SOME CAUSES AND MEASUREMENTS OF COLOR CHANGES IN FRESH RETAIL MEAT CUTS

By

Ogbourne Duke Butler, Jr.

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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AN ABSTRACT

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ABSTRACT

Discoloration of prepackaged fresh meats limits their shelf-life in self-service cases, and results in economic losses.

Some causes for the discoloration were investigated in this study with the purpose of extending the shelf-life.

Color was measured by use of disk colorimetry and by spectrophotometric estimation of metmyoglobin. A comparison of the two methods was made.

Steaks cut from the longissimus dorsi muscles of cattle grading commercial, good, and choice were used.

Though some references in the literature stated or implied that bacterial growth caused discoloration of fresh meats, little or no data were given, and objective color measurements were not usually employed. To investigate the effect of bacteria on color, paired surfaces adjacent to one knife cut were used, with one surface being inoculated with a culture of Pseudomonas sp., which is commonly found on fresh meat cuts. This report included data obtained on 114 pairs, with storage in the cooler at 32°F, and in a self-service case at 34°F, and at 40°F. Storage conditions were characterized by measurement of temperature, relative humidity, and air movement.

A commercial slicing machine was used to obtain surface samples for color analysis and bacterial study.

The following results were obtained:

1. During the period of increasing formation of metmyoglobin, there was a high correlation between the index of fading calculated from Munsell hue, value, and chroma determined

by disk colorimetry, and the percent metmyoglobin determined spectrophotometrically.

2. Bacteria commonly found on meat cuts caused discoloration. The main effect was to increase the rate of metmyoglobin formation. This effect was greatest during the logarithmic growth phase.

3. The color of freshly cut beef was not significantly correlated with the percent metmyoglobin.

4. Desirable color was maintained longer at 34°F than at 40°F in a self-service case. A temperature of 32°F in a cooler was more effective than a rack level temperature of 34°F in the self-service case.

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I. INTRODUCTION

Meat retailing by the self-service method has been widely accepted by retailers and consumers. Many shoppers enjoy the opportunity to examine unhurriedly an open display containing a wide selection of retail cuts before deciding on the main course for a meal. This examination is likely to be rather thorough and critical. Shoppers are prone to be somewhat suspicious, and most of them are relatively inexperienced and uninformed concerning meat technology. Any abnormality, such as variable color of lean in the packages of one display, may be magnified in importance, and interpreted as denoting poor quality meat. It is essential for the retailer to have an attractive display.

The meat retailer must keep a self-service case well stocked with many kinds of retail cuts. It is impossible to estimate the exact sales of any item for a day, so carryover packages are a certainty. If the carryover packages are less attractive than fresh ones, buyers will continue to reject them, and frequently the retailer must rework such packages into cheaper items such as ground meat.

A major factor affecting the attractiveness of prepackaged retail cuts is the color of the exposed lean. Myoglobin, the muscle pigment, is chiefly responsible for the color. Freshly cut lean meat does not appear at its best, for the most attractive color is developed only after the myoglobin has been oxygenated. This occurs normally and without special handling for most meats. Further color changes due to drying of the surface and to deterioration of the oxymyoglobin limit the shelf-life of fresh meat packages.

The purpose of this study was to investigate causes for color changes in fresh meats, and to find treatments which would prolong attractive shelf-life for self-service packages.

Though the color of lean is a perfectly evident characteristic, it is difficult to describe objectively. A measure of the color is essential in order to determine the effect of treatments. Several systems have been proposed for measuring the color. Two of these systems, the use of Munsell spinning disks, and spectrophotometric estimation of the percent metmyoglobin, have been applied and compared in this study.

An influence of bacteria on the color of lean of fresh (uncured) meats has been suspected by several investigators. Some have stated that bacteria cause color deterioration, but the supporting evidence has been incompletely presented or entirely absent. Accordingly, experiments were designed and conducted to measure the effect on color of certain bacteria commonly found growing on retail meat cuts.

II. REVIEW OF LITERATURE

A knowledge of the chemistry of meat pigments, and of the scientific aspects of color is needed for proper experimental design and interpretation of results. A minimum of literature concerning economic factors and general information on self-service meat retailing was reviewed. It was necessary to review the literature on bacteriology of fresh meat cuts to evaluate the results obtained on color changes when samples were inoculated with bacteria.

Voegeli (1952) made a rather complete survey of the literature on the economics of self-service meat retailing, and consumer reactions to this method of merchandising. Consumer opinions of the advantages and disadvantages compared to butcher service retailing were given. Voegeli obtained the estimates that eight to ten percent of the daily input of packages for self-service cases were reworked items previously removed from the case for a variety of causes. Discoloration was the cause for removal of approximately 26 percent of the packages which were later rewrapped or converted into a different retail item. This would entail a significant amount of labor expense, as well as a loss due to a lower selling price for the reworked product.

Urbain (1952) of Swift & Co. addressed a meeting of the American Meat Institute. He emphasized the importance of desirable color in self-service retail meat cuts. He stated that oxygen is the key to the color in meat. Oxygen is essential for desirable color formation in fresh meat cuts, but is a cause for color deterioration in cured

meats. He recognized that browning resulting from the oxidation of myoglobin to metmyoglobin was the primary cause for discoloration of fresh prepackaged retail cuts, and pointed out the possibility that bacteria might be involved. The latter possibility was considered to be of minor importance by him, however, because he stated that bacteria are normally present and their activities are controlled by lower temperatures. In fact, he considered the entire problem of color deterioration in fresh meat cuts to be minor compared to the color problem in cured meats. There is little doubt about that conclusion from the viewpoint of the packer, for the packer has a certain responsibility for the color of cured meats. Packer operations are not likely to exert much influence on the color of fresh (i.e., uncured) retail meat cuts, and the packer probably receives very few complaints from his wholesale customers concerning the deterioration of color in fresh meats.

Urbain also remarked on the importance of illuminating the meat display case with lights which transmit the wavelengths needed to bring out the desirable color of meat.

A. Chemistry of Meat Pigments

Myoglobin, the principal pigment of red meats, is a chromoprotein with an iron porphyrin prosthetic group. The porphyrin family includes two very vital members, chlorophyll and hemoglobin, both essential for life. Lemberg and Legge (1949) authored a book dealing with this family of compounds, and found it necessary to cite 3182 references. The literature is rich in research reports on the members of the

family, but most of the research has been directed toward their physiological behavior, with relatively few papers concerning the pigments in the carcass of the slaughtered animal.

The combination of heme with the protein globin results in substances in which the iron can form a dissociable compound with oxygen, with the iron remaining in the ferrous state. Hemoglobin, the blood oxygen carrier of mammals, has a molecular weight of 66,000 - 68,000 according to Haurowitz (1950), and consists of an aggregation of four "units" of the protoheme-globin complex. Myoglobin has a molecular weight of 17,000, and is similar to one of the "units" of hemoglobin, though the protein fraction is different from the globin of hemoglobin.

It is certain now that the heme prosthetic group is the same in both of these pigments, but the aggregation, and the globin (protein) fraction are different.

The prosthetic group is protoheme, and its structure is represented by the formula shown in Fig. 1, essentially as given by Haurowitz (1950).

Though the blood pigment, hemoglobin, had been studied for many years, it was not until after Theorell (1932) isolated, crystallized, and characterized the muscle pigment, myoglobin, that absolute proof was obtained for the differences in the two pigments. Before the publication of Theorell's work, it was controversial whether the muscle pigment and blood pigment were the same or different compounds. Kennedy and Whipple (1926) studied muscle and blood hemoglobins and expressed doubt that they were different compounds. By 1933, however, differences were well established. Hill (1933) recognized this fact, and noted spectrophotometrically that the sharpest absorption band for muscle

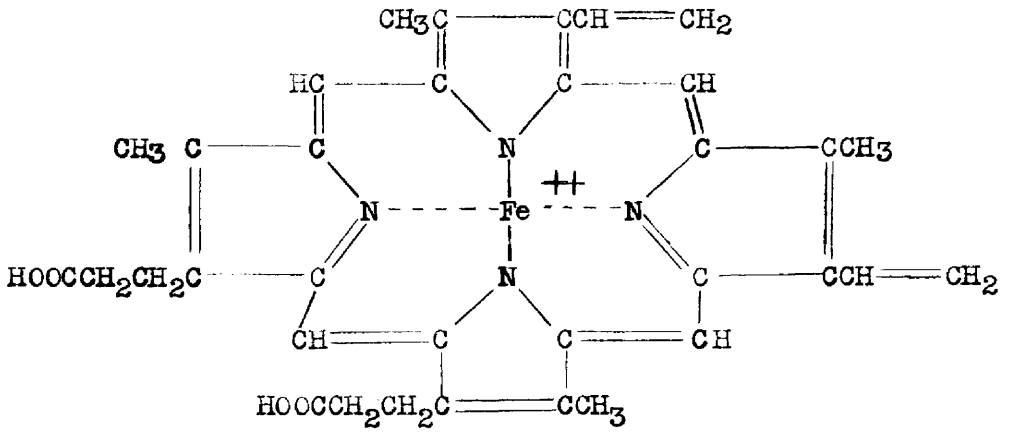

Fig. 1. Structure of protoheme

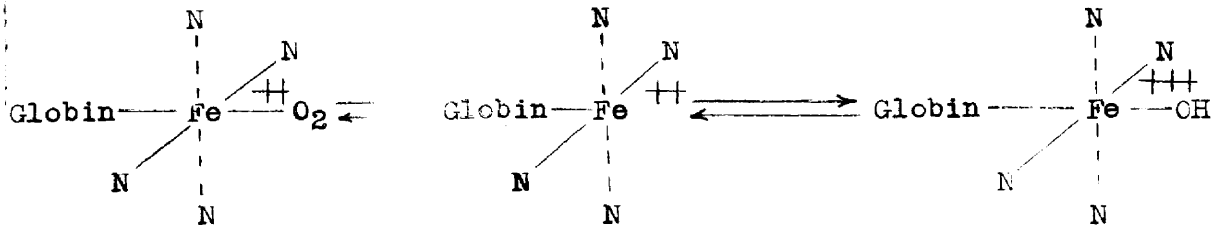

Fig. 2. Reactions of myoglobin

hemoglobin was at 5800 A while blood hemoglobin was at 5700 A. Later Hill (1936) noted the greater affinity of myoglobin (muscle hemoglobin) for oxygen than hemoglobin, and that the absorption bands for hemoglobin were sharpest at 5770 A and 5420A, compared to 5815 A and 5446 A for myoglobin. He also noted the hyperbolic oxygen dissociation curve for myoglobin compared to the sigmoid curve for hemoglobin. Millikan (1939) pointed out the same fact about the oxygen dissociation curves for the two pigments, and related the hyperbolic shape of the curve to the physiological oxygen store function of myoglobin.

The apparent discrepancy in the absorption band near 580 millimicrons as given by Hill was fixed at 582 by the work of Shenk, Hall, and King (1934), Bowen (1949), and others. Bowen's work was particularly good, and his data for extinction coefficients are used in this study for estimation of percent metmyoglobin. Austin and Drabkin (1935) published similar information for hemoglobin, and Mangel (1951) used their system in her study. In another paper, Drabkin and Austin (1935) gave the advantages of the use of methemoglobin cyanide measured spectrophotometrically as a standard for reference in preference to gasometric techniques. They showed that the reagents used for conversion of the pigments had a negligible effect on the absorption spectrum of the CN derivative at the critical wavelengths.

Myoglobin increases in concentration with increasing age of animals, and apparently increases with exercise or training of the muscles according to Millikan (1939), to Whipple (1926), and to Lawrie (1950). Whipple found a variability in dogs from 0.12% myoglobin in pups less than one month old to as much as 1.05% in a trained hunting

dog. While mature dogs in general varied from 0.52% to 0.82%, a house dog which got little exercise had only 0.37%. Kennedy and Whipple (1926) also noted that the concentration of muscle hemoglobin seemed to depend on the amount of muscular exercise. Bull and Rusk (1942), however, found that exercise did not affect the color of lean in the cattle they tested.

In meats there is some blood residue, but Shenk et al (1934) found that myoglobin usually made up at least 90% of the meat pigments, with hemoglobin usually not over 10%, and frequently much less. They proposed a method for estimation of the composition of those two pigments in a muscle extract by use of the ratio of the optical densities measured at wavelengths of 577 and 582 millimicrons.

Drabkin et al (1950) studied the distribution of hemoglobin, myoglobin and cytochrome c in the tissues of different species. They concluded that the concentration of myoglobin does not relate to body size. Myoglobin is more concentrated in the muscles of animals that run fast or work hard such as the dog and the horse. In a heifer they found the proportions of the three chromoproteins to be 1786 hemoglobin: 247 myoglobin: 1 cytochrome c. Using their figures the concentration of myoglobin in the skeletal muscles of the heifer seemed to be about 0.40% to 0.47%.

The reactions of myoglobin which are of particular importance in fresh meats may be symbolized as shown in Fig. 2, with the four nitrogen atoms of the pyrrole rings representing the porphyrin ring essentially as given by Haurowitz (1950).

Winkler (1939b), and Brooks (1933) each noted that the two main causes for surface discoloration in fresh meats were desiccation and oxidation of the myoglobin to metmyoglobin. The main effect of drying was darkening due to concentration of the pigments at the surface, with perhaps some alteration of the optical properties of the desiccated tissues.

The chemical reactions shown in Fig. 2 for myoglobin seem to account, primarily, for the changes in color of prepackaged fresh red meats, if moisture loss is minimized.

Much of the early work on the changes in the meat pigments was done by Brooks (1929, 1931, 1933, 1935, 1936, 1938, 1948). He showed that the oxidation of myoglobin was due to oxygen, for in a nitrogen atmosphere free from oxygen, the oxidation did not occur to any appreciable extent. He found the reactions to occur in the sequence shown: oxymyoglobin (MbO_2) $\underset{+O_2}{\overset{-O_2}{\rightleftharpoons}}$ myoglobin (Mb) $\underset{red}{\overset{oxid}{\rightleftharpoons}}$ metmyoglobin (Met Mb). Conant's (1923) potentiometric studies with hemoglobin agreed with the pathway noted here. Though there has been some disagreement concerning this pathway, the recent publications of George and Stratmann (1952a, b), seem to make it almost certain. In the first reaction there is no electron transfer, but the second involves the oxidation of the iron from the ferrous to the ferric state.

Oxygenated myoglobin gives the color desired in fresh meats. When first exposed to the air, the pigment is primarily in the reduced state, and the color is a shade of red purple. Oxygenation changes this to

a desirable bright red. A rather dull yellow red, characteristic of metmyoglobin results when the oxymyoglobin loses oxygen and the resulting myoglobin is oxidized.

Brooks' papers went far in explaining the factors which influence the reactions noted. The partial pressure of oxygen is of major importance. The temperature, also, is an important factor, not only because of the increased reaction rate as temperature is increased, but also because the oxygen is less soluble as temperature is increased, and the partial pressure of oxygen in the meat decreases. In one paper, Brooks (1931) found the rate constant for Hb $\longrightarrow$ Met Hb to be about four times as great at 25°C as at 15°C. The oxidation rate is fastest at low oxygen pressures, being a maximum at a pressure of about 4 mm Hg. at 0°C, according to Brooks (1938). This was estimated by Brooks by following the color changes with a microspectroscope in a tissue section. Using a more refined gasometric technique, George and Stratmann (1952b) found the maximum rate for Mb $\longrightarrow$ Met Mb to be between 1.0 and 1.4 mm oxygen pressure at 30°C. They also noted that in air the rate of oxidation of myoglobin was 4.25 times faster than the rate for hemoglobin.

The extremely long storage periods without discoloration reported by Brooks (1933) are of interest. He stopped bacterial growth by use of toluene vapor, and found that six to eight weeks elapsed before the oxidation in the surface layer of tissue was complete if drying was controlled. Later (1938) he reported that at 14°F more than sixteen weeks were required for discoloration to occur. He reported discoloration when the myoglobin was about 60% changed to metmyoglobin, but

in the present study much lower percentages of metmyoglobin gave discoloration.

