) Time of exposure. The time required for fading of picnic ham packaged in various gaseous atmospheres and exposed to Soft White fluores-

cent light having different intensities was described in a previous section. When sliced bologna was packaged with LSAT cellophane and displayed under Soft White fluorescent light at an intensity of 55 to 65 footcandles, perceptible fading occurred after 1 hour. In Figure 35, ratios of reflectance at 650 mμ to reflectance at 570 mμ for samples held in the dark and for meat exposed to light are plotted against storage time. Fading was indicated by change in intensity of color from bright pink to pale pink, and by decrease in reflectance. The most rapid change in color took place during the first 4 or 5 hours at the intensity specified. On the other hand, sliced bologna held in the dark showed little decrease in reflectance during storage; fading was not visible at the end of 21 hours, but occurred to a slight extent by the end of 55 hours.

(3) Transmission of light by materials. As shown in Figure 36, LSAT cellophane transmitted a higher proportion of light than did either polyethylene laminated to cellophane or Saran laminated to cellophane. LSAT cellophane demonstrated a rather sharp decline in the region below 380 mμ, while the less transparent laminated materials evidenced a more gradual decrease in light transmitted. Since fading is proportional to light intensity, it may be expected that the use of highly transparent wrappers would result in rapid discoloration of cured meats packaged with such materials and exposed to light. With all films tested, the amount of visible light transmitted was greater than that of ultraviolet light.