The change to metmyoglobin occurs only in the surface area where oxygen has penetrated. The partial pressure of oxygen decreases from the surface inward. Brooks (1938) found that muscle retains a residue of respiratory activity long after slaughter of the animal, and that dissolved oxygen is present only in a superficial layer a few millimeters thick -- almost never more than six millimeters from the surface. Brooks (1938) proposed a formula for estimating the depth of oxygen penetration as determined by the relative rates of oxygen diffusion and uptake.

$$d = \sqrt{\frac{2C_oD}{A}}$$

Where
- d = depth of oxygen penetration
- C_o = pressure of oxygen at tissue surface
- D = coefficient of diffusion of oxygen through tissues
- A = oxygen consumption

The value for A is roughly 10^{-4}cc per gram per minute at $0^{\circ}C$.

Brooks (1933) estimated that the surface color was affected by about a two millimeter layer of tissue, for no light was reflected from layers deeper than this.

After the chemistry of the red meat color change had become fairly well characterized, some workers began trying to influence the change. Winkler (1939b) noted that increasing the pH above 5.5 caused darkening, while lowering the pH to between 4.5 - 5.5 caused lighter colors. This agreed with observations on dark cutting beef made by Hall, Latscher, and Mackintosh (1944), though they concluded that the main reason the beef is dark in dark cutters is that the demand for oxygen is greater

than can be supplied by normal transfusion into the tissues, resulting in robbing oxymyoglobin of its oxygen. Hill's (1928) work on oxygen diffusion into the fatigued muscle might offer some explanation for this.

Bate-Smith (1948a,b) also made observations on the effect of pH which agreed with the work of Hall's group.

Certain chemical additives have been observed to affect the myoglobin ——→ metmyoglobin reaction. Coleman (1951), Watts and Lehmann (1952a, b) and Gibson (1943) have noted the desirable effect of ascorbic acid. Coleman also noted that nicotinic acid and its esters transform the interior color of meat from red purple to bright red. He stated that salt increases the rate of oxidation of meat pigments. Garner, Mills, and Christman (1951) studied the degradation of hemoglobin in living tissues, and concluded that ascorbic acid seemed to catalyze the degradation of hemoglobin to choleglobin, a green pigment, but thought that the glutathione of living red blood cells inhibited this degradation.

B. Color Science

Color is the response in the brain to the wavelengths of light picked up by the eye. Humans and birds have color perception, but most animals do not, according to Abbott (1947).

In the brain the response is due to nervous energy transmitted from the eye, so the basis for color understanding probably should begin with a study of the human eye, and of light, which initiates the nervous energy.

Wright (1944) explained the reception in the eye of light from the visible spectrum. He showed a drawing of a horizontal section of the eye, as reproduced in Fig. 3.

He explained that light energy is converted into nervous energy in the retina. There are two types of nerve cells which are actual light receptors - the rods, and the cones. In relation to color, rods are inactive. They function mainly to give vision when the amount of light is slight.

The cones are responsible for color perception, and they operate at higher illumination levels. It is thought that the initial reaction in the cones is a photochemical one, with light sensitive chemical substances being decomposed. The products formed are thought to initiate the train of impulses which travels the short distance to the brain.

The fovea is a concentrated area of cones, and here color perception is keenest. This completely rod-free area subtends an angle of only one to two degrees in the visual field. The retinal area away from the fovea is increasingly less acute for color resolution as the distance from the fovea is increased. Obviously, then, color discrimination is maximal in the center of the field of view.

Light is admitted to the eye through the cornea. Refraction in the cornea and in the crystalline lens serves to focus the light on the inner wall of the eyeball. The iris compensates for sudden changes in light intensity.

Abbott (1947) gave a rather understandable explanation of the nature of light, and information given here was taken from his publication.

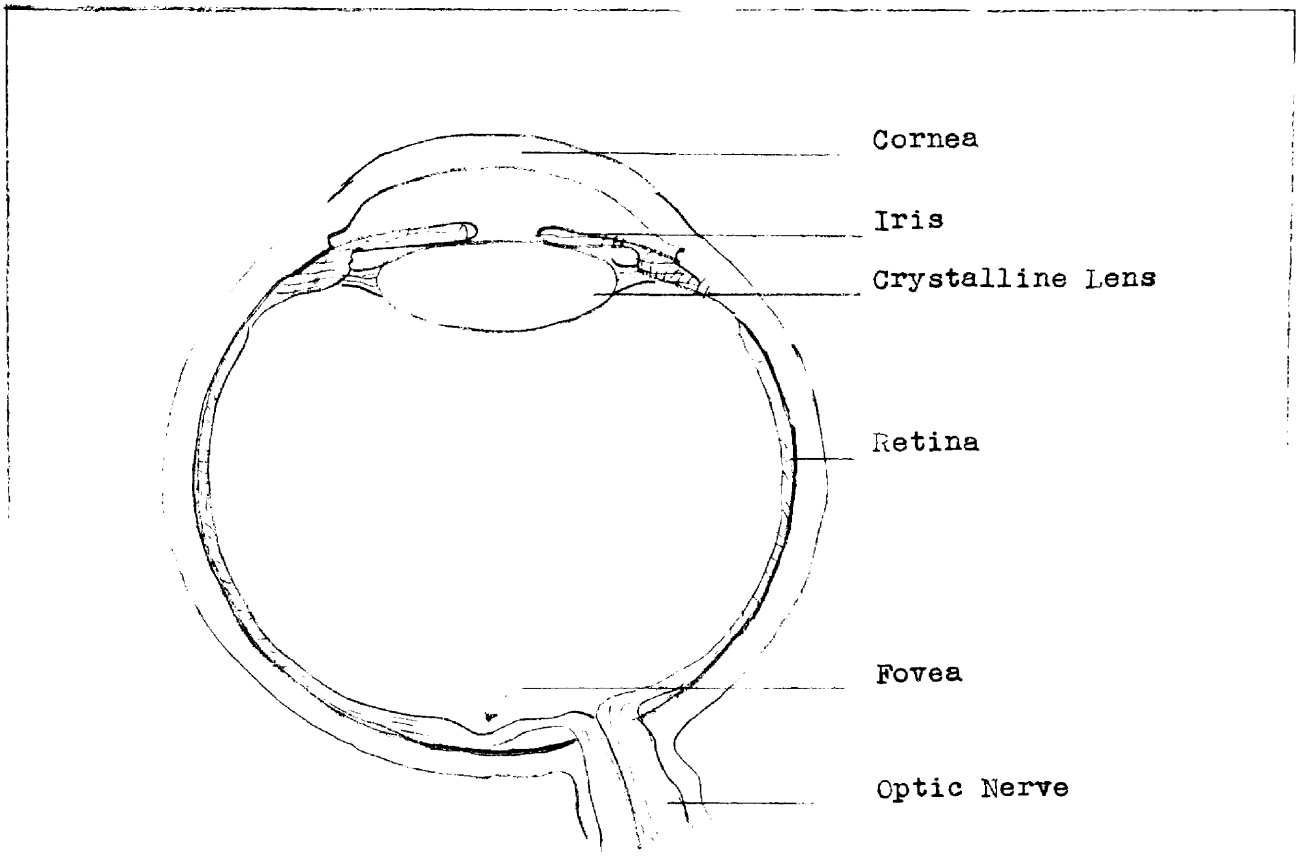

Fig. 3. Horizontal section of human eyeball

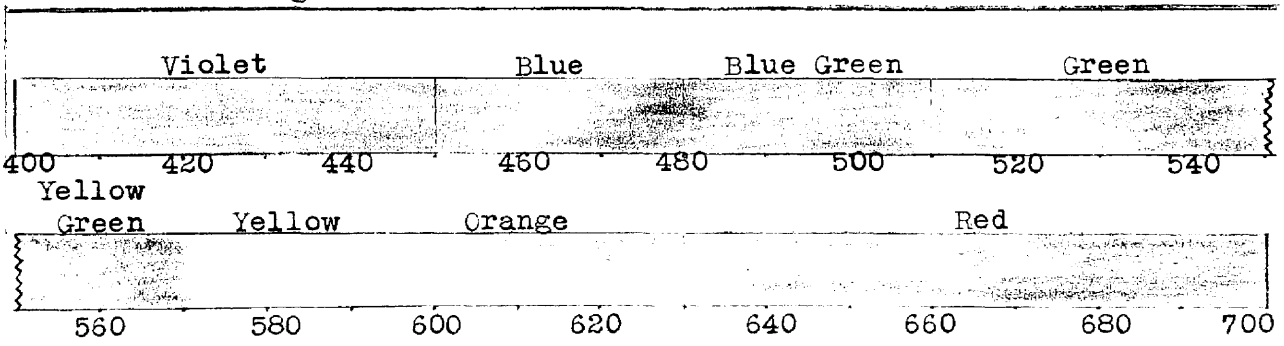

Fig. 4. Hues in the visible spectrum
Wavelengths of light from 400-700 millimicrons

Without light there would be no color. Sunlight is known as electromagnetic radiation. Electrons revolve about the protons and neutrons in an atom at a speed of about 1500 miles per second. This creates heat, and in turn causes one or more electrons to leave the atom (ionization). The protons, however, promptly recapture such electrons, and do so millions of times each second. Every time an electron is recaptured, the energy of its former motion is set free as a unit beam, or "quantum" of radiation (photon). These photons fly off into space at a speed of about 186,300 miles per second.

Light travels in waves. It travels straight unless it meets with interfering matter, which may cause the light beam to be bent, broken, or scattered, depending on the molecular composition of the matter.

The length of a light wave depends on the amount of energy that created it, and determines the color sensation produced by it. Visible rays measure about 400 to 770 millimicrons. The shorter waves vibrate more, and thereby create a different color sensation.

Radiant energy emanates from all luminous bodies. All matter absorbs part of the light, and part of the heat transmitted. The elements of the matter, and their molecular arrangement, determine the amount of light absorbed, reflected, or transmitted. The nature of the matter also determines whether it will bend (refract), break (diffract), or scatter (disperse) the rays.

Jacobson (1948) explained that when light strikes an object and is reflected to the eyes a color sensation is obtained. The wavelengths not reflected are "absorbed", that is, they are converted to heat.

When light strikes a white surface, about 90% of the light is reflected including long, medium, and short wavelengths. When these enter the eye together they stimulate the red, green, and blue receptors of the retina, which convey the total stimulation to the brain where it is recognized as white.

When the beam of white light strikes a red surface, almost all of the short wavelengths are absorbed. The long ones are reflected, and stimulate the red receptors, which convey the stimulation to certain areas of the brain to produce the sensation of red.

Wright (1944) presented the Young-Helmholtz theory of color vision. To reproduce any color, according to this theory, it is necessary to take only three radiations, such as red, green, and blue, and mix them in the correct proportions. An excellent presentation of this color concept was made in Life magazine (1944), as well as an excellent illustration of the colors of the visible spectrum, the functions of filters, the Munsell color system, and the basic structure and use of spectrophotometers. In fact, the presentation in that magazine summarized quite graphically the fundamentals of color.

The hues in the visible spectrum may be divided as shown in Fig. 4, according to Wright (1944).

Actually there is no sharp break between hues of the spectrum, but they blend gradually into each other. Red, green, and blue are the basic colors, and the others can be considered to be mixtures of them.

The hue sensation depends on the selective reflection of the wavelengths illustrated. Hue alone, however, cannot determine the entire color sensation. Other factors which are important are the

"lightness", and "saturation". The lightness depends on the amount of total reflection of light, with a white surface being highly efficient in reflecting all wavelengths, and a black surface being highly efficient in absorbing all wavelengths. The shades of gray lie intermediate between the black and white.

The saturation of a color depends upon the extent of dominance of wavelengths within any selected hue of the spectrum.

A three dimensional figure as given by Wright (1944) can serve to illustrate the color characteristics mentioned. Any color perceptible to man could be located within the boundaries of such a color solid, as shown in Fig. 5. Note that black or white objects have no hue.

Red, green, and blue are the basic colors which will give all other color sensations if mixed in the proper proportions. This concept formed the basis for scientific color reporting. According to Nickerson (1946), at the 1931 meeting of the International Commission on Illumination (I.C.I.), agreements were made which have been put to practical use all over the world. The specifications for any color as how much red, white and blue light of specified intensities were required to match it formed a sound basis. Practical application of this proved to have difficulties, however, and the I.C.I. agreed upon a mathematical transformation from the RGB to the XYZ system as explained by Wright (1944). Values for X, Y, and Z were selected so that all coefficients would be positive, and so that the base line XZ of the XYZ triangle would have zero luminance. The advantage of the latter selection was that the Y stimulus then would also express the reflectance.

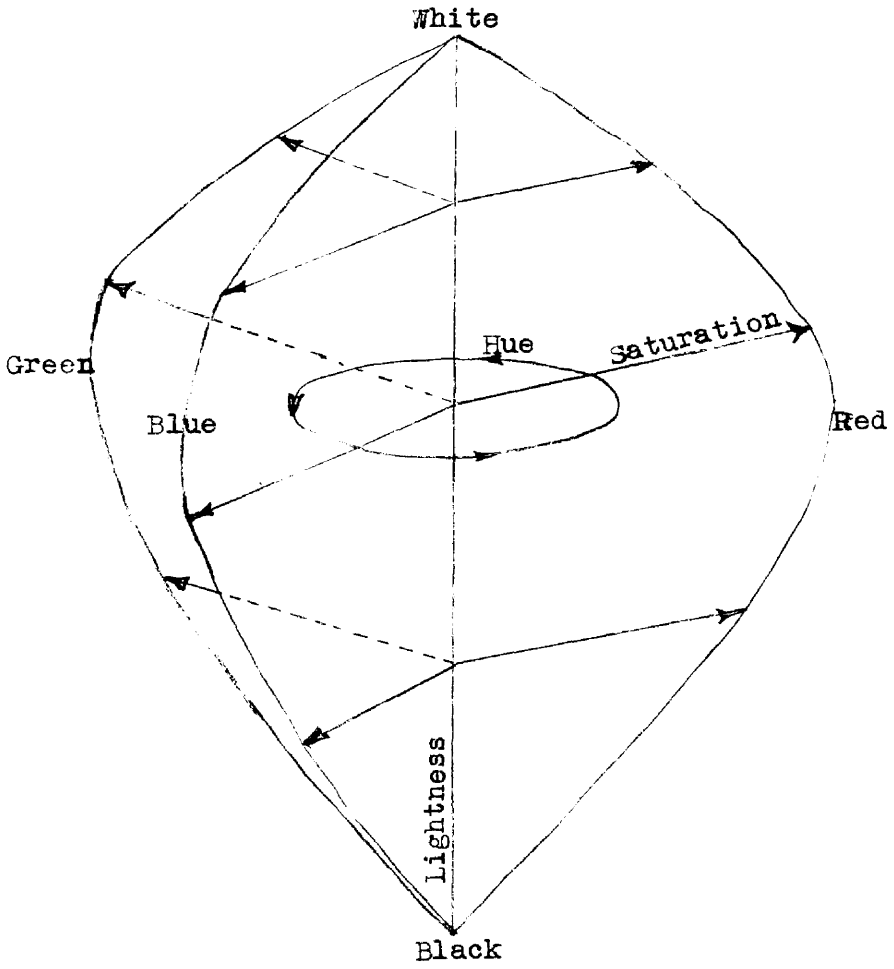

Fig. 5. A theoretical color solid

Fig. 6 shows the locus of the spectrum colors in the XYZ chromaticity diagram, as illustrated by Wright. The locus illustrated in Fig. 6 is the mean curve derived from measurements by a number of observers and transferred to XYZ tristimulus values, according to Wright. He stated that the curve is always either straight or convex, never concave, so that all colors must lie on the locus or within the area bounded by it.

Point S_E in Fig. 6 is an arbitrary intensity of white light. Point C is a certain color. If a straight line is drawn through C to intercept the locus at wavelength λ, it follows that the proportionate mixture of S_E and the wavelength at λ would produce the color C. λ as used here may be called the dominant wavelength, and the distance from the S_E toward the locus represents the saturation as shown in Fig. 5. Since the XYZ triangle is in the one plane, any point within its boundaries can be specified by two coordinates, called x and y.

The Munsell color system is probably the most widely used today of any system for color notation. In this system, any color may be described by its hue, value, and chroma. Munsell (1929) defined hue as "The distinctive characteristic of any chromatic color, distinguishing it from other hues, such as are found in the spectrum or between the ends of the spectrum." He also defined value as the lightness or darkness of any color, and chroma as the strength or weakness of a chromatic color. Saturation and intensity (purity) were given as synonyms for chroma.

Nickerson and Newhall (1943) observed that when using a good viewing instrument, the scale units for hue represented about 20 just perceptible increments per hue designation, or 2.5 hue steps represented

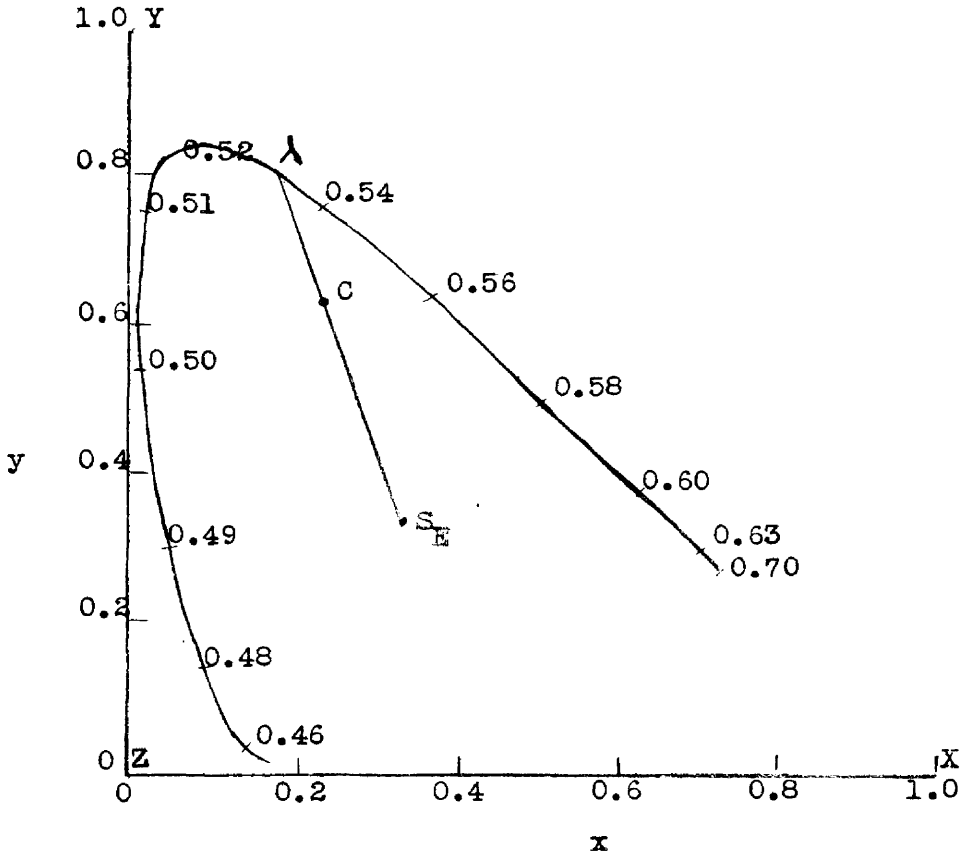

Fig. 6. The locus of the spectrum colors
Description of a color by reference to the locus
and the white point S_e

about 5 just perceptible increments. One value step represented about 50 just perceptible increments, and one chroma step about 5 just perceptible increments. They calculated that under the best observational conditions, about 7,500,000 surface colors could be distinguished.

Newhall, Nickerson, and Judd (1943) reported that the standard I.C.I. Illuminant C which approximated the light given by an object heated to 6700° absolute temperature had been taken as a neutral origin for both hue and chroma loci. This corresponds to the point S_E shown in Fig. 6.

The standard illumination used in this study was approximately that given by Illuminant C.

The handbook by Nickerson (1946) provided the basis for application of the method of disk colorimetry using the Munsell system of notation. This system was used successfully by Voegeli (1952) in following color changes in fresh beef cuts.

Other workers have found other devices to be suitable for measuring the color of meats. Winkler (1939a) used a reflectance device. Younkin (1950) employed the Hunter Color Difference Meter, developed by R. S. Hunter (1948), to measure color in tomato products, and this device has been used in meat color studies at Rutgers University. Mangel (1951) used spectrophotometric estimation of metmyoglobin as a basis for measuring meat color differences. Hall et al (1944) used color paddles. Bull, Olsen, and Longwell (1930) used a Keuffel and Esser spectrophotometer, and calculated the brilliance, hue, and saturation from measurements made directly on samples of meat instead of solutions. The relation of color to some different ages and grades of cattle was given.

Black, Warner, and Wilson (1931) first applied the method of disk colorimetry to the measurement of meat color. Mackintosh (1932, 1935) published color data obtained on several classes and grades of cattle with the spinning disks and the Munsell system. Included, also, were the packer classifications of the exposed rib eyes as bright, dark, or black. From these data Mackintosh proposed a set of standards based on the percentages of the four disks required to match the samples, and later published the value and chroma corresponding to these disk percentages. The hue was not shown for each, but it fell within the narrow range of 5.0R - 5.8R for all samples. The disks used were Red 11/4, Yellow 6/8, Black N/1, and White N/9.

By taking I.C.I. tristimulus values for those disks as given by Kelly and Gibson (1943) for illuminant C, the daylight illuminant, calculations were made for the average reading for eight normal carcasses as given by Mackintosh (1932). While the hue given by Mackintosh (1935) for those data was between 5.0R and 5.8R, by the present system the hue calculates to 4.5R. The value and chroma by Mackintosh were 3.40/5.41, but are 3.69/6.9 under the present system. Nickerson (1946) compared the early method, as used by Mackintosh, with the present method, and emphasized the increased accuracy of the present method. The hues obtained by Voegeli (1952) fluctuated rather closely around 10.0R, and seldom were less than 8.0R. The data obtained in this study agreed rather closely with those of Voegeli.

Johnson and Bull (1952) used a General Electric recording spectrophotometer to measure the percentage of light reflected in the visible region of the spectrum to compare the color of lean and fat in cured

bacon. They believed brightness of lean as measured by reflectance to be closely related to the attractiveness of sliced bacon.

This study involved the use of disk colorimetry and of spectrophotometric estimation of metmyoglobin with a comparison of the two methods. The methods applied were essentially as used by Voegeli (1952) and by Mangel (1951). Further explanation will be given in the section on experimental procedure.

C. Bacteriology Relating to Fresh Meat Color

The connection between bacterial growth and surface color changes in fresh meat cuts has not been clearly defined in the literature. Jensen (1945) stated that microorganisms, both living and dead, and their enzymes on the surface of meat may oxidize both fresh meat pigments and cured meat pigments to methemoglobin, a dark brownish compound. The trade characterization "loss of bloom" was said to describe the effect of formation of methemoglobin through bacterial enzymes and metabolic end products acting with atmospheric oxygen. This was intensified by "sweating" of the meat followed by drying. Allen (1948) stated that there is a type of meat discoloration where a gray-brown color develops when the coloring matter is destroyed by bacteria.

Allen (1949) also reported on the bacterial growth on prepackaged boneless round steaks held at 34° - 40°F. The bacterial counts were made every three days. The count rose rapidly until the ninth day, after which it remained rather constant.

Urbain and Greenwood (1940) worked with dilute hemoglobin solutions at 10°C. One set of tubes was treated with toluene to control bacterial

growth, and in the other set of tubes, bacteria were allowed to grow. The tubes were shaken in air every day for maximum aeration. There was no methemoglobin in the bacteria free tubes after sixty days storage, but the contaminated tubes showed reduced oxygen capacity after eleven days, and the oxygen capacity was reduced to fifty percent at sixty days. This was attributed to methemoglobin formation.

The solution changed color as the percent methemoglobin increased. The authors stated that this demonstrated that meat color in general was affected by the presence of microorganisms acting upon the sensitive heme pigments.

Kraft and Ayres (1952) noted that in many instances discoloration of samples was observed before disagreeable odors or other evidences of spoilage became apparent at 40°F. They observed the influence on surface color of several different wrapping materials, and found that films that permitted good oxygenating conditions allowed the most rapid bacterial growth. DuPont Cellophane MSAT 80 was effective in preserving good color in their samples for the first three days of storage, and off odors developed in about eight days, followed by the appearance of slime in about eleven days. The surface bacterial counts per sq. cm. obtained by a swabbing technique showed that a count of about 2×10^6 organisms correlated with the first detectable off odor. Use of carbon dioxide gave increased storage life. In their opinion discoloration was not correlated with organoleptic or microbiological evaluation as a means of determining storage end points.

Niven (1951a) stated that the first criterion as to the acceptability of a food by the consumer is its color. Niven (1951b) studied

discolorations of bacterial origin in cured meats and sausages, and found the responsible organisms to be of the genera Lactobacillus and Leuconostoc principally, with possible implication of Micrococcus. The main effect was a greenish discoloration. Apparently there is no close connection between the problems he studied and those of fresh meats in a self-service case.

Ayres (1951) reviewed the literature, and reported some of his research findings, in an address to the Third Conference on Research of the American Meat Institute. He recognized the need for determination of the contribution of specific organisms to the ultimate spoilage. Level of initial contamination, as well as the temperature of the self service case, were reported to have great influence on storage life. He observed a four day longer storage period for short steaks before the onset of off odor at 40°F when the bacterial count was very low. A swabbing technique indicated the normal initial load to be between about one hundred and twelve hundred organisms per sq. cm. for the beef cuts made in their laboratory.

He reported that many papers indicated that slime formation in meats was due to Acromobacter and Pseudomonas types of organisms. Due to a change in the official description, many organisms formerly classified in the genus Achromobacter now fall under Pseudomonas. At spoilage time in one test more than 98% of the flora was comprised of the two genera mentioned. He used off odor as the principal criterion for spoilage detection, and noted that it correlated well with the slime point and increase in production of carbon dioxide. He plotted the bacterial growth curves for meat stored at 40°F, and his curves showed a sample of round steak to have a keeping time of about fourteen days before the appearance of off odor.

Sulzbacher (1952a) noted generation times of 4.50 hours in ground beef, and 4.81 and 5.99 hours in ground pork for Pseudomonas type organisms inoculated into the meat before grinding, with storage at 44.6°F (7°C). The inoculation was rather heavy, with about 10^4 to 10^6 organisms per gram apparently having been added.

Kirsch, Berry, Baldwin, and Foster (1952) made microbiological studies of a considerable number of commercial hamburger samples. Initial counts varied from 1,400,000 to 95,000,000 per gram. Storage in a household refrigerator at 0° - 2°C gave rapid growth, with 500 million or more organisms being present after six days storage. Off odor was apparent in most samples after eight to twelve days storage. The maximum counts obtained ranged from 500 million to 10 billion per gram. A putrefactive odor was present in only one case, with the off odor being of a typical stale, sour, non-putrefactive type in all other cases. The great majority of bacterial isolates were nonpigmented, motile, gram negative, nonspore forming rods classified as Pseudomonas sp. The only color observation made was that fading of the color at the surface exposed to the air (in loosely covered containers) was noticeable in four to six days.

Voegeli, Bratzler, and Mallmann (1952) made bacterial counts on the equipment used for processing meats in two stores having self-service retailing, and also made counts on packages of meat having been displayed for periods of time ranging from zero to 120 hours. The equipment showed bacterial counts ranging from one hundred to 12 million organisms per square inch. For beef cuts, the average count per square inch of surface in store one was 131,000; in store two,

595,000 per square inch immediately after storage. The counts were up to 675,000 in store one, and 3,035,000 in store two for beef cuts stored four days. The store with the higher counts on equipment also showed the higher counts on the meat.

It is noteworthy that none of the reports reviewed made any mention of the use of good systematic, objective, color observations.

In regard to the specific effect of bacteria on meat pigments, Neill (1925a, b, c, d), Neill and Avery (1924), and Neill and Hastings (1925) made a series of studies using several organisms, with hemoglobin substrates. Pneumococci and other organisms were observed to be capable of speeding the formation of methemoglobin, and also to be capable of reducing methemoglobin to hemoglobin, depending on the available oxygen in the cultures. When oxygen was absent, reduction occurred. Their proposed pathway for oxidation of hemoglobin to methemoglobin involving peroxides and other compounds as the oxidizing agents seems to have been refuted by the work of Conant (1923), and of George and Stratmann (1952a, b).

Other evidences of the oxidation-reduction capabilities of bacteria were presented by Eddy, Ingram and Mapson (1952) on the reduction of dehydroascorbic to ascorbic acid by Escherichia coli, and by Hewitt (1950). Hewitt noted that it is commonplace for bacterial cultures to develop reducing conditions, with the lowest oxidation-reduction potential being reached when the metabolic activities of the bacteria are most intense during the logarithmic growth phase. He stated that once growth is established in a culture, oxygen donators and hydrogen acceptors are seized upon avidly by the cells as they

carry on their normal metabolic activities. As activity wanes after the logarithmic growth phase, air slowly diffuses into most cultures, and the oxidation-reduction potential drifts upward again.

Jensen (1945) presented a graph of the normal growth curve for bacterial cultures, along with factors likely to exert influence on the different phases, with particular regard to meat operations.

In this study, the influence of microorganisms on the color of self-service meats has been observed by plate counts of the microorganisms present, and by measurement of surface color of wrapped steaks by means of spinning disks and by spectrophotometric estimation of the percent metmyoglobin.

III. EXPERIMENTAL PROCEDURE

A. Sampling

The beef used was obtained from the longissimus dorsi muscle of the rib cut from commercial, good, and choice grade cattle as specified. Steaks were cut approximately one-half inch thick across the grain. All packaged steaks were wrapped in du Pont 300 MSAT-80 cellophane, a common wrapper for self-service fresh meats. The experimental surface of the steaks was covered with one layer only, with excess wrapper folded and sealed on the other side. The wrap was applied with the wettable side to the meat as prescribed by the manufacturer.

For the bacterial studies the longissimus dorsi was dissected out as aseptically as practical, with the knives used being cleaned and sanitized at frequent intervals. Chlorine solution, approximately 200 parts per million, was found to be satisfactory as a sanitizing agent for equipment. Parke-Davis liquid germicidal detergent was used to sanitize the hands of the operator.

Steak surfaces were paired, that is, the surfaces adjacent to one knife cut were assigned, one as an untreated control, and the other inoculated with bacteria. This pairing technique was used to overcome color differences in the same muscle due to variable marbling, as well as other factors.

Bacterial cultures used were obtained from the American Meat Institute Foundation. The organisms were isolated from beef obtained from retail meat counters. Exploratory testing of the effect of each of five cultures classified as Pseudomonas sp. by the Foundation showed

that the effect on surface color of the inoculated meat samples was almost the same for each, so one culture was selected and used for all other tests. A twenty-four hour nutrient broth culture incubated at room temperature (approx. 25°C) was used for all inoculations. For lot H steaks, stored in the cooler at 32°F, the organisms were applied by streaking the steak down the center longitudinally with several loops of culture. Other lots of steaks were inoculated by direct spraying using compressed air with a spraying device. The broth culture was diluted one to ten with sterile 0.85% saline solution just before application. The cultures were well shaken to provide a uniform suspension of the organisms.

Steak pairs were removed from storage in a random manner, with the order of removal obtained by use of the table of randomly assorted digits found in the book by Snedecor (1946).