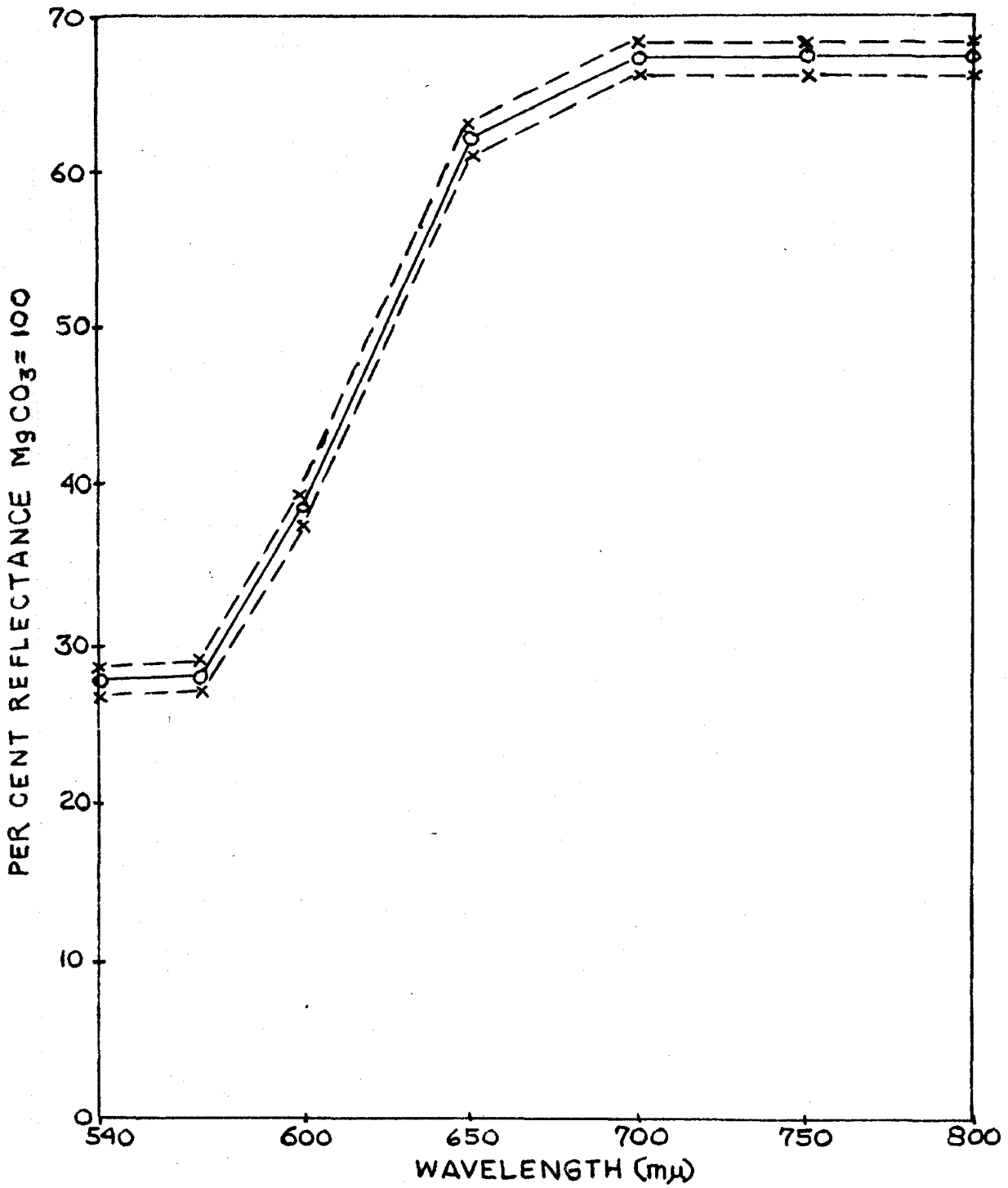


Figure 34. Spectral reflectance of fresh sliced large bologna

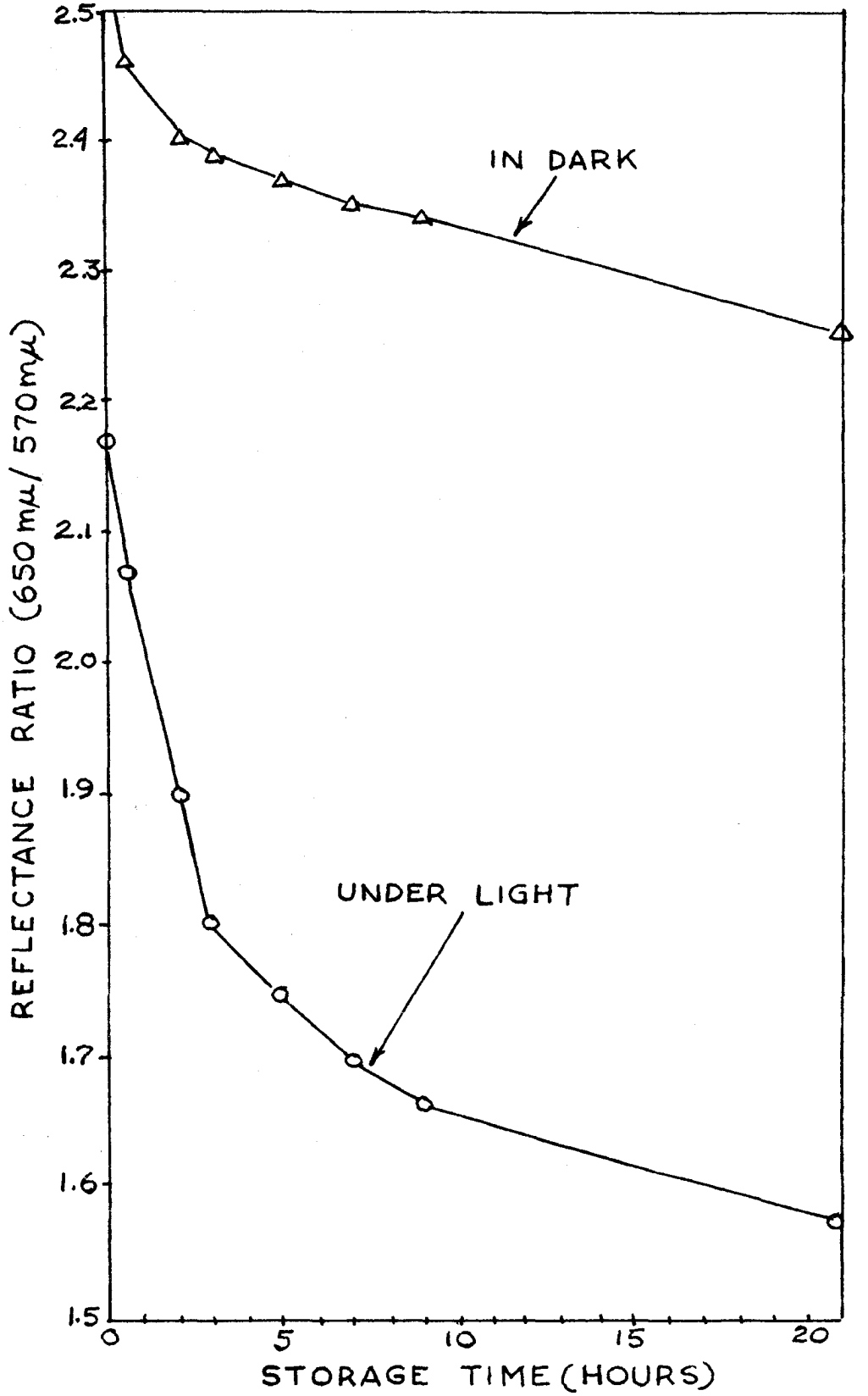


Figure 35. Relation of light and time of exposure to fading of sliced bologna

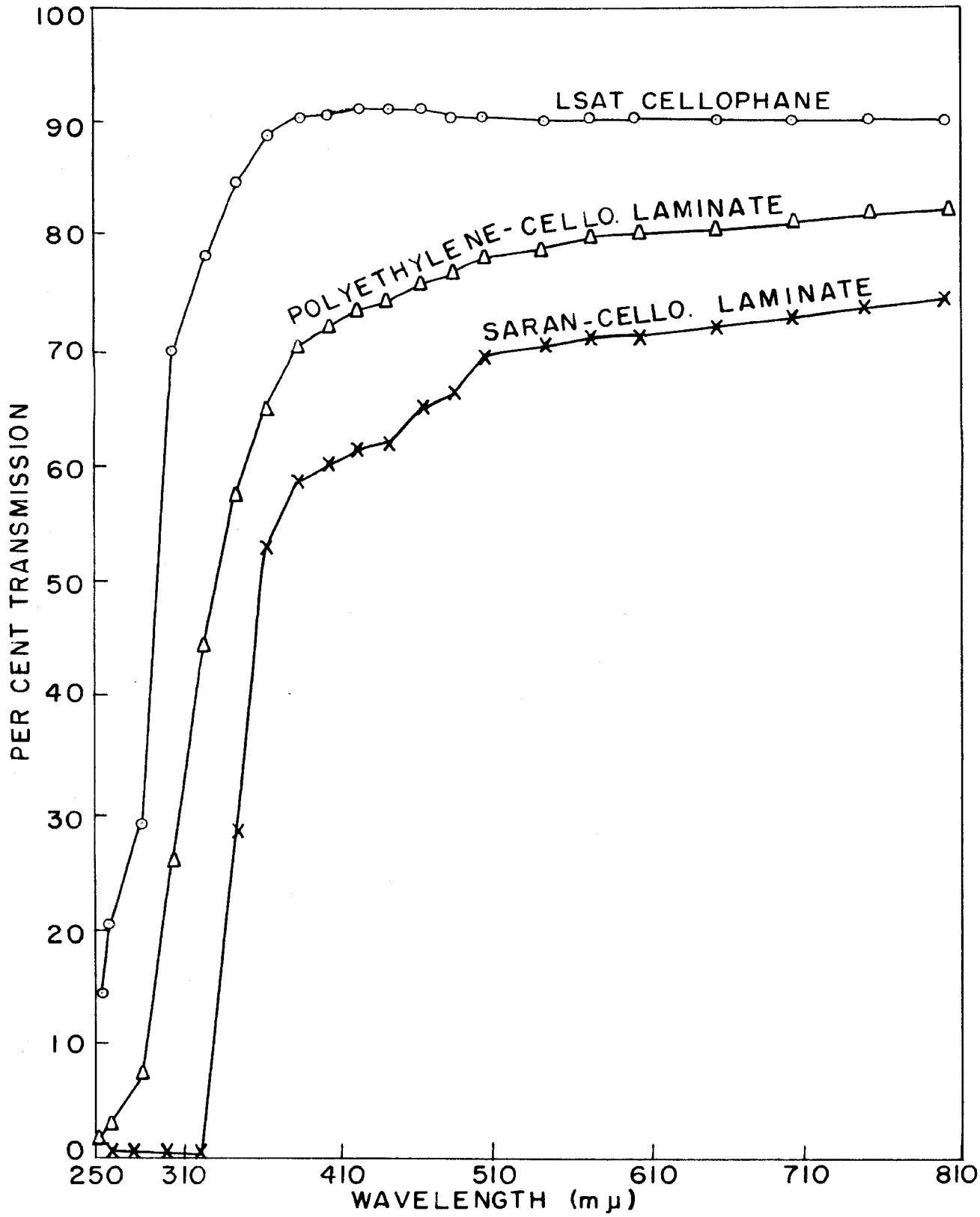
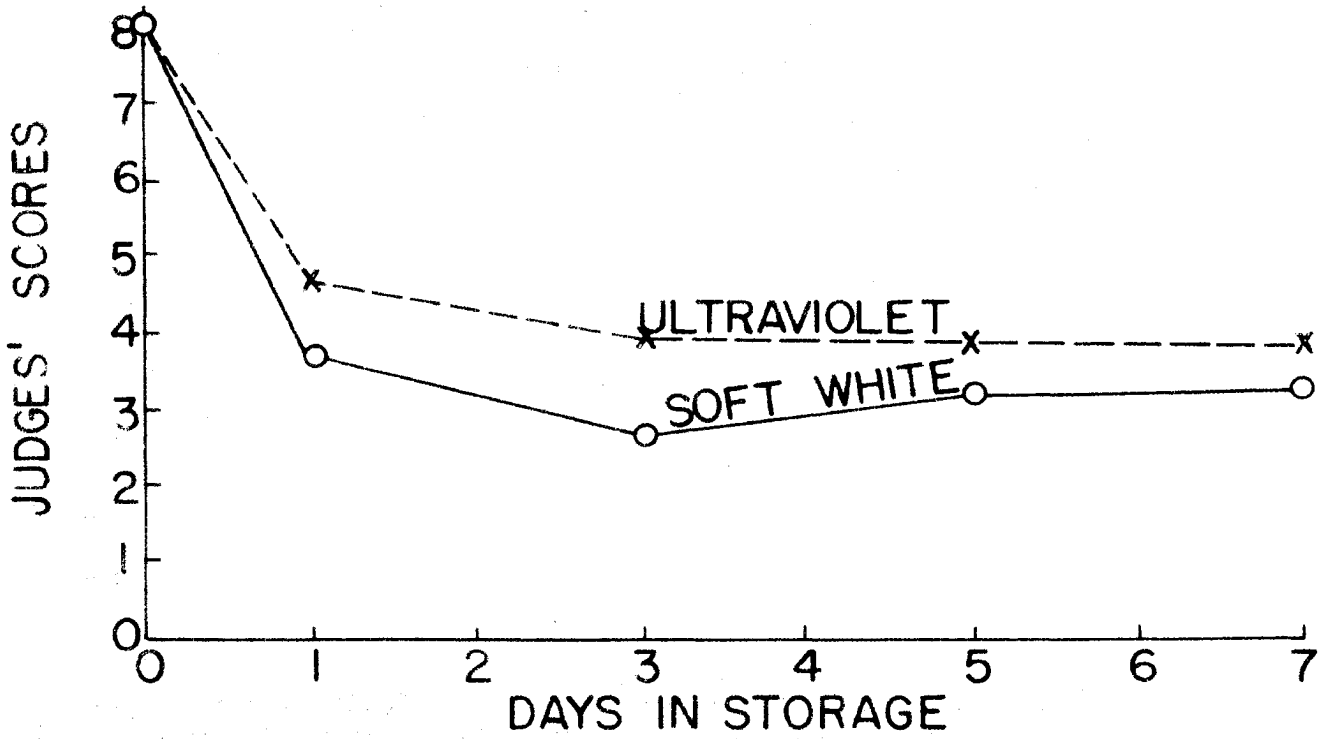


Figure 36. Transmission of light by LSAT cellophane, polyethylene laminated to cellophane, and Saran laminated to cellophane

(4) Type of light. The effect of ultraviolet light compared with that of Soft White fluorescent light in producing changes in color and reflectance of sliced bologna is depicted in Figure 37. Again, it should be mentioned that intensity of ultraviolet radiation on meat samples was less than that furnished by fluorescent light. When bologna was packaged with polyethylene daminated to cellophane and stored under both types of lights, fading was less pronounced and proceeded at a slower rate on exposure to germicidal ultraviolet light than was observed after display under Soft White fluorescent light. Similar to the results obtained with the laminate, sliced bologna packaged with LSAT cellophane and exposed to ultraviolet light showed less fading than that resulting from display under fluorescent light.

Picnic ham packaged with Saran-coated cellophane did not discolor as rapidly on exposure to ultraviolet light as it did when displayed under Soft White fluorescent light. After exposure to ultraviolet radiation, initial fading was evidenced by a pale pink color which was observed at the time when meat held under fluorescent light appeared faded brown. With either type of light, fading was noticeable after about 2 days. Average reflectance values for picnic ham stored for 28 days are given in Table 26. In general, reflectance was higher for meat displayed under ultraviolet light than it was when Soft White fluorescent light was used.