Surface bacterial counts were obtained on a slice three mm. in thickness taken from the treated surface, without removal of the wrapper. A Hobart model 400 slicing machine was used. Repeated swab tests showed that the areas of this machine contacted by the meat were rendered sterile by thorough washing with a good detergent in hot water followed by washing with hot chlorine solution. This procedure was followed after each steak was sampled, and the machine was allowed to run to clear off residual chlorine solution before the next sampling.

Slices were handled with sterile forceps. The cellophane was removed and the slice was weighed on a torsion balance to the nearest one-hundredth gram. The pan of the balance was sanitized in the same manner as the slicing machine.

After weighing, the slice was transferred to a sterile Waring Blendor jar, which had been sterilized in the autoclave. Sterile, cooled, distilled water was measured in a sterile 100 ml. graduated cylinder and added to give a one to five dilution. The meat was cut in the Waring Blendor jar for one minute. By use of a sterile 2 ml. pipette, a sample was removed and transferred aseptically to sterile saline dilution blanks (0.85% saline), or to sterile petri dishes as desired to give the necessary dilutions.

Tryptone-glucose extract agar was the plating medium used, and incubation of the plates was at 10°C, with counts made after 96 hours incubation.

The bacterial techniques employed were patterned after the recommendations of the Subcommittee on Microbiological Methods of Meat Research as reported by Sulzbacher (1952b).

B. Storage Conditions

Lot H steaks were stored in the cooler of the meats laboratory. The temperature and relative humidity were measured by use of a Foxboro filled system recording thermometer with a Dynalog Dewcel used for recording the dewpoint. Records obtained were as shown in Fig. 7. Air movement across the samples was approximately twenty-five feet per minute as measured by a Hastings Air Meter. The steak packages were weighed into and out of storage in order to determine the loss in weight of each package.

A Hussman self-service case was used to store the lots of steaks observed under self-service conditions. Storage conditions were

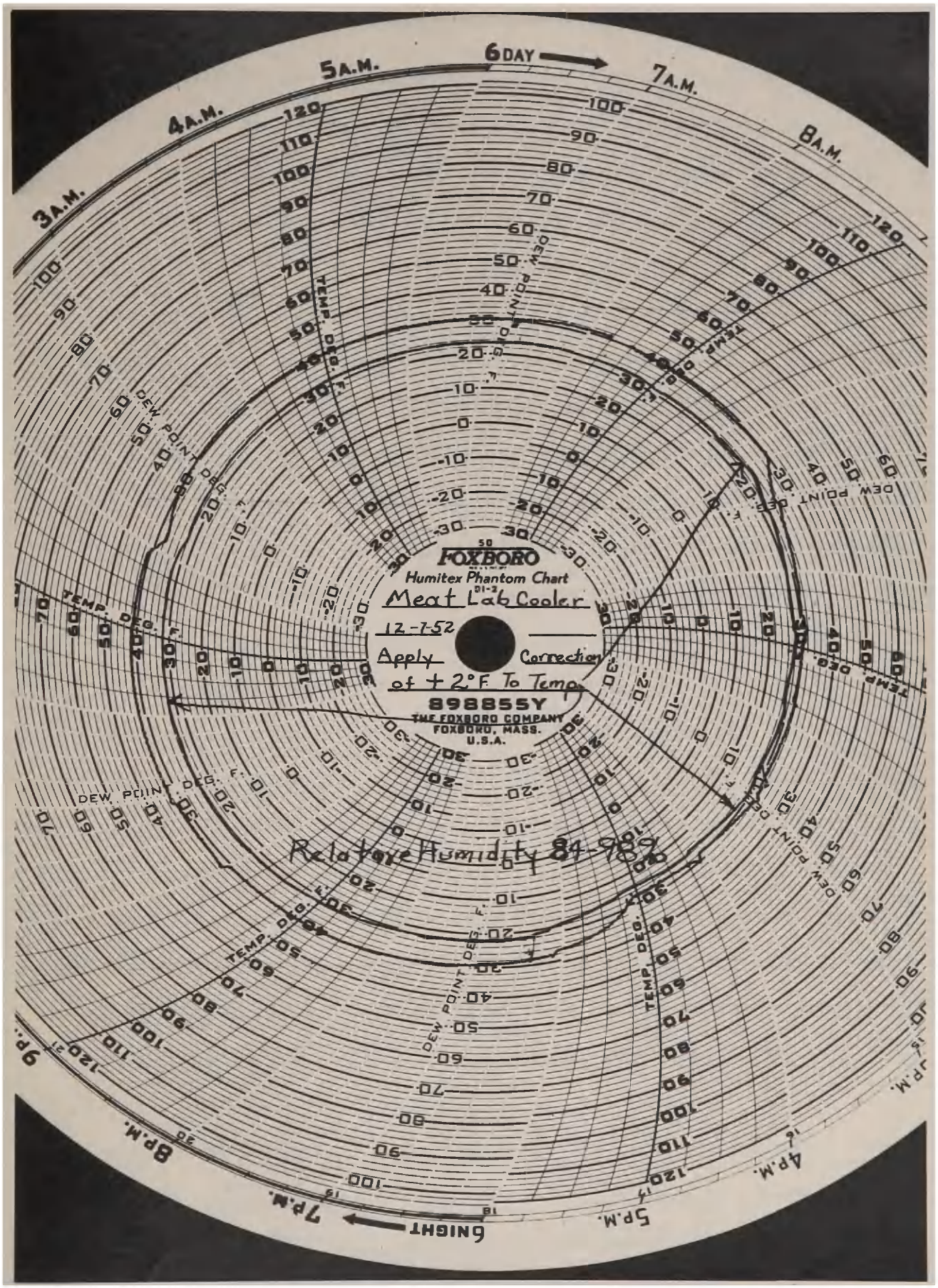

Fig. 7. Temperature and relative humidity of cooler used (apply correction of + 2°F to temperature)

measured with the same instruments as noted for the cooler. The air movement across the samples was about twenty-five feet per minute. The temperature and relative humidity for lots T and U were as shown in Fig. 8. The room housing the self-service case had a temperature of about 70°F and a relative humidity of about 30%.

The dewpoint fluctuation pattern as shown in Fig. 8 was shown to be not unusual for self-service cases by using the same instrument to obtain a record for a similar case in service in a retail store as shown in Fig. 9. The dewpoint cycle corresponded to the compressor cycle. The short-time temperature changes were much less evident, as shown in Figs. 8 and 9. A Honeywell recording potentiometer was used, also, to check the extent of the short-time temperature changes in the self-service case, and the records obtained from it also showed negligible short-time temperature fluctuations.

The temperatures given for the different lots in the self-service case were measured at the storage rack level in the area where steaks were stored. Records obtained by use of the potentiometer showed that the temperature varied as much as five degrees F. from the front of the case to the back, with the middle area having the highest temperature. The steaks were arrayed from side to side of the case across the middle, as this was shown to be an area of rather uniform temperature and air movement. The pairs of steaks were kept together. The steaks were not stacked.

The air temperature at the upper surface of the steaks was from two to four degrees warmer than at the lower surface as shown by the potentiometer. The average increase in temperature from lower to

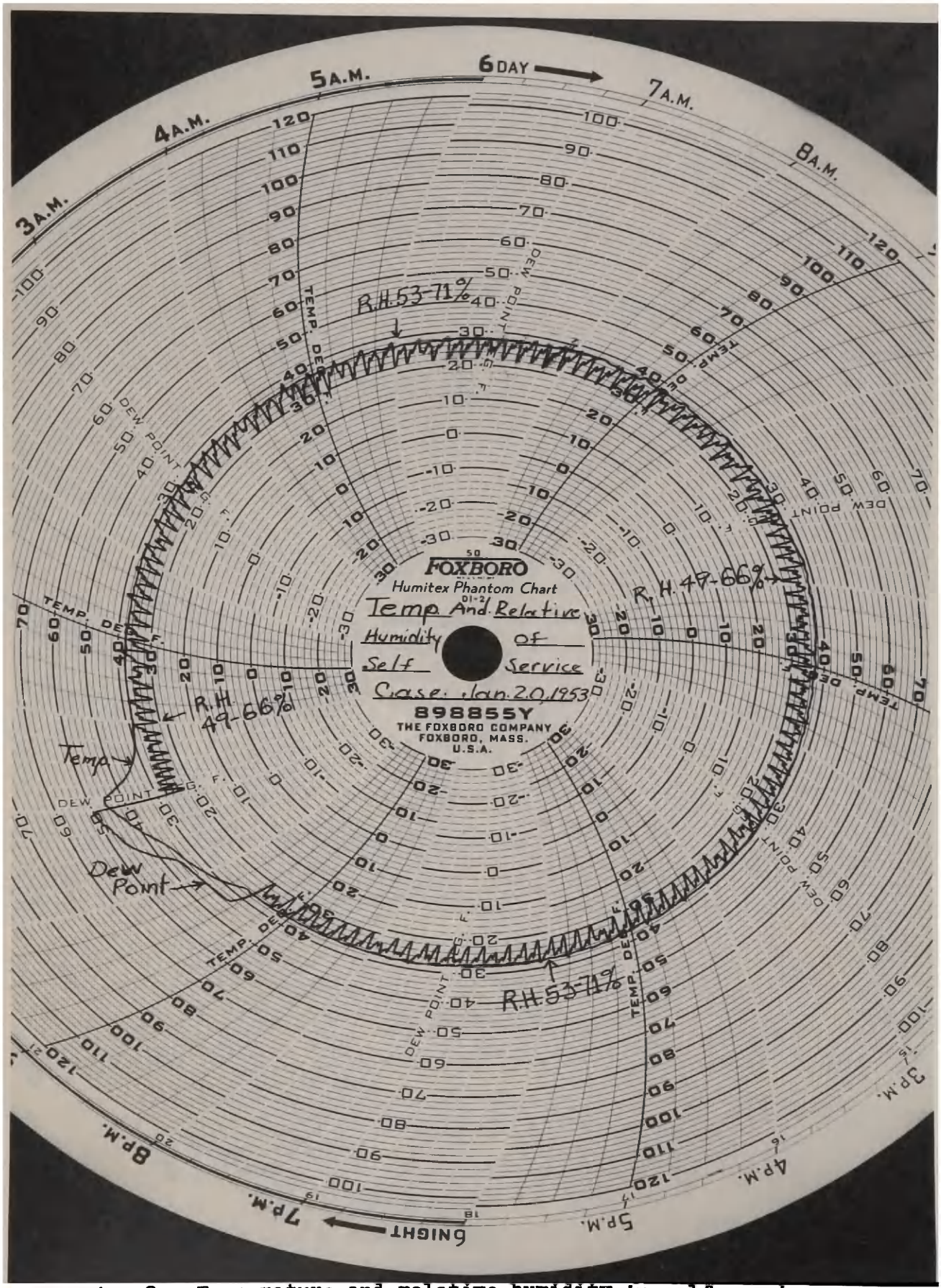

Fig. 8. Temperature and relative humidity in self-service case for lots T and U

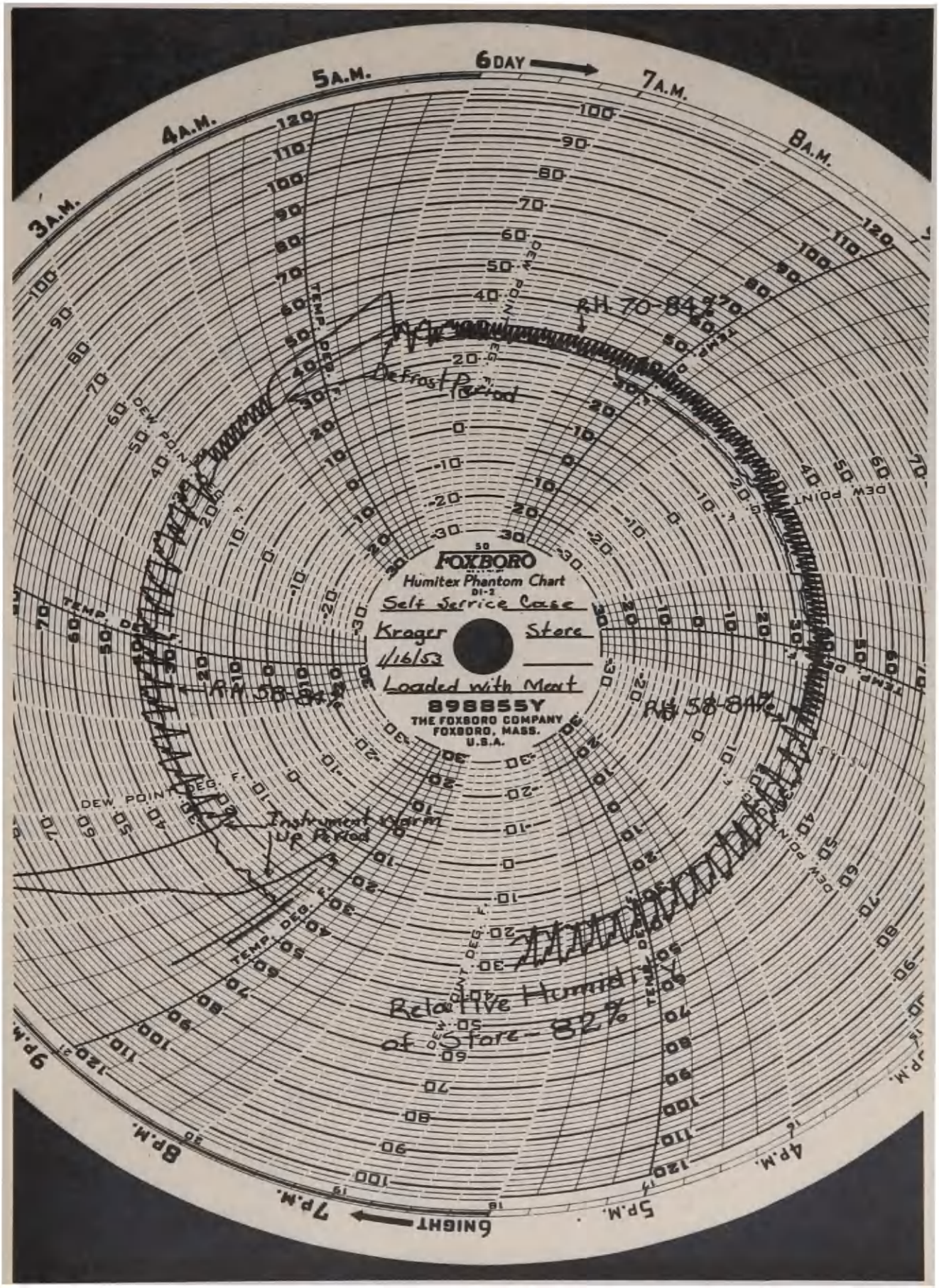

Fig. 9. Temperature and relative humidity of a commercially operated self-service case

upper steak surfaces was three degrees. The storage temperature given for a lot of steaks agreed closely with the temperature at the lower surface of the steaks.

C. Color Measurement

Detailed instructions were given by Voegeli (1952) for the measurement of the surface color of meats by use of Munsell spinning disks, and the procedures he found to be successful were employed in this study, using the same equipment.

The standard color employed for calculation of the index of fading (I) by the formula of Nickerson (1946) as applied by Voegeli (1952) was 7.0 Red in Hue, 4.0 in Value, and 8.0 in Chroma. This was much redder and higher in chroma than the 1.0 Yellow Red, 4.0 Value, 6.0 Chroma standard used for packaged samples by Voegeli (1952). The standard used in this study was close to the color specifications obtained on steak samples which had been oxygenated under an oxygen pressure of 30 pounds per sq. in. for one hour, with color readings made soon after oxygenation. This color, then, was believed to approximate closely the color of fully oxygenated myoglobin. Because the percent metmyoglobin was to be compared with the index of fading as a description of the surface color of the samples, it seemed logical to use a standard such as selected which was based on the color of well oxygenated pigments. The spectrophotometric method used for estimation of percent metmyoglobin assumed a mixture containing only oxymyoglobin and metmyoglobin, with oxymyoglobin as the "standard".

Estimation of the percent metmyoglobin was essentially as applied by Mangel (1951). Steak slices were made, weighed, and extracted as described previously in the procedure for determining bacterial counts. A portion of the same extract was used for the bacterial count, spectrophotometric estimation of metmyoglobin, and pH determination. The Waring Blendor extract was centrifuged six minutes at 5000 r.p.m., decanted, and filtered through Whatman no. 40 filter paper. Further dilution followed before spectrophotometric reading, with solutions of 0.02 to 0.03 millimolar concentration being the concentrations sought. Solutions of such concentrations had optical densities falling on a desirable area of the scale.

Extracted myoglobin is somewhat unstable, and the metmyoglobin percentage increases with time after extraction as shown by Mangel (1951). For this reason, results obtained are somewhat empirical, and standard procedure is necessary. Immediate reading in the spectrophotometer after extraction would give best estimation of the percent metmyoglobin in the sample, but such procedure does not fit well into routine determinations for multiple samples. In this study, readings obtained one hour after extraction worked out best, as the intervening time was needed for preparation of the final dilutions, and for making the agar plates for bacterial counts.

Optical density was measured in a Beckman model B spectrophotometer at wavelengths of 544 and 582 millimicrons. The total pigment concentration was obtained by conversion of the pigments to the CN derivative by adding a trace of potassium ferricyanide and of potassium cyanide to each cubit after readings were obtained. The optical density of the

resultant metmyoglobin (and methemoglobin) cyanide derivative was measured at 540 millimicrons, and the millimolar extinction coefficient of 11.3 given by Bowen (1949) for metmyoglobin cyanide was used to calculate the concentration. By use of the dilution factor and the molecular weight of myoglobin, an estimation of the concentration of pigment in the original sample, expressed as percent myoglobin, was obtained.

The calculation of concentration of the solution whose optical density was measured was based on the Lambert-Beer law as given by Bowen (1949).

$$\log_{10} \frac{I_o}{I} = d = \epsilon cl$$

Where I_o = intensity of incident light
I = intensity of emergent light
d = density from spectrophotometer
ϵ = molar extinction coefficient
c = molar concentration
l = length of light path through the solution in cm (= 1.0 for the cubits used)

$$\therefore \quad c = \frac{d}{\epsilon} \text{ where } l = 1.0$$

and for this study

$$c = \frac{d}{11.3 \times 10^3}$$

$c \times 10^{-3} \times 17000$ (M.W. of myoglobin) = grams of myoglobin per liter of solution

grams/liter X dilution = grams/1000 grams of meat

$$\frac{\text{grams/1000 grams of meat}}{10} = \text{grams/100 grams of meat} = \% \text{ myoglobin}$$

Although c (molarity of the solution measured) as noted above was accurately obtained, the percent myoglobin in the muscles was only approximately measured. The loss of accuracy in measuring the percent

myoglobin was due mainly to the uncorrected variability in fat content of the slices. If the weight of the muscle extracted had been determined more accurately, a more accurate method of measuring the dilution water would have been employed.

To estimate the percent metmyoglobin, the differences between the extinction coefficients of oxymyoglobin and metmyoglobin at 544 and 582 millimicrons were taken as the maximum possible changes at these wavelengths. These figures were 9.6 at 544, and 12.1 at 582 millimicrons as given by Bowen (1949). At each wavelength, the change due to metmyoglobin was equal to the extinction coefficient of oxymyoglobin minus that of the sample. By dividing the figure obtained by the total change in extinction coefficients from oxymyoglobin to metmyoglobin and multiplying by a hundred, an estimation of the percent metmyoglobin was obtained. The estimates of the percent metmyoglobin at 544 and 582 millimicrons were averaged to obtain the final estimate of the percent metmyoglobin.

IV. RESULTS AND DISCUSSION

A. Comparison of Spinning Disk Method with Spectrophotometric Estimation of Metmyoglobin for Determination of Surface Color of Fresh Meats

All present objective methods of meat surface color determination have some disadvantages. The greatest handicap in the use of the spinning disk method is that human judgement influences results. Nickerson (1946) recognized that the color vision of individuals varied. Munsell color data obtained by disk colorimetry are somewhat inexact due to differences between operators, and to the repeatability error for each operator.

Color determination by physical-chemical means using instruments would eliminate the judgement errors. Analyses on the pigments responsible for the color should indicate causes for color changes. Disk colorimetry gives only a description of the color.

Spectrophotometric estimation of the percent metmyoglobin as applied by Mangel (1951) was used. A large number of determinations were made to compare the efficiency of it with the spinning disk method. Figs. 10 through 14 present comparative data on five lots, totaling 114 samples. Correlation coefficients between the index of fading and the percent metmyoglobin were obtained by the method of Snedecor (1946), and ranged from 0.830 to 0.882.

By reference to the section on color science, it can be seen that fundamentally both methods are based on the light absorbed, reflected, or transmitted at different wavelengths of the visible spectrum. A very high correlation was expected. The correlation coefficients were