JUDGES' SCORES
8
7
6
5
4
3
2
1
0
ULTRAVIOLET
SOFT WHITE
0
1
2
3
4
5
6
7
DAYS IN STORAGE

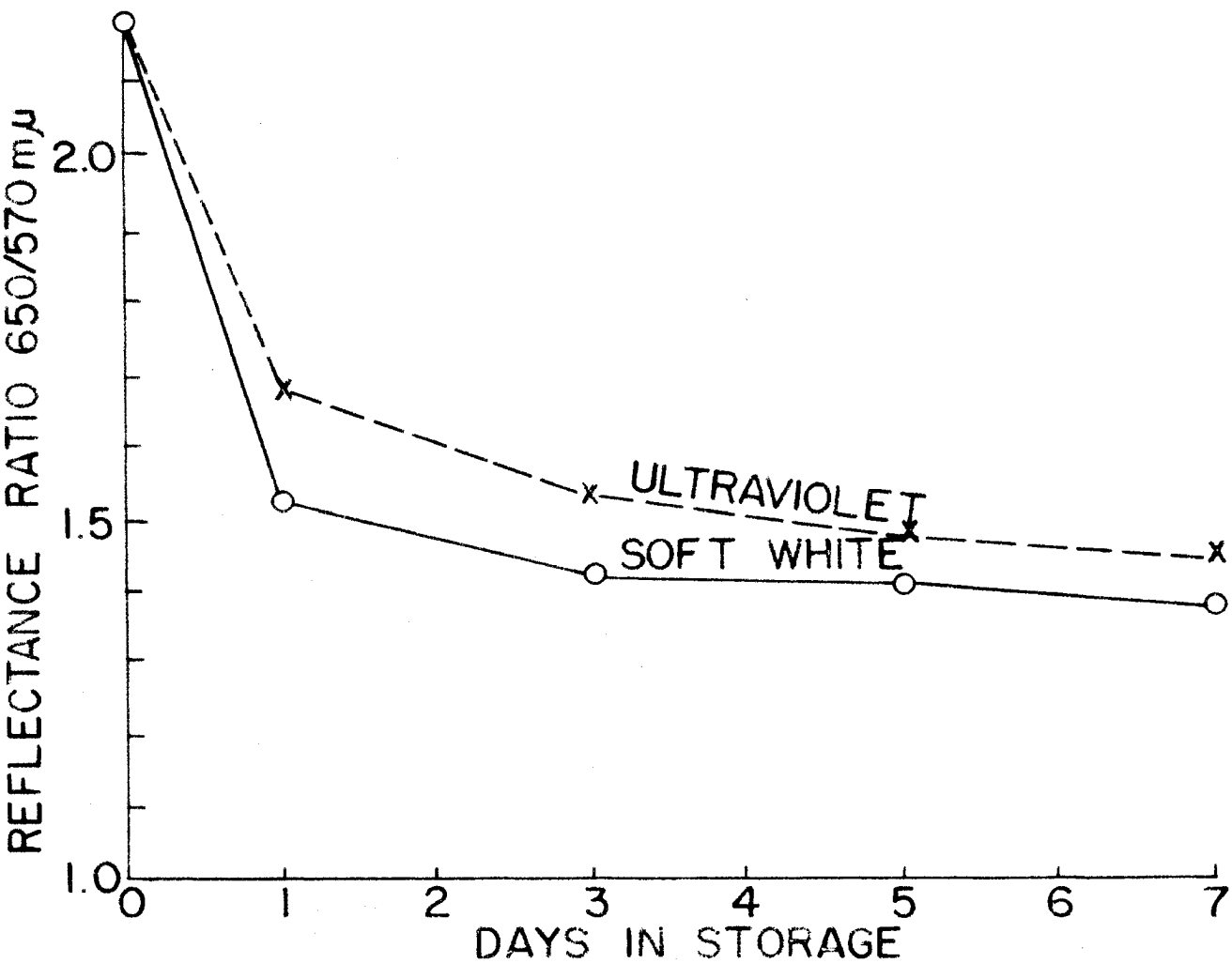

REFLECTANCE RATIO 650/570 mμ
2.0
1.5
1.0
ULTRAVIOLET
SOFT WHITE
0
1
2
3
4
5
6
7
DAYS IN STORAGE

Table 26. Effect of type of light on reflectance* of packaged picnic ham**

Type of light	Wavelength (mμ)						
	540	570	600	650	700	750	800
Ultraviolet	22.6	23.3	28.6	42.2	49.7	48.1	48.0
Soft White	21.4	23.9	27.4	36.3	44.0	46.2	47.0

*Values are means of reflectance of four samples measured at 2, 7, 14, 21, and 28 days

**Storage conditions:
Ultraviolet light intensity 7 to 10 footcandles
Soft White light intensity 40 to 45 footcandles
Percival open-type display case
Temperature 2.5°C. ± 1°
Relative humidity 40 to 75%
Packaging material Saran-coated cellophane

Discoloration of the fat of slices of picnic ham was more pronounced after exposure to ultraviolet radiations than was noted after display under Soft White fluorescent light. The color of fat of ultraviolet-irradiated samples was brown after exposure for 28 days; with meat held under fluorescent light, the fat appeared cream-white.

Desiccation was not appreciably influenced by the type of illuminant employed. Although picnic ham treated with germicidal ultraviolet light lost more moisture than similar meat did after exposure to fluorescent light, drying did not advance to the point where color was visibly affected. Table 27 presents weight losses for picnic ham and sliced large bologna exposed to ultraviolet and fluorescent lights. The effect of desiccation in relation to packaging materials will be discussed in the next section.

Table 27. Weight losses* of picnic ham and sliced bologna exposed to ultraviolet and fluorescent lights**

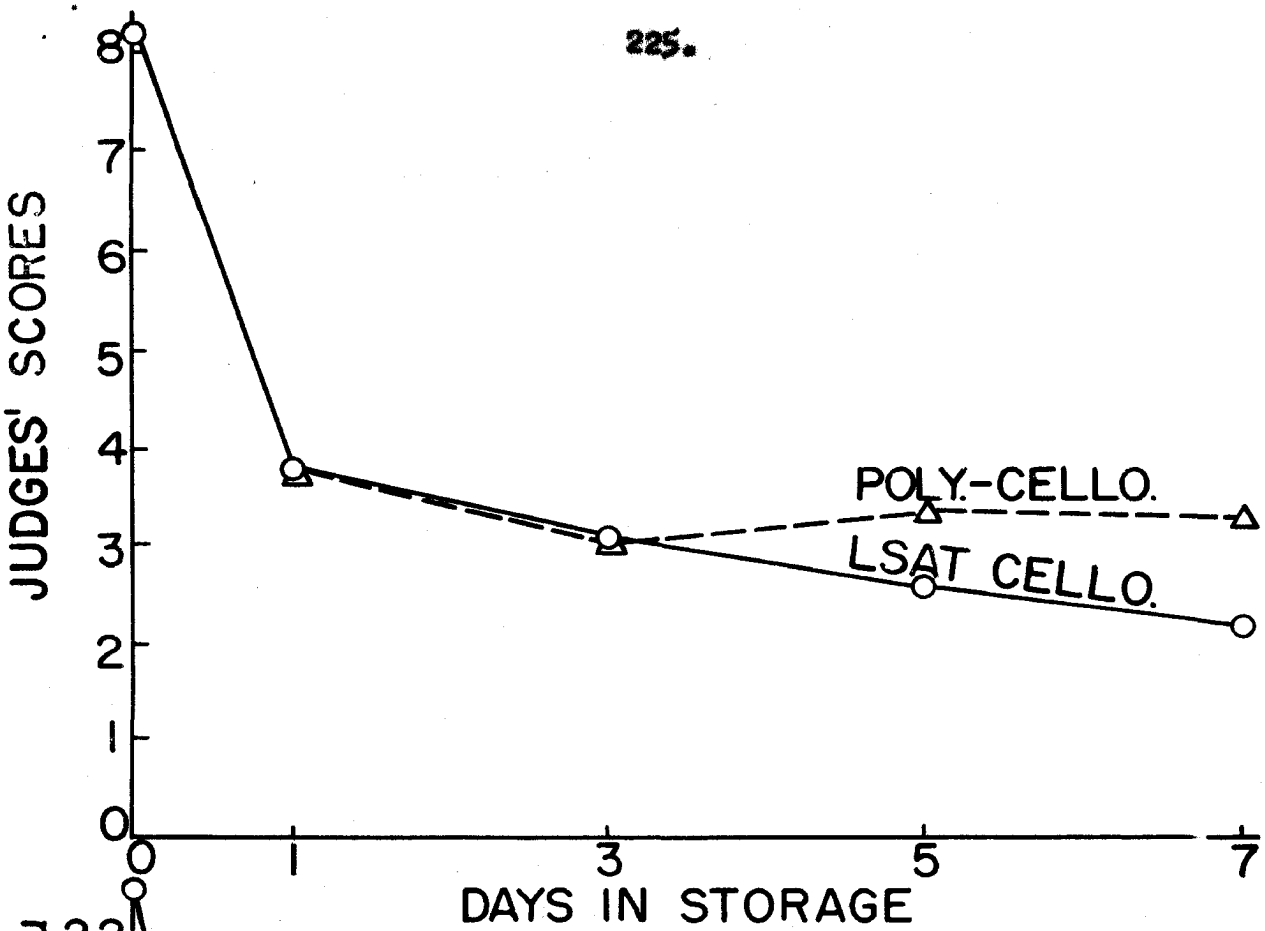
Type of meat	Packaging material	Days in storage	Weight loss	
			Ultraviolet	Soft White
Picnic ham	Saran-coated cellophane	2	0.00	0.00
		7	0.73	0.09
		14	3.54	0.58
		21	4.56	1.25
		28	5.82	1.93
Large bologna	LSAT cellophane	1	2.85	2.70
		3	9.68	8.76
		5	15.8	15.1
		7	21.8	20.9
Large bologna	Polyethylene-cello. laminate	1	0.00	0.00
		3	0.00	0.01
		5	0.01	0.00
		7	0.01	0.00

*Mean of weight losses for four samples, expressed as % initial wet weight

**Storage conditions:
Ultraviolet light intensity 7 to 10 footcandles
Soft White light intensity 40 to 45 footcandles for picnic ham
30 to 45 footcandles for large bologna
Percival open-type display case
Temperature 2.5°C. ± 1°
Relative humidity 40 to 75%

(5) Permeability of materials to oxygen and moisture vapor. In addition to transparency, other properties of packaging films of importance in discoloration of self-service cured meats include permeabilities to oxygen and water vapor. The influence of these factors on discoloration of bologna packaged with LSAT cellophane and with the polyethylene-cellophane laminate is demonstrated in Figures 38 and 39. Little difference was observed in judges' scores for meat displayed under 35 to

Figure 38. Relation of packaging materials to judges' scores and reflectance ratios of bologna exposed to 35-55 foot-candles of Soft White fluorescent light


JUDGES' SCORES
8
7
6
5
4
3
2
1
0
POLY.-CELLO.
LSAT CELLO.
0
1
3
5
7
DAYS IN STORAGE

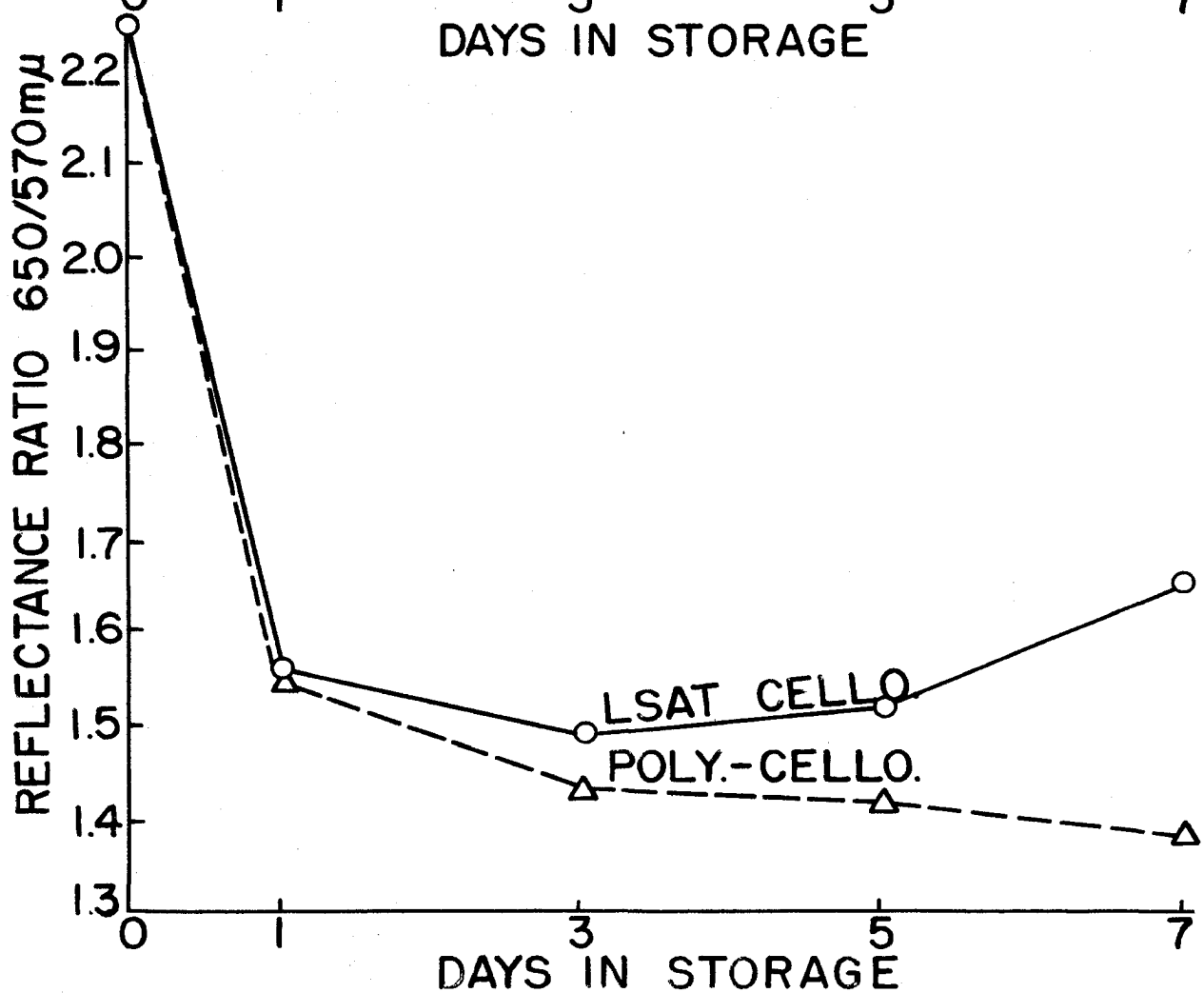
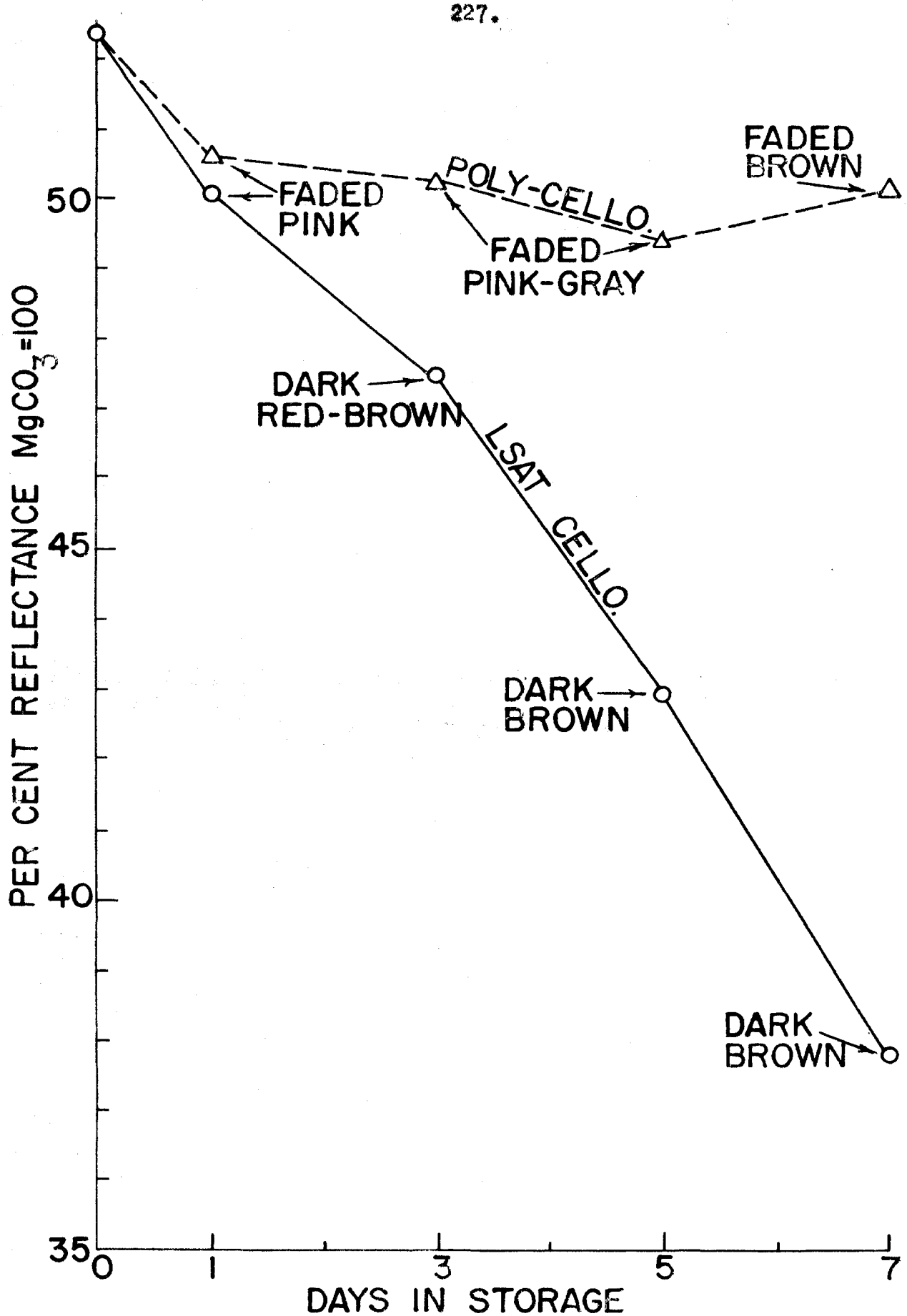

REFLECTANCE RATIO 650/570mμ
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
LSAT CELLO
POLY.-CELLO.
0
1
3
5
7
DAYS IN STORAGE

Figure 39. Relation of packaging materials to changes in color and reflectance of bologna stored under 35-55 footcandles of Soft White fluorescent light


FADED
BROWN
POLY-CELLO.
FADED
PINK
FADED
PINK-GRAY
DARK
RED-BROWN
LSAT CELLO.
DARK
BROWN
DARK
BROWN
50
45
40
35
PER CENT REFLECTANCE MgCO3=100
0
1
3
5
7
DAYS IN STORAGE

55 footcandles of Soft White fluorescent light for 1 and 3 days, but after 5 and 7 days, bologna wrapped with the laminate was scored higher than was similar meat packaged with LSAT cellophane. At the end of 1 day in storage, the ratio of reflectance at 650 mμ to reflectance at 570 mμ was higher for bologna packaged with the cellophane than it was for meat wrapped with the laminate, and increased during further storage. Increase in reflectance ratio may have been caused by browning of the meat packaged with cellophane; although reflectance in the range 540 to 800 mμ (Figure 39) showed a decline, the ratio of values at 650 mμ to those at 570 mμ became higher (Figure 38). On the other hand, changes in reflectance of bologna wrapped with polyethylene laminated to cellophane were negligible after initial fading occurred. At the end of 1 day, samples were described as faded pink in color; bologna packaged with LSAT cellophane appeared slightly darker than that wrapped with the laminate. During additional storage, cellophane-packaged meat continued to darken and presented a dark brown appearance at the end of the seventh day of display. Bologna packaged with the polyethylene-cellophane laminate was described as faded brown in color after a similar period of storage.

Differences in color changes of bologna packaged with the above materials may be ascribed in part to dehydration. Weight losses of samples are illustrated in Figure 40. LSAT cellophane permitted appreciable desiccation to occur; at the end of 3 and 5 days, bologna lost about 10 and 20 per cent respectively of its original weight.

As noted previously, such meat was considered to have an undesirable dark brown color and was scored lower than were samples held in the laminate of polyethylene and cellophane. Excessive loss of moisture effected changes in color different from the fading due to the action of light and oxygen.

(6) Light intensity. An indication of the effect of light intensity on fading of picnic ham packaged in air may be gained from Table 18 under the section on time for color fading. Discoloration of large bologna exposed to 50 and 150 footcandles of Soft White fluorescent light was determined by the judging panel.

In these experiments, the meat was packaged with Saran laminated to cellophane and with LSAT cellophane. Marked and unmarked controls were wrapped with the laminate, overwrapped with aluminum foil, and stored in the dark. Judges gave scores for the top, second, and third slices in cellophane packages, and for the top slices of meat wrapped with the laminate; these data are presented in Table 28.

In all cases, control samples were scored highest throughout the storage period. At the end of 1 day, little difference was noted between top slices of bologna packaged with the laminate or with cellophane and stored at the same light intensity. At later examinations, meat wrapped with the laminated material received higher scores than that packaged with LSAT cellophane. This was observed for meat stored at each light intensity, but with both packaging materials, discoloration of bologna was greatest at the higher light intensity. Exposure

Table 28. Effect of light intensity on color scores* for packaged large bologna**

Packaging material	Light intensity (footcandles)	Position of slice	Days in storage 1	5	8	14
Saran-cellophane laminated overwrapped with foil (marked control)	0 (in dark)	Top	9.9	10.0	9.8	9.8
Saran-cellophane laminated overwrapped with foil (hidden control)	0 (in dark)	Top	9.8	9.8	10.0	9.6
LSAT cellophane	50	3rd	8.2	8.5	8.3	8.2
		2nd	8.4	7.5	7.2	6.8
		Top	5.6	4.7	3.4	2.8
	150	3rd	8.0	8.7	8.0	7.8
		2nd	7.4	7.2	6.4	5.8
		Top	4.3	3.6	2.7	1.8
Saran-cellophane laminated	50	Top	5.3	6.0	5.1	4.6
	150	Top	4.3	4.3	3.5	3.5

*Mean of scores given by six judges for two samples

**Storage conditions:
Victor refrigerator with top open
Temperature 1.5°C. ± 1°
Soft White fluorescent light
Relative humidity 50 to 65%

of sliced bologna to light resulted in discoloration before storage for 1 day regardless of light intensity or packaging materials employed: it was shown previously that cellophane-wrapped bologna exposed to 55 to 65

footcandles of Soft White fluorescent light gave evidence of fading after about 1 hour. None of the top slices exposed to light were judged as acceptable at the end of display for 1 day.