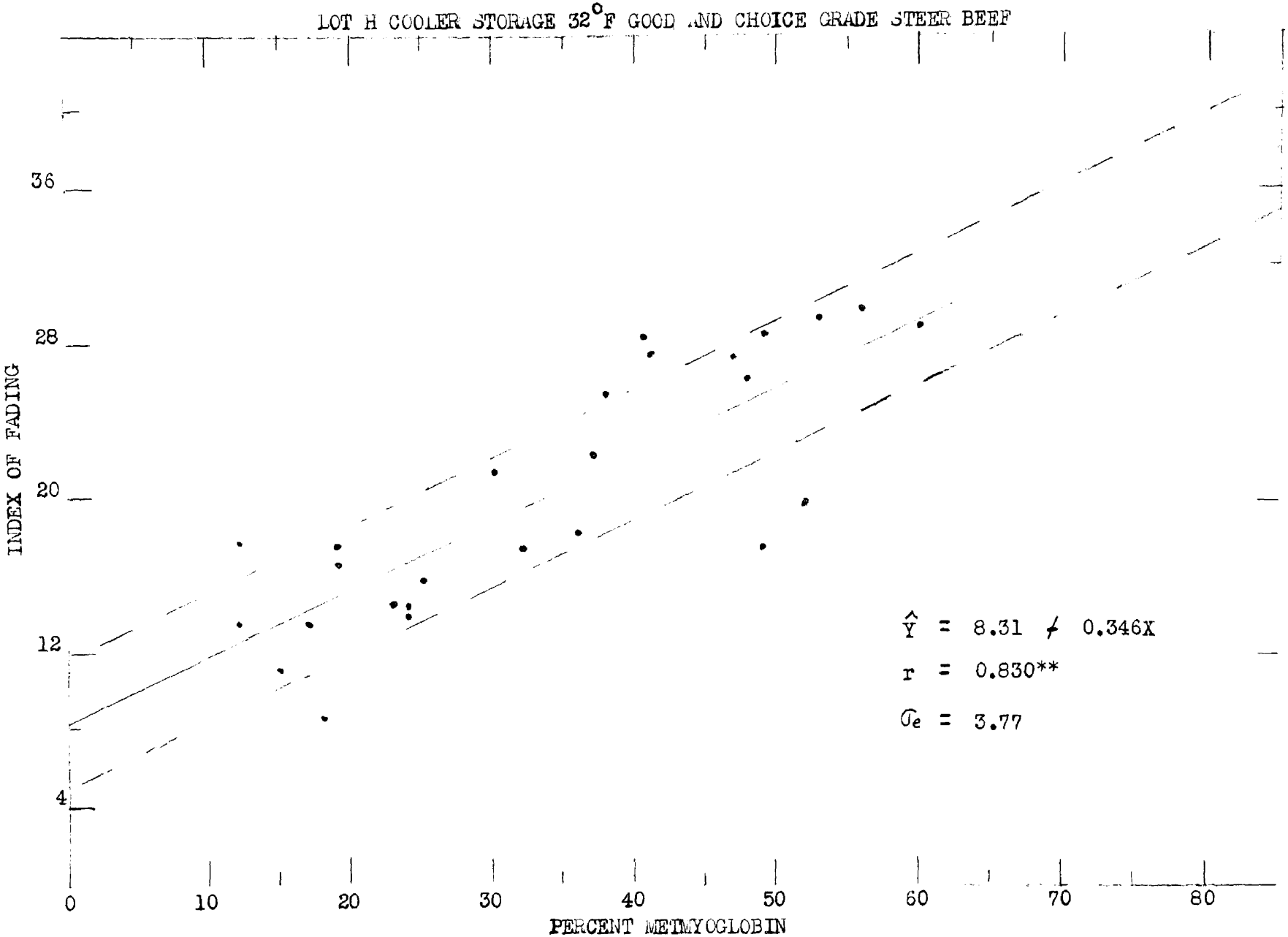

Fig. 10. Correlation between index of fading and percent metmyoglobin

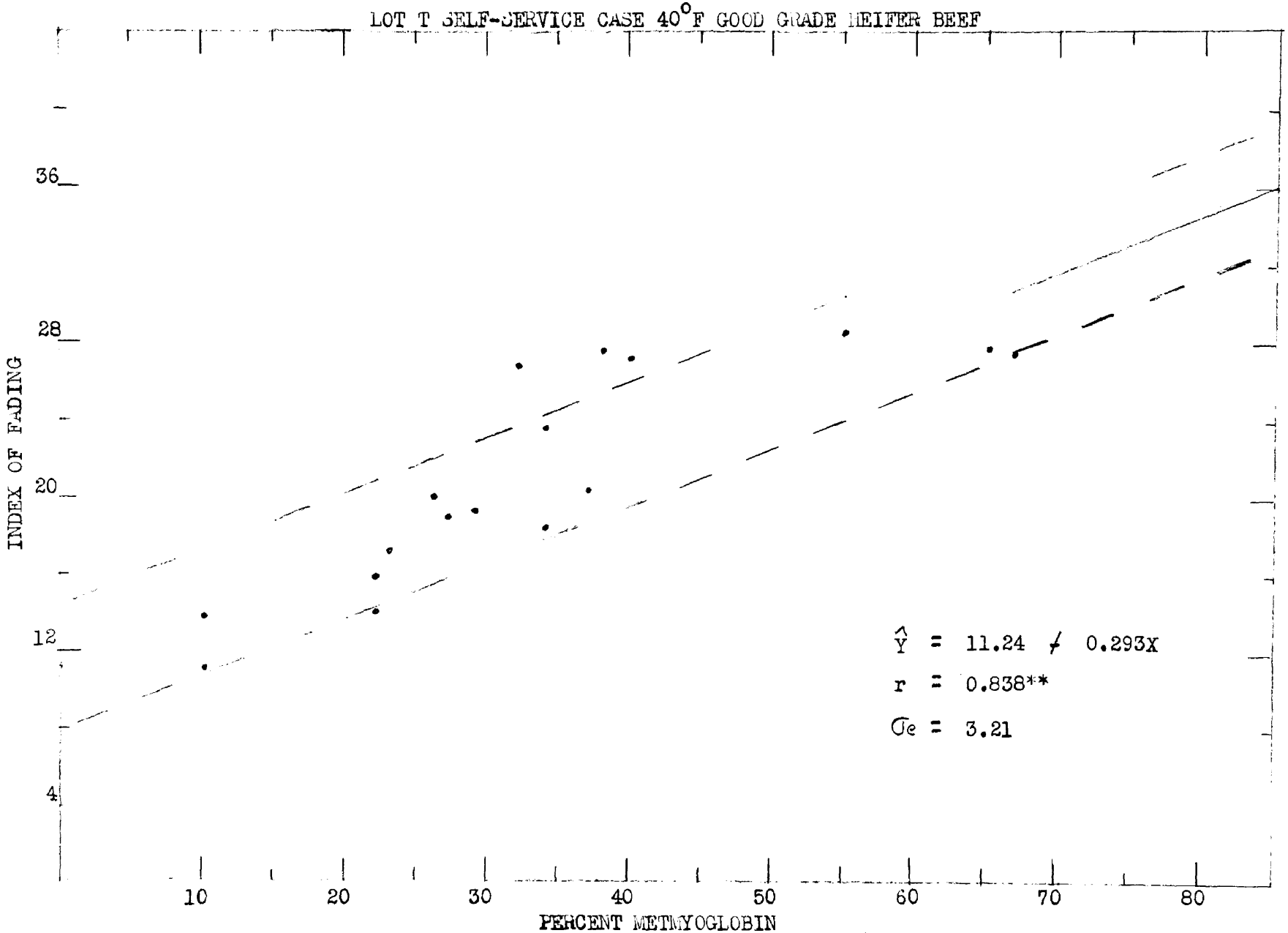

Fig. 11. Correlation between index of fading and percent metmyoglobin

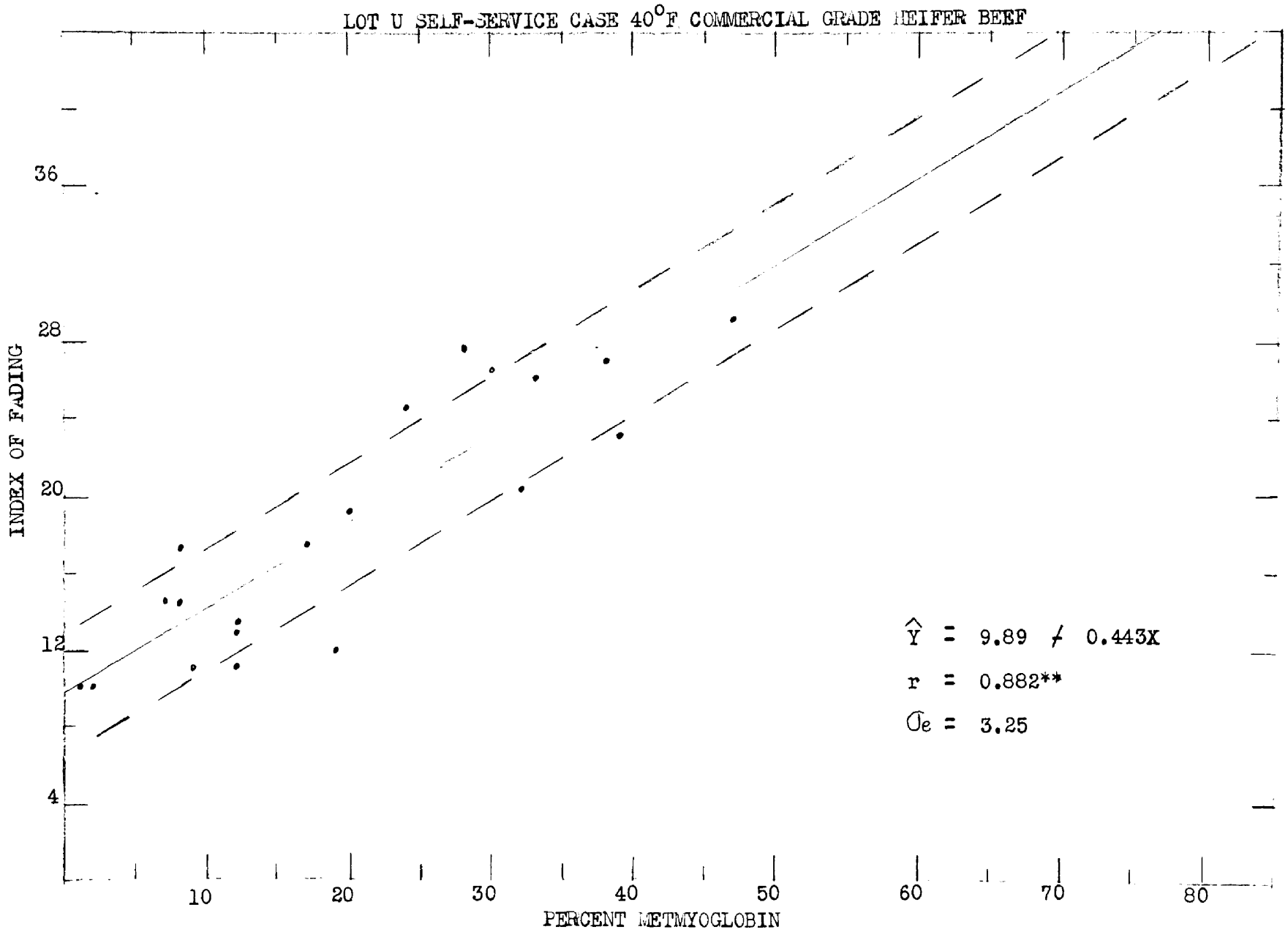

Fig. 12. Correlation between index of fading and percent metmyoglobin

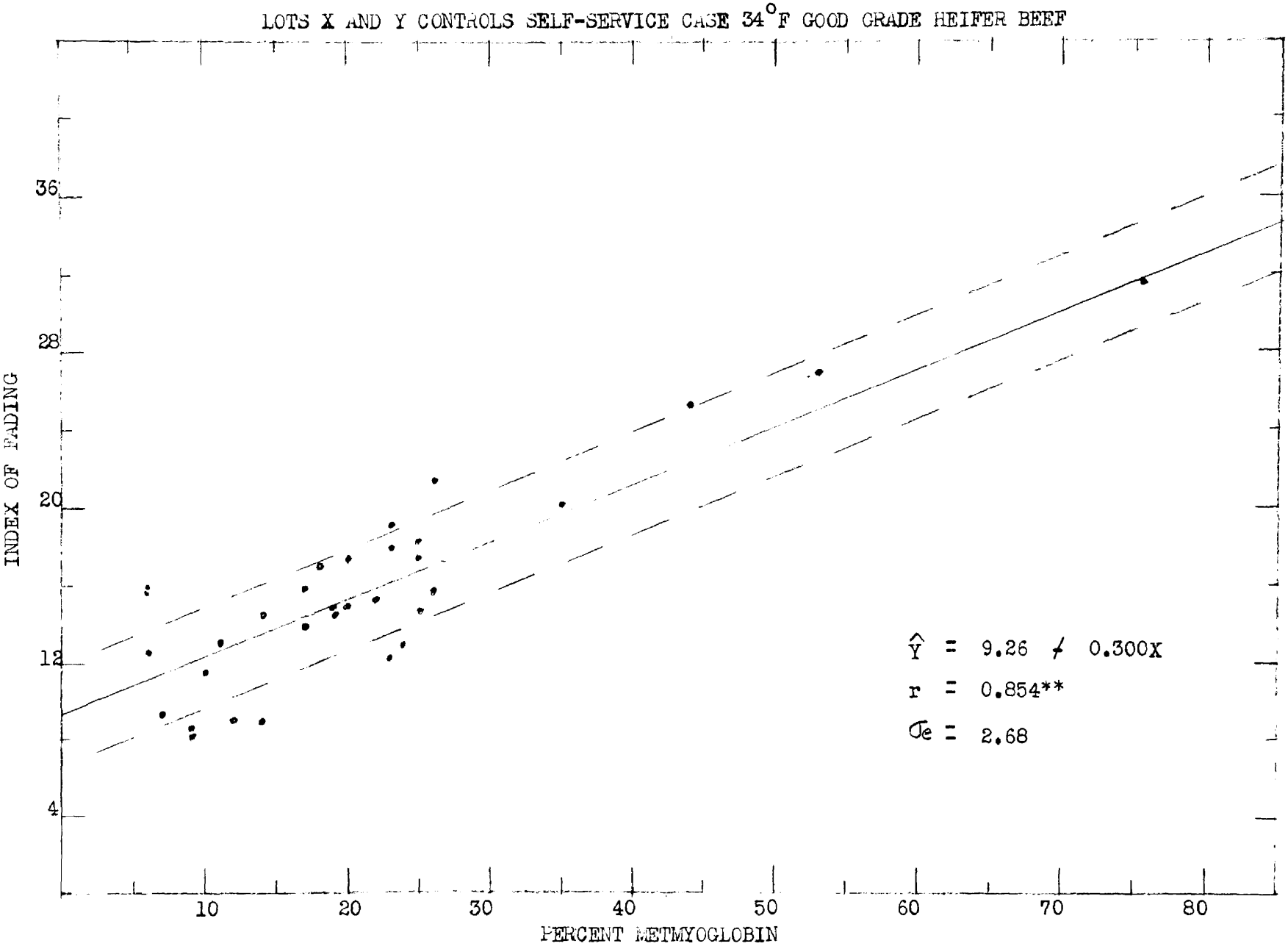

Fig. 13. Correlation between index of fading and percent metmyoglobin

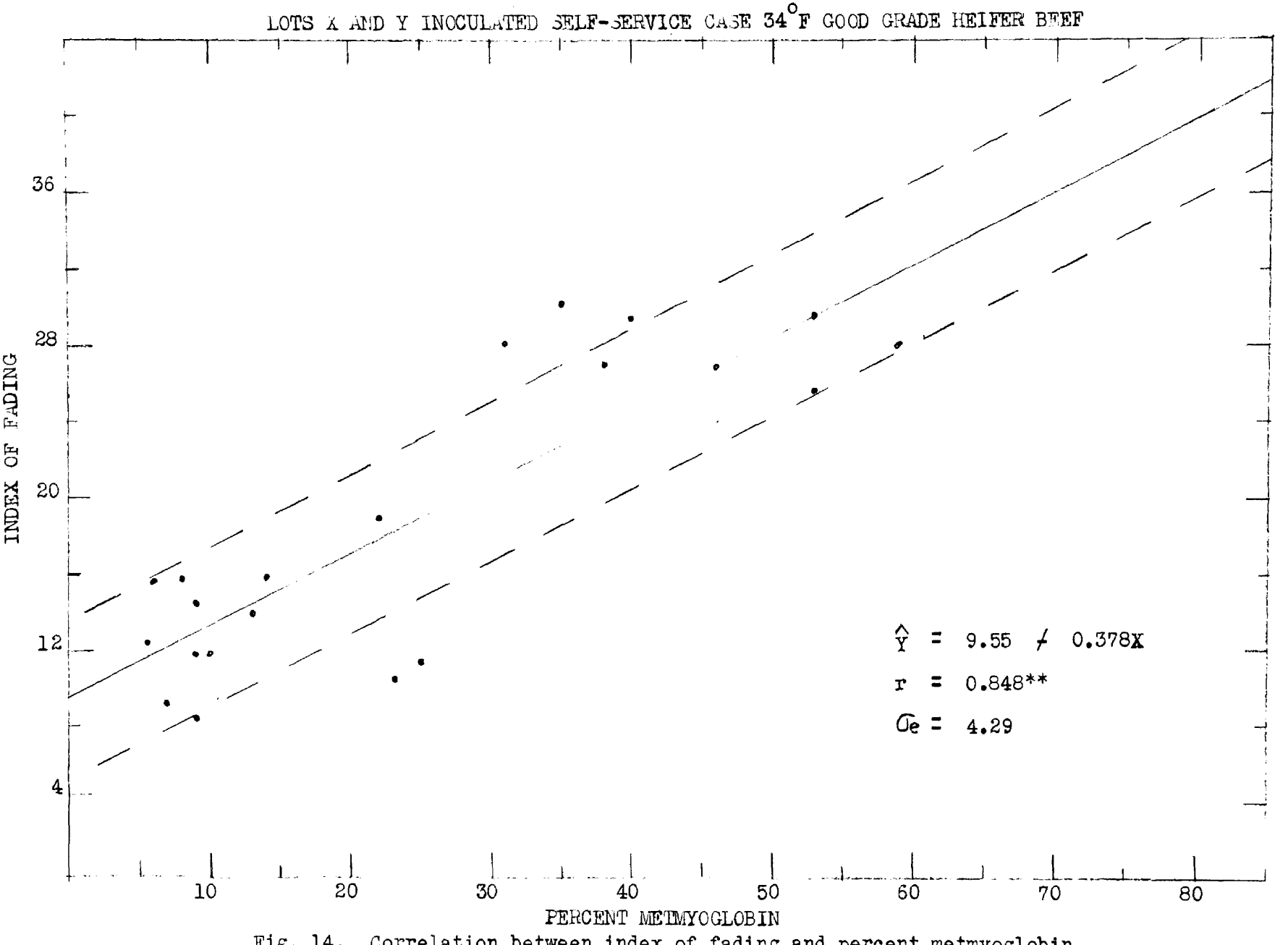

Fig. 14. Correlation between index of fading and percent metmyoglobin

well above requirements for the one percent level of significance given by Snedecor (1946), yet almost a third of the variability ($1-r^2$) between the two methods was unaccounted for. Factors responsible for part of the uncorrelated variability were observed.

The index of fading based on Munsell hue, value, and chroma obtained from spinning disk color matching is subject to the human errors noted previously. There is also a slight error inherent in the application of the Nickerson (1944) formula for the index of fading, $I = \frac{C}{5}(2\Delta H) + 6\Delta V + 3\Delta C$. The formula assumes that at chroma /5 one unit change in value has the same effect on the overall color as two units change in chroma or three units change in hue. These relationships are only approximations.

In disk colorimetry an area of approximately 12 sq. cm. was scanned. This was only about one fifth of the surface area of the samples used. There was some variability in surface color in each sample. Though the color of the area scanned could be determined rather accurately, the color of adjacent areas was not necessarily the same. This factor was important when color changes occurred in areas which could not be scanned, such as around the edges of the samples. Metmyoglobin discoloration usually appeared first at the edges of the steaks which were inoculated by spraying.

Some variables were found in the determination of the percent metmyoglobin. The empirical nature of the results obtained has been explained previously. Percentages obtained on duplicate samples varied as much as two percent, though most samples checked within one percent.

The greatest handicap to the use of metmyoglobin as a color description, however, is that the method does not apply to freshly cut meat. Using steaks freshly cut from the longissimus dorsi muscle of sixteen good and choice grade steers, a non-significant correlation coefficient of -0.1 was obtained between the spinning disk method and percent metmyoglobin. The percent metmyoglobin averaged 6.4% with little variability while the values for the index of fading ranged from 8.1 to 16.4 with an average of 13.0. The range in index of fading showed that there were actual color differences, but the lack of correlation showed that metmyoglobin was not responsible. Data are shown in the appendix.

The color of freshly cut meat depends upon many factors, including the concentration of pigments present, marbling, fat color, density of the tissue, and particularly upon the state of oxygenation of the heme pigments. The percent metmyoglobin had little effect until values above ten percent were obtained.

As shown in the section to follow, bacterial growth resulted in reducing conditions which converted metmyoglobin to myoglobin when the bacterial population became high. Though the color was quite dark and the index of fading was high for steaks in which this had occurred, the percent metmyoglobin was low, sometimes lower than obtained on the freshly cut steaks. Each lot of samples was composed of paired steaks from one carcass. Sequential analyses were made on pairs removed in a random manner from the lot. Samples which showed the typical color of myoglobin (reduced), along with metmyoglobin percentages much lower than had previously been measured, were not included in the calculations

to compare the two color methods. The percent metmyoglobin had little relation to the color of those samples.

During the period of increasing metmyoglobin formation, which included the important sales period for self-service meats, the percent metmyoglobin gave valuable information. There was no practical significance attached to the storage period in which bacteria caused reduction of metmyoglobin to myoglobin, for the meat was considered unsalable due to discoloration, off odor, and usually slime.

Results reported here involved only the longissimus dorsi. A difference in the rate of color change of other muscles was observed. In one lot the small spinalis dorsi muscle was left attached to the longissimus dorsi on most of the stored steaks. Consistently, the spinalis dorsi discolored much sooner than the longissimus dorsi.

The knuckle portion of lamb legs was cut into boneless steaks and used for some observations. The vastus intermedius muscle in those steaks always developed a brighter, more desirable color than the muscles adjacent to it, and maintained desirable color for a longer storage period. When this muscle was dissected from the stored steaks and compared to the remaining muscles, a lower percent metmyoglobin, lower myoglobin concentration, and a slightly higher pH (about 0.5 pH unit) were observed. Investigation of the reasons for the differential storage response of muscles might give important information.

B. Effect of Bacteria on Color

Bacterial growth reduced the salable shelf-life of fresh meat packages in all cases observed. Changes which were caused or speeded

by bacterial growth included discoloration due to increased rate of metmyoglobin formation, production of off odors, and slime formation. Usually the changes appeared in the order mentioned. Any one of these could render the meat unsalable.

Figs. 15, 16, and 17 illustrate the increased rate of discoloration due to bacterial inoculation. Using an index of fading of 20.0 as the value at which the packages became unsalable due to discoloration, the control samples had an increased shelf-life compared to the inoculated samples of five days for the samples stored in the cooler at 32°F, and of about four days at 40°F, and seven days at 34°F for the samples stored in the self-service case. The increased shelf-life of the control steaks at 34°F compared to the control steaks at 40°F was due partially to the lower initial contamination of the samples, as well as to the slower bacterial growth rate at the lower temperature.

Fig. 18 shows the growth curves found for the bacteria on the steaks stored in the self-service case at 40°F. The curves are remarkably similar to normal growth curves as illustrated by Jensen (1945). The bacteria on the inoculated steaks apparently entered the logarithmic growth phase after about one day. The values for metmyoglobin increased rapidly during the logarithmic growth phase.

Differences between the bacterial growth on control and inoculated steaks are shown in Fig. 19. The bacterial counts shown in Fig. 18 for lot U are not as dependable as those for lots X and Y. The steaks of lot U were used to make photographs showing color changes. It was necessary to remove the cellophane before photographing. Subsequent transfer of the exposed samples to the laboratory for bacterial sampling allowed some opportunity for contamination.