The second and third slices of bologna wrapped with LSAT cellophane were scored lower than were controls, but still remained acceptable to the judging panel after the meat was stored for 14 days. After 1 day, differences in scores for these slices were only slight, but thereafter the third slice was judged best for color retention. Light intensity apparently affected fading of meat not directly exposed to light, since third and second slices in packages displayed under 50 footcandles generally were ranked higher respectively than similar slices held at 150 footcandles.

Less fading was produced by light intensities in the range 30 to 35 footcandles than that observed when bologna packaged with LSAT cellophane was exposed to 50 to 60 footcandles of Soft White fluorescent light (Figure 41). As in other trials, fading was greatest during the first day of display. At intervals of 1, 2, 3, and 7 days, bologna held at the lower range of light intensity received higher scores and gave higher values for the reflectance ratio than those noted for meat displayed at 50 to 60 footcandles. The shape of the reflectance ratio curves in Figure 41 indicates that the rate of discoloration was slightly lower at 30 to 35 footcandles than it was at the higher level of light intensity.

Figure 40. Influence of wrapping materials on weight losses of packaged bologna

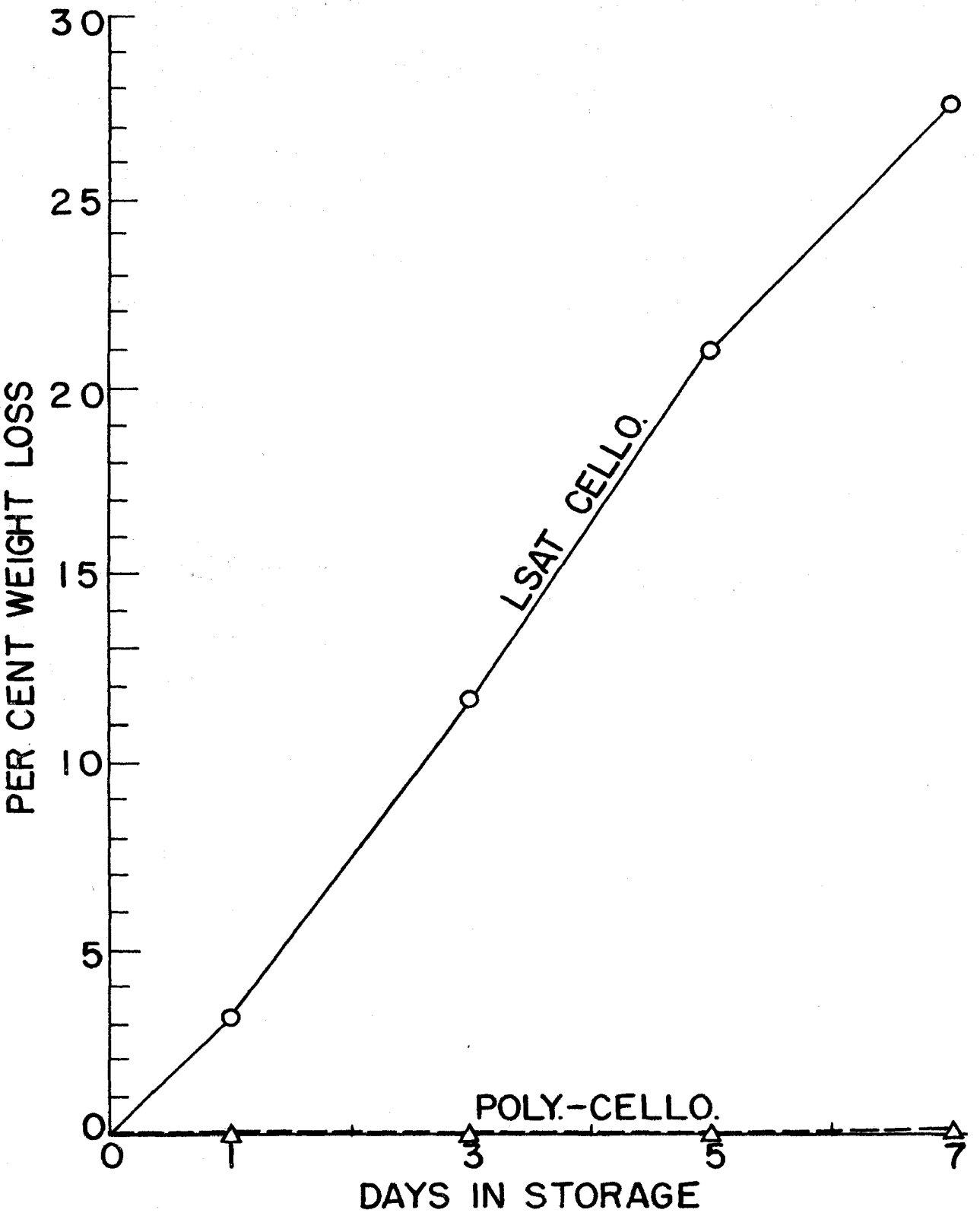
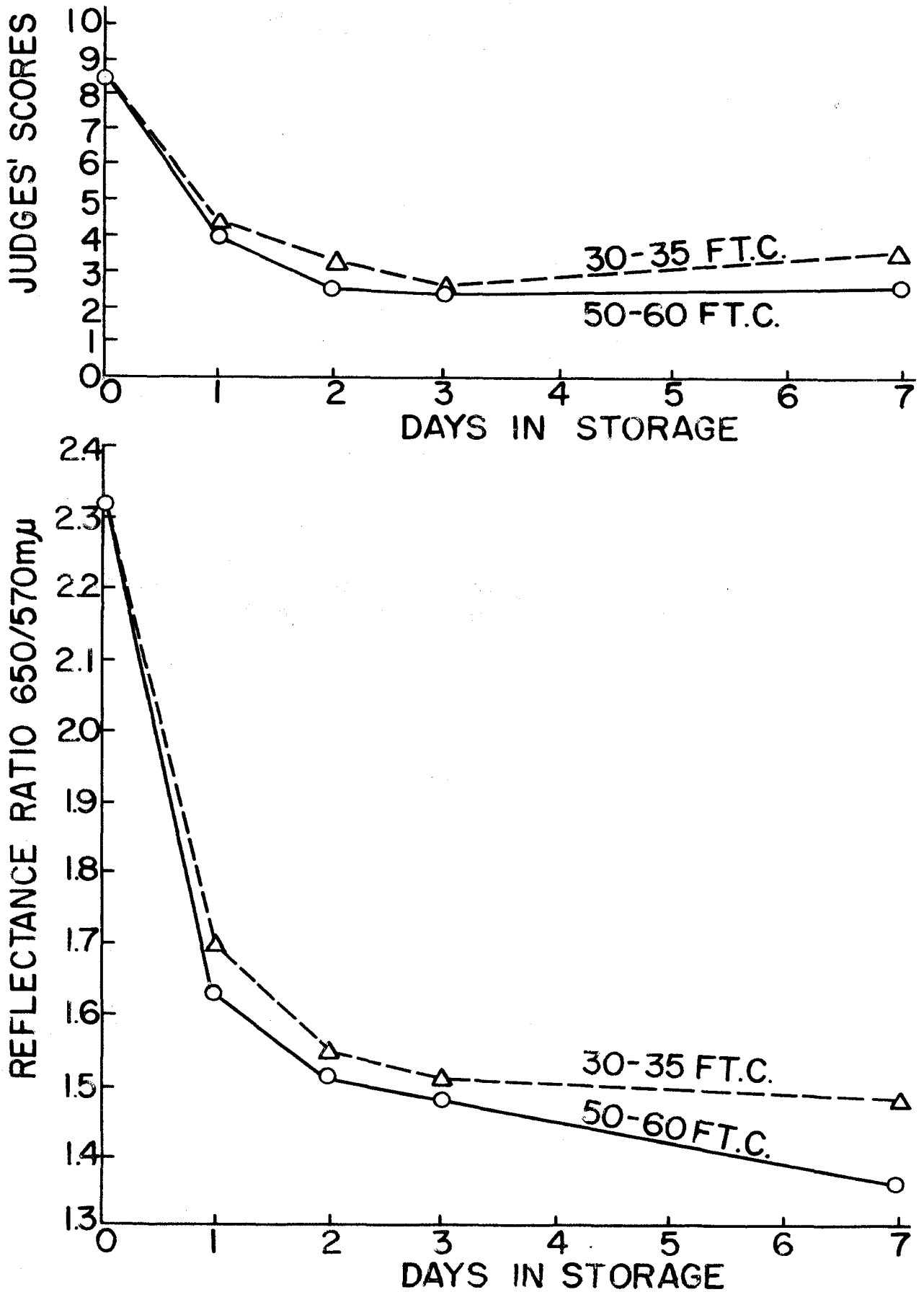

30
25
20
15
10
5
0
PER CENT WEIGHT LOSS
LSAT CELLO.
POLY.-CELLO.
0
1
3
5
7
DAYS IN STORAGE

Figure 41. Effect of light intensity on scores and reflectance ratios of bologna packaged with LSAT cellophane


JUDGES' SCORES
10
9
8
7
6
5
4
3
2
1
0
30-35 F.T.C.
50-60 F.T.C.
0 1 2 3 4 5 6 7
DAYS IN STORAGE
REFLECTANCE RATIO 650/570mμ
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
30-35 F.T.C.
50-60 F.T.C.
0 1 2 3 4 5 6 7
DAYS IN STORAGE

(7) Deterioration of materials. In studies wherein picnic ham was packaged with Saran-coated cellophane and exposed to ultraviolet radiations, it was found that the packaging material became light brown in color during storage. Light transmission of the coated material before and after 16 days' exposure to Soft White fluorescent and ultraviolet lights was determined; curves are given in Figure 42. Transmission of light was markedly decreased by treatment with ultraviolet radiation; fluorescent light had little effect on transparency. It would seem, then, that the intensity of ultraviolet radiation on meat wrapped with the material would become less as storage time was prolonged.

b. Effect of type of light on bacterial growth. In initial experiments, it was found that bacterial growth on picnic ham was not influenced by different types of fluorescent light. Later work was concerned with the effect of germicidal ultraviolet light in comparison with that of visible light; the latter was provided by the Soft White fluorescent lamp.

Bacterial counts from packaged picnic ham displayed under the two types of light mentioned above are given in Table 29. The use of ultraviolet radiations resulted in a slight decrease in numbers of surface organisms throughout the storage period of 4 weeks. As shown in Table 27, desiccation of ultraviolet-irradiated picnic ham was not important in influencing the development of surface organisms.

Figure 42. Effect of type of radiation on light transmission of Saran-coated cellophane

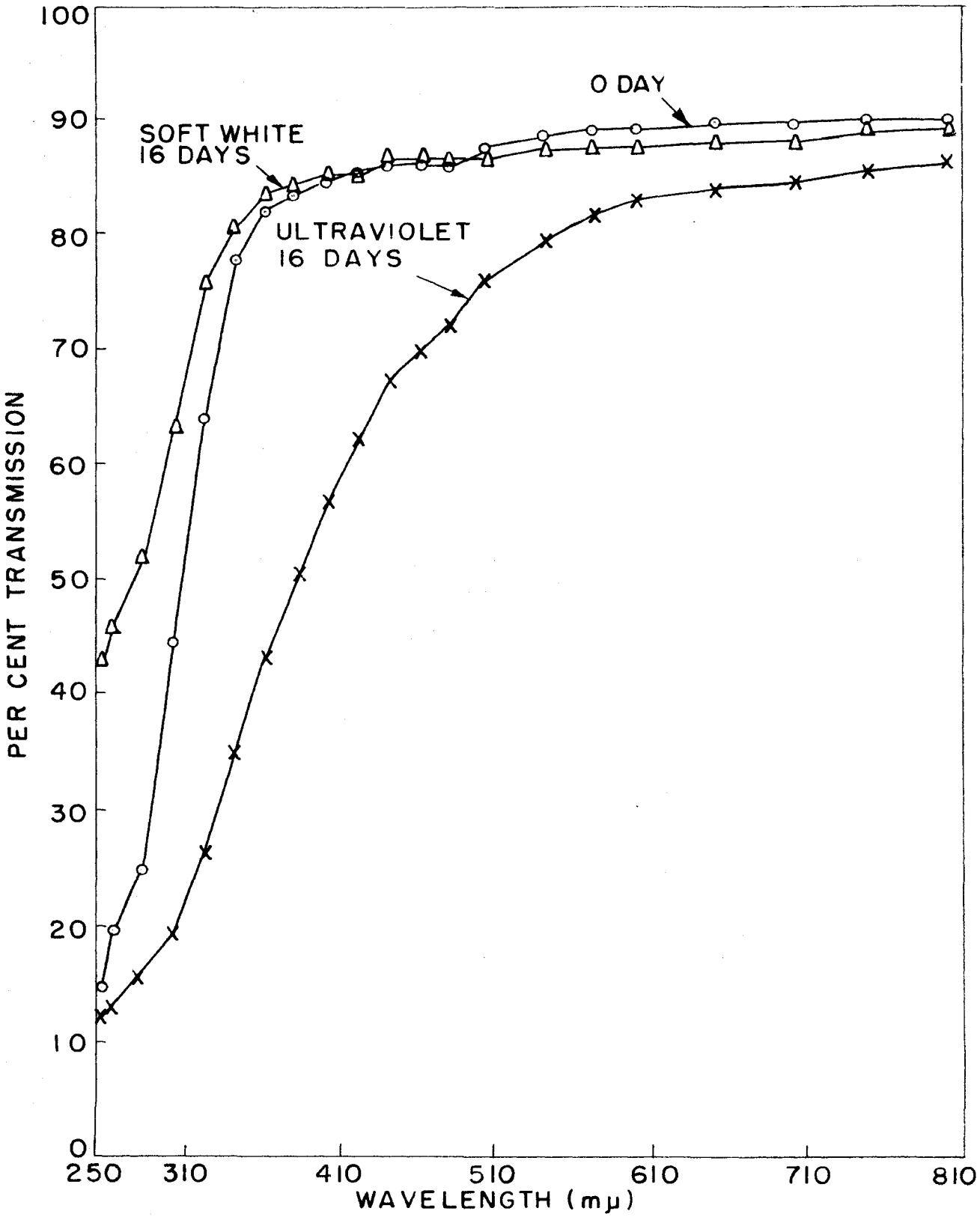

100
90
80
70
60
50
40
30
20
10
0
PER CENT TRANSMISSION
0 DAY
SOFT WHITE
16 DAYS
ULTRAVIOLET
16 DAYS
250
310
410
510
610
710
810
WAVELENGTH (mμ)

Table 29. Logarithmic averages of bacterial counts* from packaged picnic ham exposed to germicidal ultraviolet and Soft White fluorescent lights**

Type of light	Days in storage					
	0	2	7	14	21	28
	Logarithmic averagers of counts					
Ultraviolet	4.827	3.779	5.028	6.103	8.166	9.014
Soft white fluorescent	4.827	4.425	6.282	7.388	9.411	9.175

*Mean of counts obtained from four samplings of meat

**Storage conditions:
Percival open-type display case
Light intensity 7-10 footcandles ultraviolet light, 40-45 footcandles Soft White fluorescent light
Packaging material Saran-coated cellophane
Temperature 2.5°C. ± 1°
Relative humidity 40 to 75%

In common with results obtained in fresh meat studies, during the lag period the bacterial population demonstrated a greater decrease in numbers when germicidal ultraviolet light was employed than when fluorescent light served as the illuminant. Off-odor developed after 14 days on meat held under the fluorescent light, and at the end of 18 days on ultraviolet-irradiated meat.

It was shown in a previous section that the packaging material became less transparent to ultraviolet radiation as storage time progressed. Reduction in light intensity may have decreased the effectiveness of the radiation in inhibiting bacterial growth.

In contrast to its value for display of fresh meats, germicidal ultraviolet light appears to be beneficial both for retention of color of the lean and for prolongation of keeping time of cured meats. One disadvantage originating from the use of this type of light is the lack of sufficient illumination to properly display the meat. In other words, the low level of light intensity responsible for improved color may also cause decreased sales appeal. The effect of ultraviolet radiation in accelerating development of rancidity of fat was not evaluated in this study, but should receive attention before consideration is given to application of the light in meat display cases in retail stores.

c. Influence of light intensity on bacterial growth. Table 30 lists bacterial counts from picnic ham exposed to 150, 50, and 30 footcandles of Soft White fluorescent light. Intensity of this type of light did not affect development of surface organisms; bacterial counts were quite similar at all intensities tested.

As a further check on the relation of light intensity to growth of microorganisms on cured meats, counts were made from sliced large bologna stored at 150 and 50 footcandles of Soft White fluorescent light. Samplings were taken from the top slice and from the second slice in packages. Counts are given in Table 31.

Little difference was found between counts obtained from meat held at high and low light intensities. When LSAT cellophane was used, bacterial numbers generally were slightly higher on second slices than

Table 30. Effect of light intensity on bacterial growth* on packaged picnic ham**

Light intensity (footcandles)	No. of samplings	Days in storage			
		7	14	21	28
		Logarithmic averages of counts			
150	7	5.189	6.706	7.053	6.336
50	7	5.318	6.571	7.414	6.529
30	4	6.058	6.779	7.371	-

*Each value is the logarithmic average of counts for number of samplings specified; logarithmic average initial count 4.806 bacteria per sq. cm.

**Storage conditions:
Victor refrigerator with top open
Soft White fluorescent light
Temperature 1.5°C. ± 1°
Relative humidity 50 to 65%
Packaging material Saran-coated cellophane

they were on cuts directly exposed to light. However, these differences were not pronounced. Possibly one reason for the lower counts on top slices was greater drying of the meat surface than that which occurred with second slices. With Saran laminated to cellophane, a material highly impermeable to moisture, the numbers of bacteria on second slices were usually no greater than those on top slices.

d. Discussion. It is evident that the action of ultraviolet light on color of cured meats differs markedly from that on fresh meat color. The reasons for this were not determined in the present work. Apparently, the addition of curing salts and/or heat renders the pigments more stable to ultraviolet radiation. Since heat processing results in denaturation of nitric oxide myoglobin to give nitric oxide myochromogen,

Table 31. Effect of light intensity on bacterial growth* on packaged large bologna**

Packaging material	Light intensity (footcandles)	Slice	Days in storage 1	5	8	14	19
LSAT cello-phane	150	Top	1.926	2.134	2.929	5.620	6.996
		Second	2.126	2.425	3.756	5.888	6.670
	50	Top	2.121	2.405	3.165	4.589	6.866
		Second	1.939	2.940	3.329	5.024	6.515
Saran-cello-phane laminate	150	Top	2.075	2.157	2.813	4.582	6.417
		Second	1.764	2.075	2.959	4.205	6.216
	50	Top	1.651	2.725	3.475	4.598	6.726
		Second	1.382	2.724	2.343	4.612	6.399

*Each value is the logarithmic average of counts for three samplings; logarithmic average initial count 2.329 bacteria per sq. cm.

**Storage conditions:
Victor refrigerator with top open
Soft White fluorescent light
Temperature 1.5°C. ±1°
Relative humidity 50 to 65%

further denaturation by ultraviolet radiation may not occur or may be limited so that color is not appreciably affected by the radiation. With cured meats, the effect of different types of light appears to be subordinate to the influence of light intensity. Fundamental work is needed concerning the differences in stability of pigments of fresh and of cured meats to different intensities of visible light. Also, additional information of a more basic nature would be necessary for explaining the mechanisms whereby ultraviolet light causes discoloration of fresh meats but does not appreciably affect the color of cured meats.