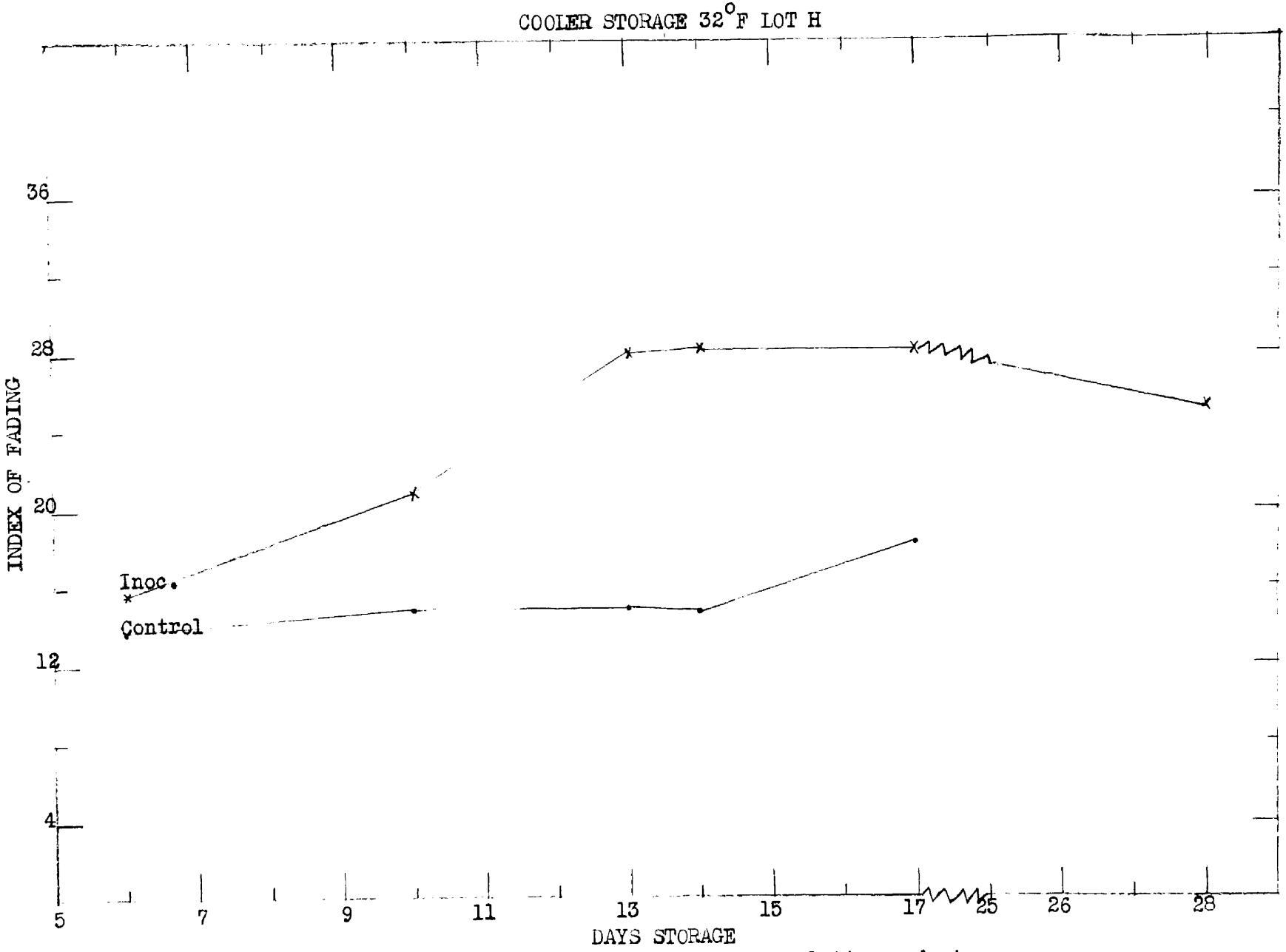

Fig. 15. Effects of bacterial inoculation and storage on rates of discoloration

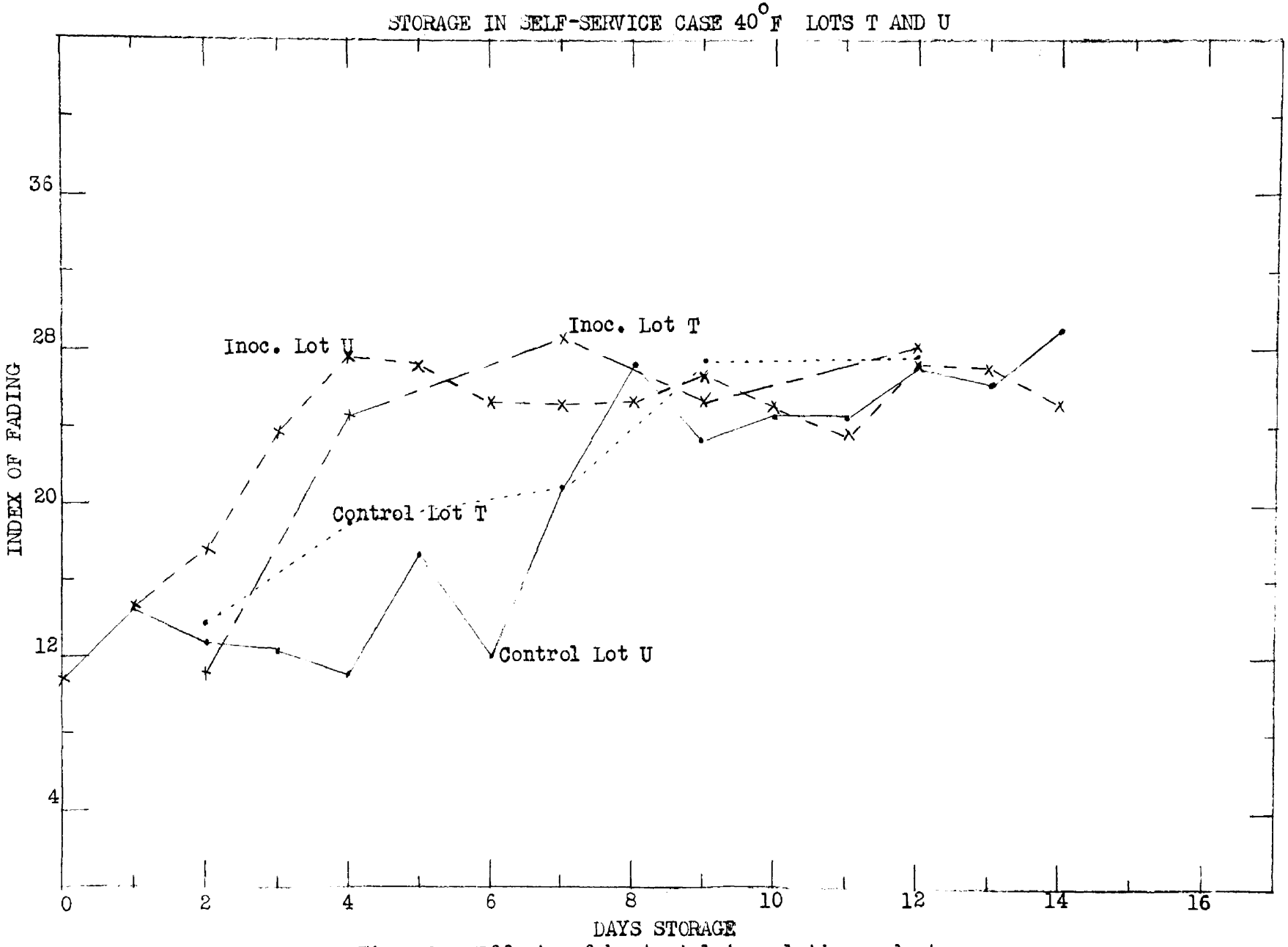

Fig. 16 Effects of bacterial inoculation and storage on rates of discoloration

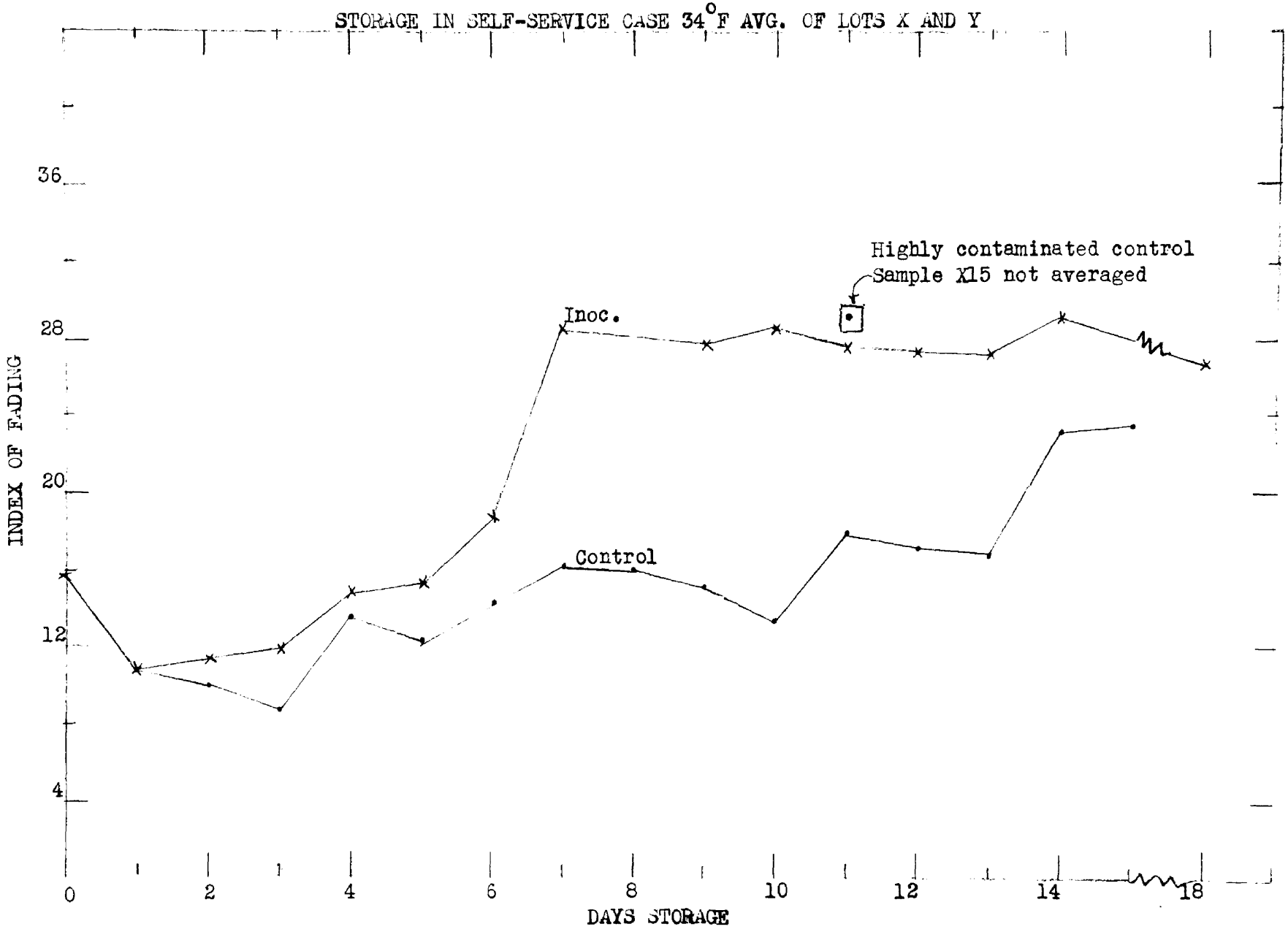

Fig. 17. Effects of bacterial inoculation and storage on rates of discoloration

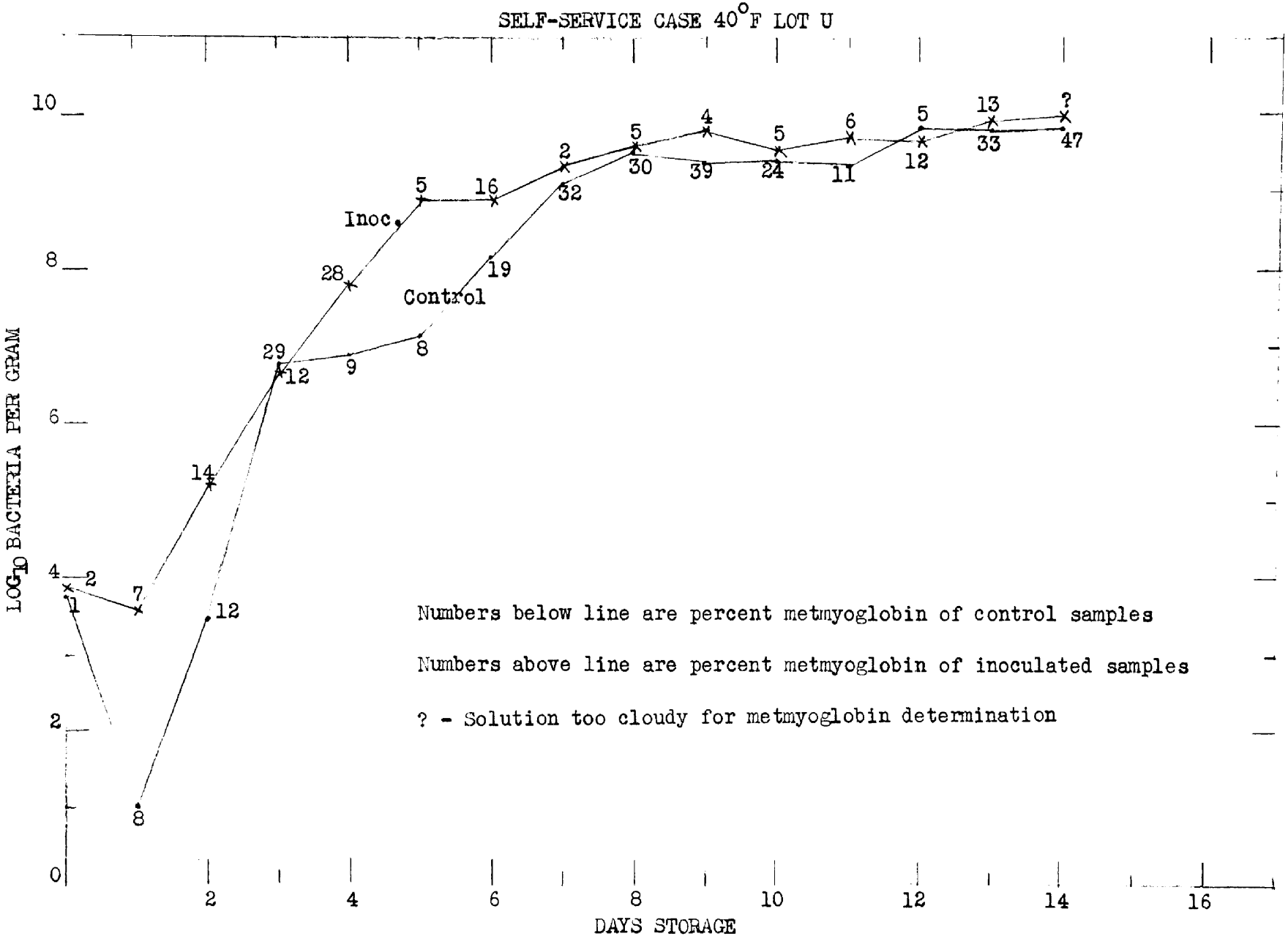

Fig. 18. Growth curves of bacteria on inoculated and control samples

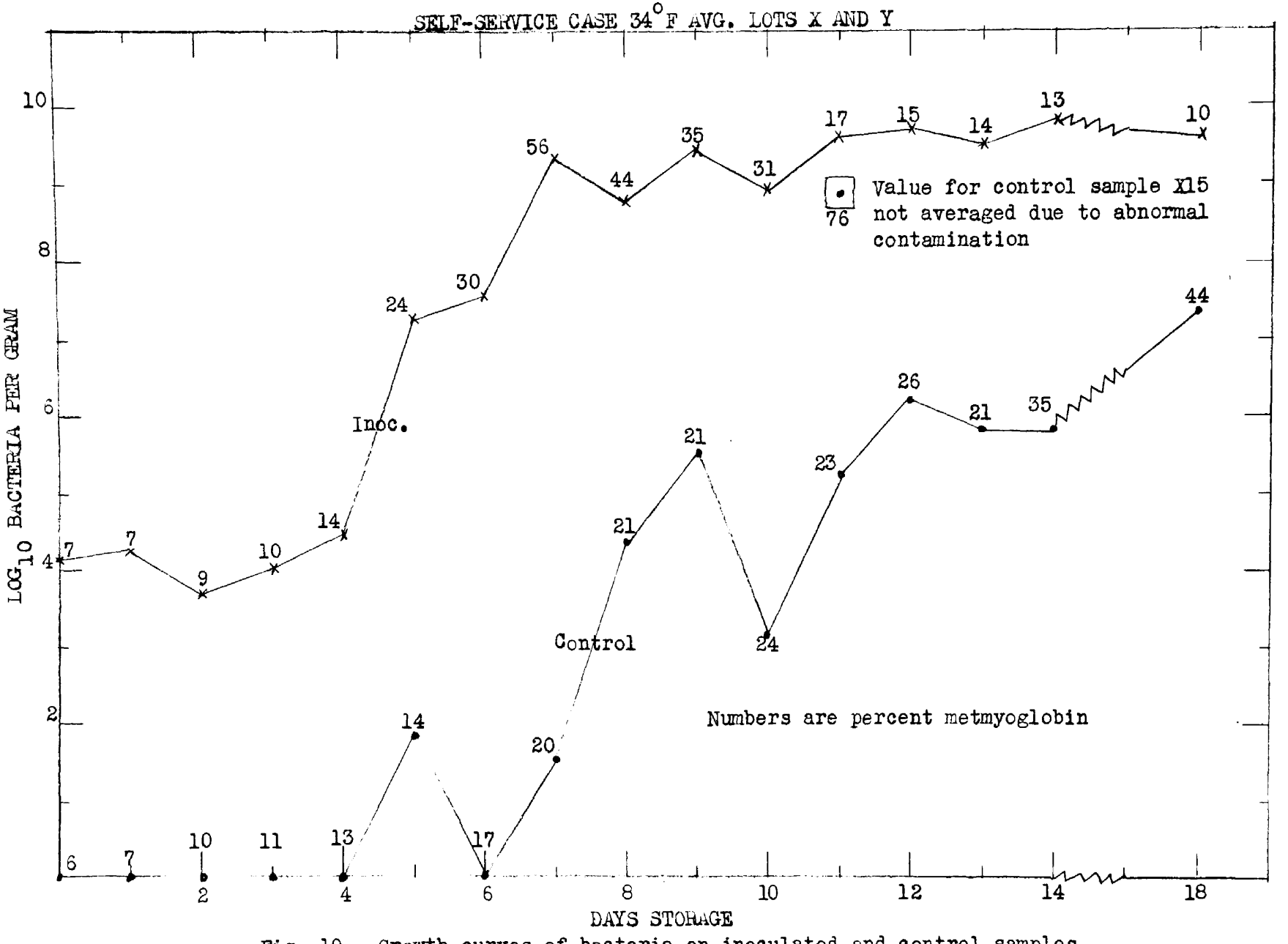

Fig. 19. Growth curves of bacteria on inoculated and control samples

The series of color photographs showed well the faster discoloration of the inoculated steaks.