The absorption of ultraviolet radiation by wrapping materials reduces the intensity of ultraviolet light incident to the surface of of packaged meats; reduction in light intensity may account for improvement in color retention by the use of ultraviolet light as compared with fluorescent light. Since discoloration due to the former type of light was less than that brought about by visible radiation, further evidence may be added to the findings of Taylor and Pracejus (1950) and Ramsbottom et al. (1951) that ultraviolet energy caused no greater fading of cured meats than that produced by the action of visible light.

Pracejus (1950) and Ramsbottom et al. (1951) have indicated that discoloration is limited to the top layer of sliced cured meats. In the work here, slices of bologna other than the exposed piece were noticeably changed in color during storage of packages. Of course, this is not conclusive evidence that light may cause discoloration of meat not directly exposed to light rays, but it seems more than coincidental that third slices in packages were less affected than second slices. Further, second and third pieces in packages held at high intensity of light were more discolored than were similar slices displayed at low intensity. In no case, however, did the degree of discoloration approach that evidenced by top slices.

With regard to packaging materials, fading of sliced bologna on exposure to light was not prevented by the use of a laminated material having low permeabilities to oxygen and to moisture vapor. This type of discoloration resulted from the reaction of the cured meat pigment

with oxygen initially trapped in the packages and from the action of oxygen transferred through the wrapper. Laminated films were sufficiently impermeable to moisture vapor that darkening of the meat did not occur.

In considering the scores noted by the judges, and the moisture losses of bologna packaged with LSAT cellophane (Figures 38 and 40), considerable desiccation (between 10 and 20 per cent weight loss) was required before darkening of the meat became objectionable. In this connection, an interesting feature noted was that in the earlier phases of storage, some of the judges preferred the darker color of bologna packaged with the cellophane to the faded pink or grayish pink of meat wrapped with the less-permeable laminate. When darkening became more advanced, the ratio of reflectance at 650 mμ to reflectance at 570 mμ was not as closely related to judges' scores as were values for reflectance at wavelengths in the range 540 mμ to 800 mμ.

Discoloration of cured meats packaged with transparent materials appears to be an inevitable consequence of exposure to light. A practical approach toward minimizing this problem would involve the use of laminated materials having low permeabilities to oxygen and to moisture vapor; even greater benefits might result if vacuum or gas packaging were employed with these materials.

Intensity of visible light exerted little influence on development of surface organisms on cured meats, but the type of cured product seemed to be important in determining the bacteriological quality of these meats. Initial contamination of bologna was less than that of

picnic ham. During storage, numbers of bacteria on picnic ham (Table 30) were higher than those found on sliced bologna (Table 31) regardless of light intensity. Evidently, picnic ham furnished a better medium for growth of surface organisms than did sliced bologna; bacterial growth was affected to a greater degree by the type of meat than it was by intensity of illumination.

V. CONCLUSIONS

1. Bacterial growth on the surface of packaged fresh meats is most rapid with films having high permeabilities to oxygen and moisture vapor; such films permit retention of red color for periods of time longer than those found when less permeable materials are used.

2. Laminated materials, coated films, and Saran films are effective in prolonging storage life of fresh meats. Reduction of myoglobin to the purple pigment is associated with the use of these materials.

3. Initial bacterial loads and temperature of storage affect keeping time of packaged fresh meats.

4. Improvement in storage life of fresh and of cured meats is achieved by the use of CO_2 when materials having high or low permeability to the gas are used as wrappers. High concentrations of CO_2 cause brown discoloration of packaged fresh beef.

5. The effect of relative humidity on color changes, desiccation, and bacterial spoilage of packaged meats varies with wrapping materials employed. With films having high permeability to moisture vapor, inhibition of bacterial growth on meat held at low humidity is not attainable without occurrence of severe desiccation and discoloration. Changes brought about by reduction in humidity are less pronounced when fresh and cured meats are packaged with materials providing good barriers to transfer of moisture.

6. Germicidal ultraviolet light causes marked retardation of bacterial growth on fresh beef wrapped with a highly transparent material, but produces undesirable darkening and desiccation.

7. Loss of moisture from fresh beef exposed to germicidal light is responsible, in part, for decreased bacterial counts and for darkening of the pigment.

8. Intensity of fluorescent light has little effect on development of surface organisms on packaged fresh and cured meats or on discoloration of packaged beef.

9. Extent of discoloration of cured meats displayed under light is decreased by packaging in atmospheres of CO_2 or nitrogen, or by evacuation of air from packages.

10. When air is not completely removed from packages, vacuum packaging does not appreciably delay fading of color of sliced large bologna.

11. The action of germicidal ultraviolet light on color of cured meats differs from that exerted on color of fresh meats; ultraviolet radiation causes less discoloration of cured meat than that produced by visible light.

12. Fading of color of cured meats is more rapid and more advanced at high light intensities than it is when low intensities are used for display.

13. For cured meat, the ratio of spectral reflectance at 650 mμ to reflectance at 570 mμ provides a good index of fading; this ratio is not an adequate criterion of discoloration when desiccation of cured meat is

well advanced.

14. Discoloration of packaged meats is associated with decrease in spectral reflectance at wavelengths in the visible portion of the spectrum.

15. Materials having low degrees of permeability to moisture vapor prevent darkening caused by desiccation of fresh and of cured meats.

VI. SUMMARY

An investigation was made of the effects of various packaging materials, gases, lights, and relative humidities on the keeping quality of fresh and of cured packaged meats held at refrigeration temperatures. Keeping time generally was based on occurrence of off-odor or slime formation. These manifestations of spoilage were associated with definite numbers of organisms per square centimeter of meat surface. Other criteria of spoilage included production of H_2S or CO_2 by microorganisms.

Materials found to preserve the desirable red color of fresh meat in the early phases of storage permitted most rapid proliferation of microorganisms on the surface of the meat. The time for appearance of discoloration varied with wrapping materials used. Fresh beef packaged with materials having low permeability to oxygen exhibited the purple color of reduced myoglobin throughout storage periods as long as 16 days at 4.4°C. Subsequent oxygenation of the pigment with resultant bright red color occurred on exposure of the meat to air.

Of various packaging materials tested with fresh meats, a laminate of aluminum foil and Pliofilm was found to be most effective in retarding spoilage. Owing to its low gas-permeability, this material was well adapted to packaging with CO_2. With all materials, keeping time of meat was improved by the incorporation of CO_2 within the atmosphere of the packages. Initial exposure of fresh beef to CO_2 prior to packaging

was demonstrated to be of value in prolonging storage life. The level of gas for such exposure was limited by the discoloration that resulted when high concentrations were used.

Improvement in storage life resulting from the use of relatively gas-impermeable films was ascribed to low rates of exchange of gases between the outer atmosphere and the atmosphere in packages; keeping time was greater with heavy gauge Saran films than it was when thinner films of the same type were utilized as wrappers for fresh meat.

Reduction in temperature and low initial contamination favored extension of keeping time of packaged fresh meat.

When beef was wrapped with MSAT-80 cellophane or with Pliofilm FM-1, holding at low humidity was not a practical means of controlling growth of surface organisms because of accompanying discoloration and desiccation. The humidity of storage did not affect changes in beef wrapped with Saran laminated to cellophane. The use of polyethylene laminated to cellophane showed promise as a means of retarding development of surface bacteria on beef stored at low humidity; dehydration and discoloration of the meat were negligible.

Germicidal ultraviolet light caused inhibition of bacterial growth on fresh meat wrapped with a highly transparent material, but this type of light also produced darkening and desiccation of the meat. In addition, ultraviolet radiation effected deterioration of packaging films. Although ultraviolet energy <u>per se</u> exerted a bactericidal or bacteriostatic effect, accompanying dehydration of the meat appeared to be

partly responsible for discoloration and for retardation of bacterial development. When fresh beef was displayed under Soft White fluorescent light, little difference was observed in color, reflectance, or bacterial growth at different light intensities. Spectral reflectance of fresh beef showed a definite trend with regard to color changes; reflectance values decreased as discoloration progressed.

While packaging of cured meats in atmospheres of nitrogen or CO_2 did not prevent fading of color on exposure of the meats to light, less discoloration was observed than that resulting from packaging in an atmosphere of air. Color changes of cured meats packaged under vacuum resembled those of similar meats held in CO_2 or in nitrogen.

Storage life of frankfurters packaged with several wrapping materials was increased by high concentrations of CO_2 in the atmosphere of the packages, when storage was carried out at 4.4°C. and at 7.2°C. The ability of materials to retain the gas was an important consideration; however, the meat did not spoil until some time after CO_2 had been lost from the packages. Preliminary treatment of frankfurters with the gas before packaging decreased the rate of growth of surface organisms. Keeping time of picnic ham also was extended by the use of CO_2; vacuum packaging produced similar results. Packaging in an atmosphere of nitrogen gave no greater storage life than that resulting from packaging in air.

Changes in color of packaged bologna stored under light were determined by a panel of judges and by reflectance measurements. A scoring

system based on the ratio of reflectance at 650 mμ to reflectance at 570 mμ was devised for assigning scores to controls. The reflectance ratio served as an objective measure for establishing reproducible standards for subjective evaluation of color by the judging panel. Fading of color was associated with decrease in reflectance ratio; however, when appreciable desiccation occurred, the reflectance ratio was not as closely related to judges' scores as were values for reflectance at wavelengths between 540 and 800 mμ.

Reduction of humidity caused pronounced discoloration and desiccation of sliced bologna wrapped with LSAT cellophane. Proliferation of surface organisms was retarded by the use of the cellophane with storage at low humidity. Materials that provided good barriers to transfer of moisture vapor prevented darkening due to dehydration of the meat. With these films, bacterial growth was not appreciably affected by relative humidity of the atmosphere external to packages.

Germicidal ultraviolet light caused some reduction in the numbers of bacteria on picnic ham. This type of light also caused less discoloration of the lean of cured meats than that found when fluorescent lighting was employed, but the fat showed brown discoloration after exposure to ultraviolet radiation. Color fading of cured meats was more rapid and more advanced at high intensities of fluorescent light than it was when low intensities were used for display of the meats; this relation was observed regardless of packaging materials or methods employed. Intensity of visible light did not influence development of bacteria on cured meats.

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IX. APPENDIX

Table 32. Absorption of CO_2 by fresh beef stored at 4.4°C.

Days in storage	Grams meat per ml. CO_2 initially	Initial CO_2 conc. (%)	CO_2 conc. at sampling (%)	CO_2 uptake* (ml./g.)
1	6.82	28.9	10.3	0.101
	8.25	26.5	9.7	0.082
2	8.42	23.2	11.1	0.066
	8.35	24.2	10.9	0.071
3	9.20	25.4	11.3	0.065
	8.51	25.5	11.7	0.068
4	6.13	26.3	17.3	0.063
	7.64	24.8	15.6	0.052

*Calculated for 25°C.

Table 33. Growth rates* of bacteria on fresh beef packaged in CO_2 and air stored at 4.4°C.

Packaging material	Atmos-phere	Log b	Log B	t (hours)	k	g (hours)
MSAT-80 cellophane	Air	6.852	3.678	72	0.101	6.84
	CO_2	7.592	3.042	216	0.048	14.34
Pliofilm FM-1	Air	6.907	4.177	72	0.087	7.96
	CO_2	7.261	3.319	216	0.042	16.52
Pliofilm FF-120	Air	7.404	4.418	144	0.048	14.55
	CO_2	6.928	3.548	216	0.036	19.28
Alum. foil-Pliofilm laminate	Air	7.242	3.386	216	0.041	16.89
	CO_2	7.041	2.952	264	0.036	19.49

*Method for calculation of growth rates:

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

b = number of bacteria per sq. cm. at the end of time t

k = velocity coefficient; $k = \frac{2.3}{t} (\log b - \log B)$

g = generation time; $g = \frac{\ln 2}{k} = \frac{0.694}{k}$

Table 34. Spectral reflectance* and variation in reflectance of fresh red beef

	Wavelength (mμ)							
	540	570	600	650	675	700	750	800
	6.90	7.30	16.0	30.0	31.5	32.5	30.5	29.5
	6.20	8.70	16.0	28.5	32.0	34.0	34.0	31.5
	5.35	5.90	16.0	28.5	30.5	31.5	28.0	30.5
	5.40	6.00	17.0	29.5	31.0	33.0	29.0	27.5
	5.25	6.20	16.0	28.0	30.5	32.5	28.5	27.0
	5.55	5.90	15.0	30.0	35.5	36.5	34.5	32.5
	5.90	6.60	17.5	33.5	41.5	44.5	42.0	39.5
	5.40	5.50	14.5	29.5	32.5	34.0	33.0	32.5
	4.40	4.40	12.5	26.5	29.5	31.5	30.0	30.0
	8.80	8.60	16.0	30.0	37.5	42.0	43.0	43.5
	9.10	8.90	18.0	31.5	38.0	42.5	43.5	44.5
	9.20	8.80	17.0	31.0	38.0	41.0	42.5	43.5
	8.70	9.00	16.5	32.0	37.5	40.0	43.0	43.5
	5.70	6.00	17.5	31.5	39.5	38.0	36.5	35.5
	6.15	6.20	18.5	32.0	40.0	45.0	42.5	41.5
Mean	6.53	6.93	16.3	30.1	35.0	37.2	36.0	35.5
Confidence interval estimates (P=0.05)	5.64	6.10	15.4	29.1	32.7	34.5	32.6	31.9
	7.42	7.76	17.1	31.1	37.3	39.9	39.4	39.1

*Standard $MgCO_3$

Table 35. Evaluation of differences in reflectance* of beef packaged with MSAT-80 cellophane and exposed to germicidal ultraviolet and Soft White fluorescent lights

Type of light	Run	Days in storage 2	5	8	
Ultraviolet	RS-30	10.50	9.45	5.20	
		12.00	5.65	5.80	
	RS-32	14.00	15.50	14.00	
		16.50	18.50	16.50	
	Mean				11.97
Soft White fluorescent	RS-30	23.50	21.50	23.50	
		18.50	22.50	17.00	
	RS-32	30.00	16.50	14.50	
		19.00	16.50	13.50	
	Mean				19.71

Type of light	No. of observations	Degrees of freedom	Mean of reflectance	Sum of squares
Ultraviolet	12	11	11.97	236.73
Soft White fluor.	12	11	19.71	241.18
		Sum = 22	Difference = 7.74	Sum = 477.91

Pooled variance = 21.723
Standard error = 1.903

$$t = \frac{7.74}{1.903} = 4.07^{**}$$

*Reflectance at 650 mμ
**Significant difference (P = 0.01, Snedecor, p.65)

Table 36. Changes in concentration of CO_2* in packages of frankfurters wrapped with Pliofilm FF-120

Days in storage	CO_2-treated packages	Air-treated packages
1	49.8	0.2
1	55.1	0.2
2	52.1	-
3	10.9	0.5
7	5.6	0.3
7	2.6	0.8
9	3.7	-
12	1.9	1.2
12	1.7	0.0
17	0.8	0.9
22	1.5	-
28	0.9	-

*Carbon dioxide concentration expressed as %
Initial CO_2 level 93%

Table 37. Changes in concentration of CO_2* in packages of frankfurters wrapped with Flexvac and with aluminum foil laminated to Pliofilm

Packaging material	Atmosphere	Days in storage		
		4	9	13
Flexvac	CO_2	84.0	66.4	65.8
	Air	1.2	18.3	16.4
Aluminum foil-Pliofilm laminate	CO_2	82.8	70.8	75.4
	Air	1.5	19.2	24.2

*Carbon dioxide concentration expressed as %
Initial CO_2 level 91%

Table 38. Changes in gas concentration* in packages of picnic ham wrapped with Saran-coated cellophane

Atmosphere		Days in storage				
		4	7	14	21	28
Nitrogen	CO_2 conc. (%)	0.4	0.6	0.8	1.6	-
		0.0	0.6	0.7	1.8	3.7
		-	0.6	2.9	4.9	11.3
		-	0.6	2.3	5.1	9.7
	O_2 conc. (%)	3.6	6.3	7.1	8.4	-
		2.0	1.6	5.6	9.9	10.4
		-	1.0	3.5	5.2	7.9
		-	1.6	2.4	5.9	6.5
	N_2 conc. (%)**	96.1	93.1	92.2	90.1	-
		98.0	97.8	93.7	88.3	85.9
		-	98.4	93.6	89.9	80.8
		-	97.8	94.3	89.1	83.8
CO_2	CO_2 conc. (%)	85.4	84.4	79.8	75.8	-
		94.5	93.2	87.6	78.4	70.5
	O_2 conc. (%)	4.7	6.9	10.2	12.1	-
		2.3	2.7	3.9	4.9	10.3
	N_2 conc. (%)**	9.9	8.7	10.0	12.1	-
		3.2	4.2	8.5	16.7	19.2
Air	CO_2 conc. (%)	0.5	2.2	2.8	3.9	-
		0.0	2.0	2.4	5.8	11.7
		-	0.8	3.8	5.0	8.9
		-	0.7	2.9	4.9	8.5
	O_2 conc. (%)	20.5	19.3	18.9	18.1	-
		21.0	19.4	18.9	16.7	12.4
		-	21.2	16.4	15.7	16.1
		-	20.5	17.6	15.2	16.7
	N_2 conc. (%)**	79.0	78.5	78.3	78.0	-
		79.0	78.6	78.7	77.5	75.9
		-	78.0	79.8	79.3	75.0
		-	78.8	79.5	79.9	74.8

*Initial CO_2 level 95%
Initial N_2 level 98%

**N_2 concentration calculated as difference between sum of CO_2 and O_2 concentrations and 100%

Table 39. Changes in pressure in evacuated packages of picnic ham wrapped with Saran-coated cellophane

	Days in storage					
	0	4	7	14	21	28
Vacuum (cm. Hg.)	74.5	73.6	73.0	73.2	72.6	-
	73.5	73.5	73.2	72.8	71.9	72.1

Table 40. Spectral reflectance* and variation in reflectance of sliced bologna

	Wavelength (mμ)						
	540	570	600	650	700	750	800
	25.0	24.5	34.5	60.0	63.0	63.0	63.0
	25.0	25.0	35.0	59.0	64.0	64.0	64.0
	28.0	28.0	39.0	63.0	69.0	69.0	69.0
	28.0	28.0	39.0	62.0	67.5	67.5	67.5
	26.0	28.0	38.5	62.0	67.0	67.0	67.0
	29.0	29.0	39.5	63.0	69.0	69.0	69.0
	27.0	27.0	37.0	59.0	64.0	64.5	64.0
	28.0	28.0	38.5	62.0	67.5	68.0	69.5
	27.5	27.5	39.0	64.5	69.5	70.0	70.0
	27.5	27.5	38.0	62.5	67.5	67.5	67.5
	29.0	29.0	40.0	64.5	69.5	68.5	68.5
	31.0	31.5	42.5	66.5	71.5	71.0	72.0
	28.0	28.0	37.5	59.5	64.5	64.0	64.0
	28.0	28.0	38.0	59.0	64.5	64.0	64.0
	28.5	29.0	40.5	63.5	68.5	68.5	68.5
	28.5	29.5	40.0	63.5	68.5	68.5	68.5
	29.0	28.5	39.0	63.5	69.0	69.0	69.0
	28.5	28.0	39.5	63.0	68.5	68.5	68.5
Mean	27.9	28.0	38.6	62.2	67.4	67.3	67.4
Confidence interval (P=0.05)	27.2	27.2	37.7	61.1	66.2	66.1	66.1
	28.6	28.8	39.5	63.3	68.6	68.5	68.7

*Standard $MgCO_3$