When the bacteria entered the stationary period at peak growth as shown by the leveling of the growth curve, reducing conditions prevailed, and the metmyoglobin percentages decreased due to the reduction of metmyoglobin to myoglobin. By this time the steaks usually had some surface slime, which increased the oxygen barrier, and the myoglobin remained in the unoxygenated state. The myoglobin could be oxygenated, however, for two sample steaks were exposed to oxygen at a pressure of 30 pounds per sq. in. for one hour, and developed again the desirable bright red color typical of oxymyoglobin. The desirable color deteriorated rapidly, however, even though the steaks were stored in the cooler at 32°F.

Preparation of control samples without bacterial contamination was quite difficult, but those for lots X and Y had extremely low contamination. Zero counts were obtained with dilutions as low as one to five until the fifth day of storage. Certainly there were a few bacteria present, however, for all control steaks eventually developed high populations. Early in the storage period the bacterial numbers apparently were so low that none survived the isolation and plating.

There was one notable exception. Control sample X15 somehow became heavily contaminated, and had a population of 800 million per gram when removed as scheduled on the eleventh day of storage. The high value of 76% metmyoglobin and complete discoloration shown by it attested to the undesirable effect of bacterial growth. No other control sample developed discoloration to any marked extent, though there was a

significant amount of drying after long storage. All data are shown in the appendix.

As shown in Fig. 19, apparently the bacteria were entering the logarithmic growth phase on the control steaks of lots X and Y when the last samples were removed.

Figs. 20 and 21 show the effect of storage on percent metmyoglobin. The effect of bacterial growth shows well. As shown in Fig. 20 the inoculated steaks did not develop high metmyoglobin percentages. The rapid bacterial growth rate apparently allowed early attainment of reducing conditions. A lower initial population on the control samples allowed a slower rate of metmyoglobin formation, but the peak values were higher. Reducing conditions were apparent, however, for the control samples analyzed on the tenth, eleventh, and twelfth days of storage. The variability in response was probably due to the variable bacterial population, particularly among control samples.

Fig. 21 illustrates the effects of bacterial contamination on the percent metmyoglobin. The typical response showing a peak followed by reduction is shown for the inoculated samples.

The correlation between bacterial populations and discoloration as shown by the index of fading is illustrated by Figs. 22, 23, 24, and 25.

All the data point to the conclusion that bacterial growth speeds discoloration by increasing the rate of metmyoglobin formation. Special investigations were not made to determine the manner in which bacteria influenced the chemical state of the pigments present. No use of the pigments for bacterial food was indicated, as the pigment concentrations

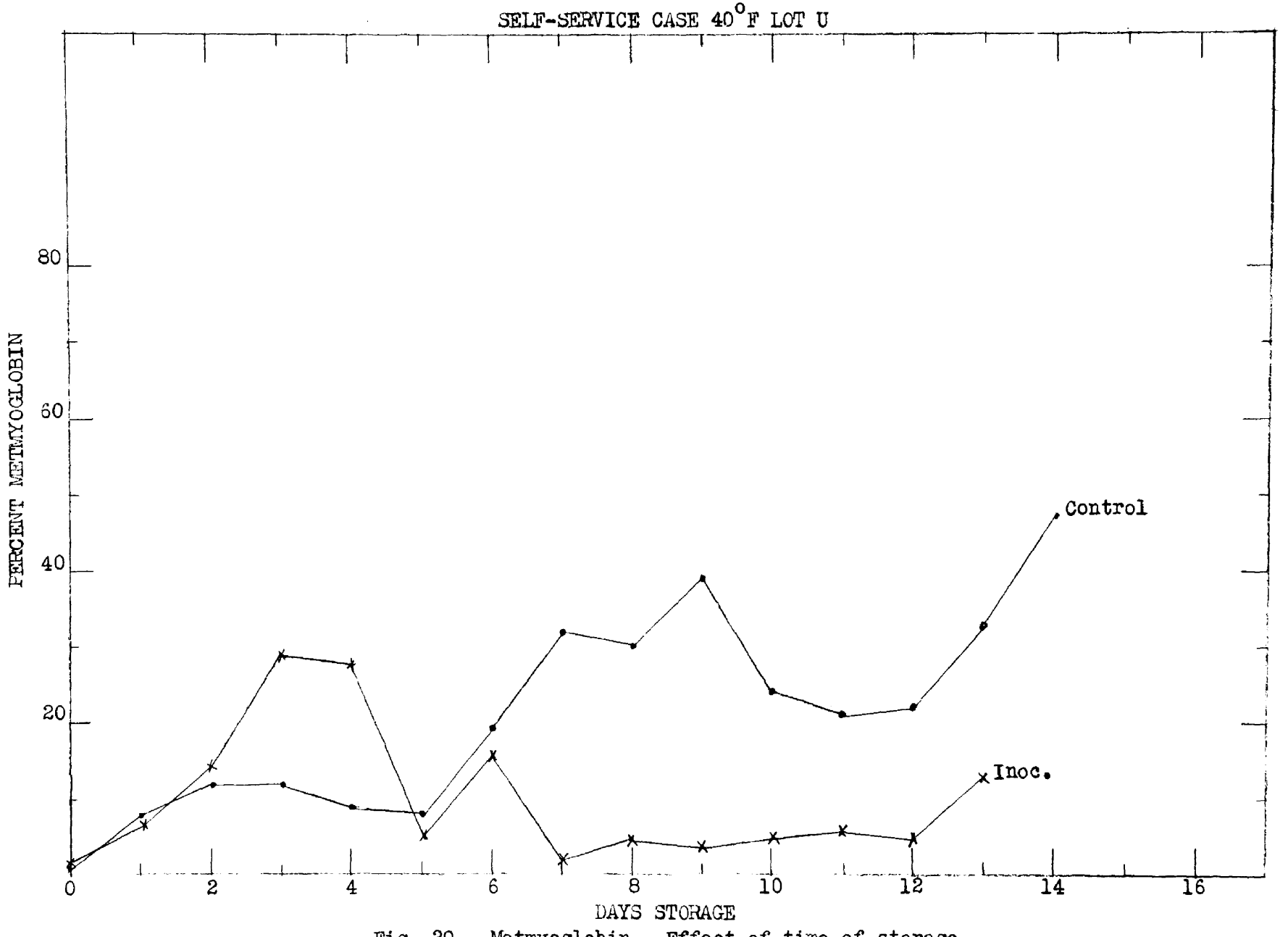

Fig. 20. Metmyoglobin. Effect of time of storage.
Responses of inoculated and control samples

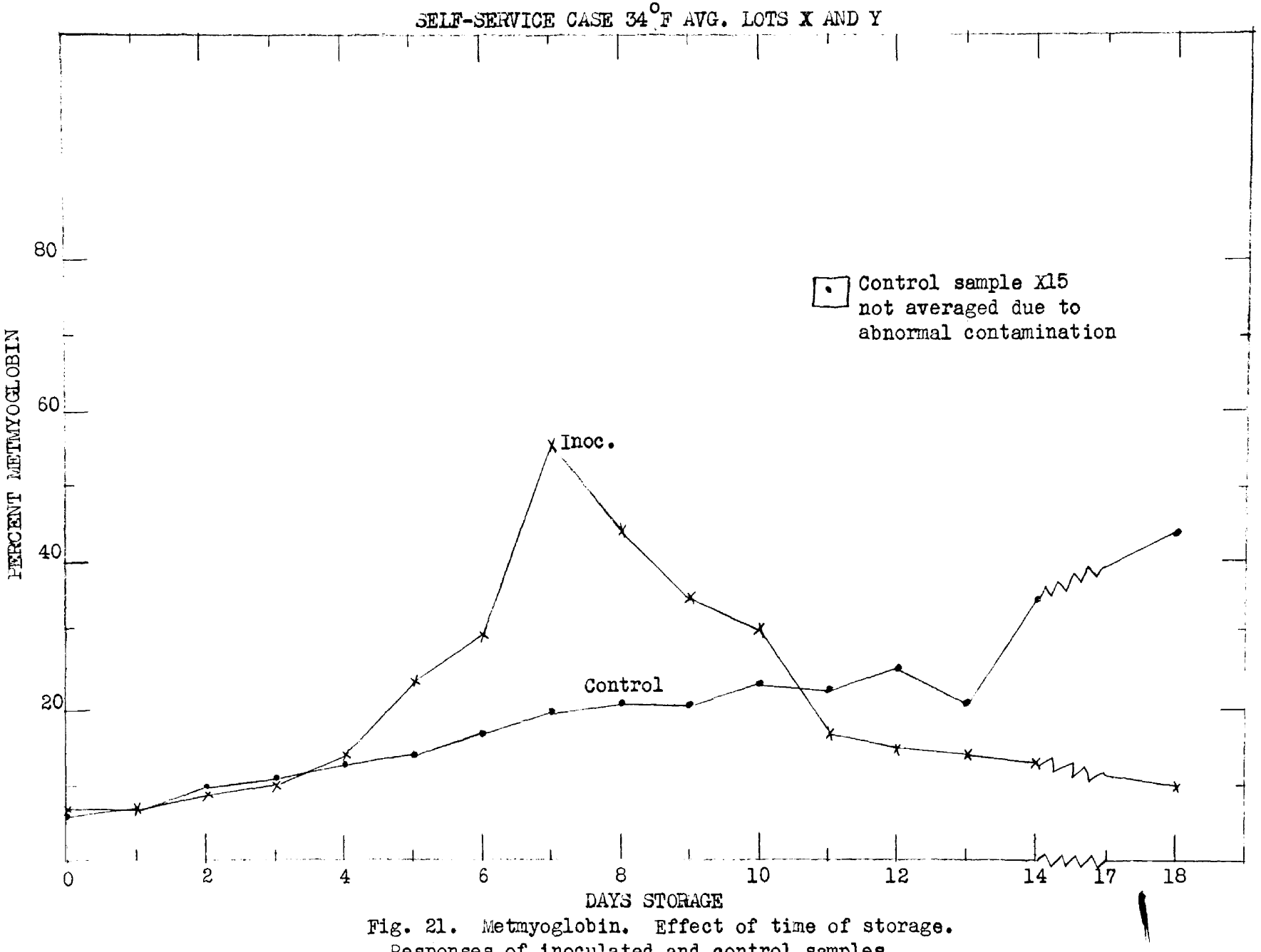

Fig. 21. Metmyoglobin. Effect of time of storage.
Responses of inoculated and control samples

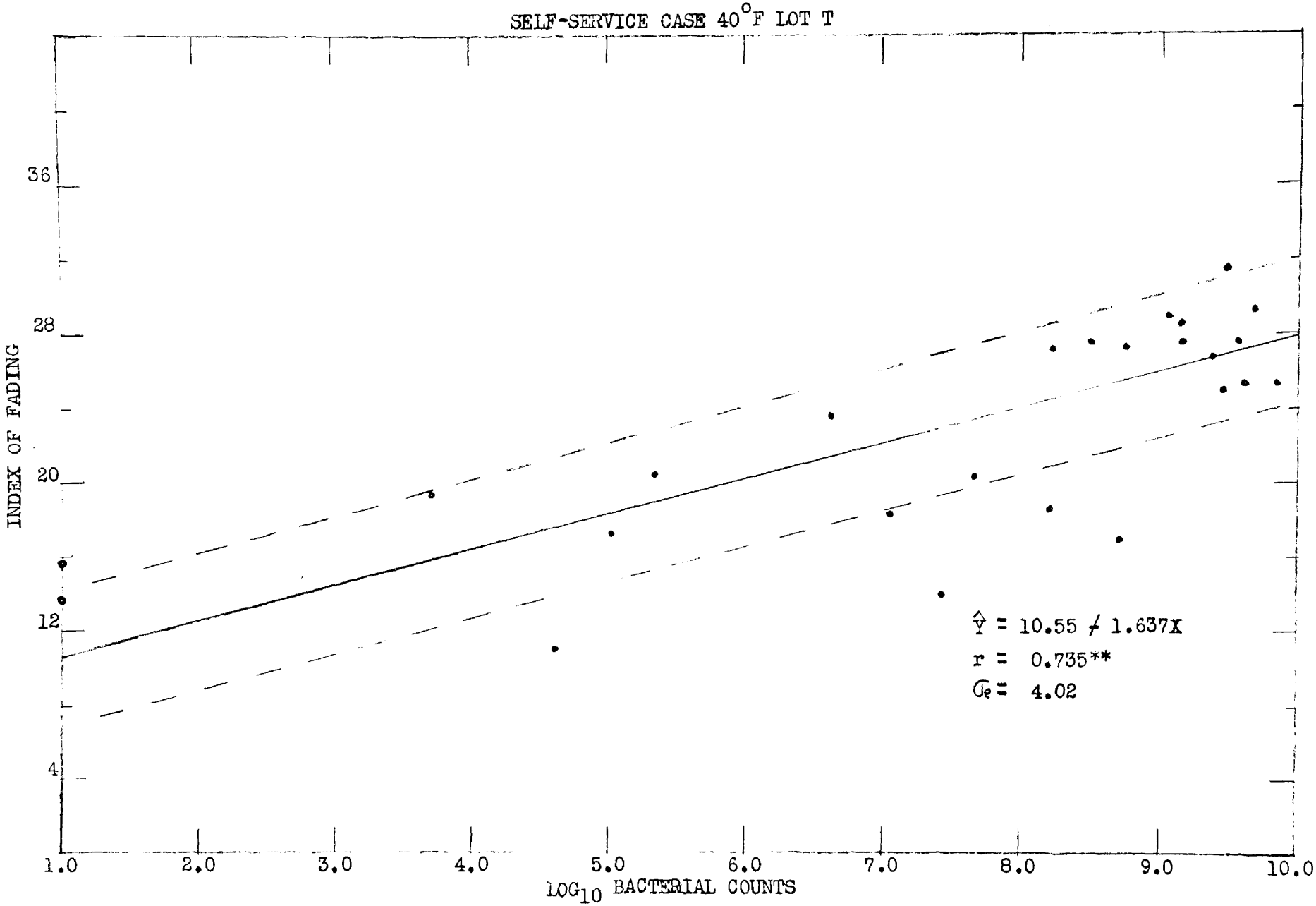

Fig. 22. Relationship between bacterial counts and surface color of meat as shown by index of fading

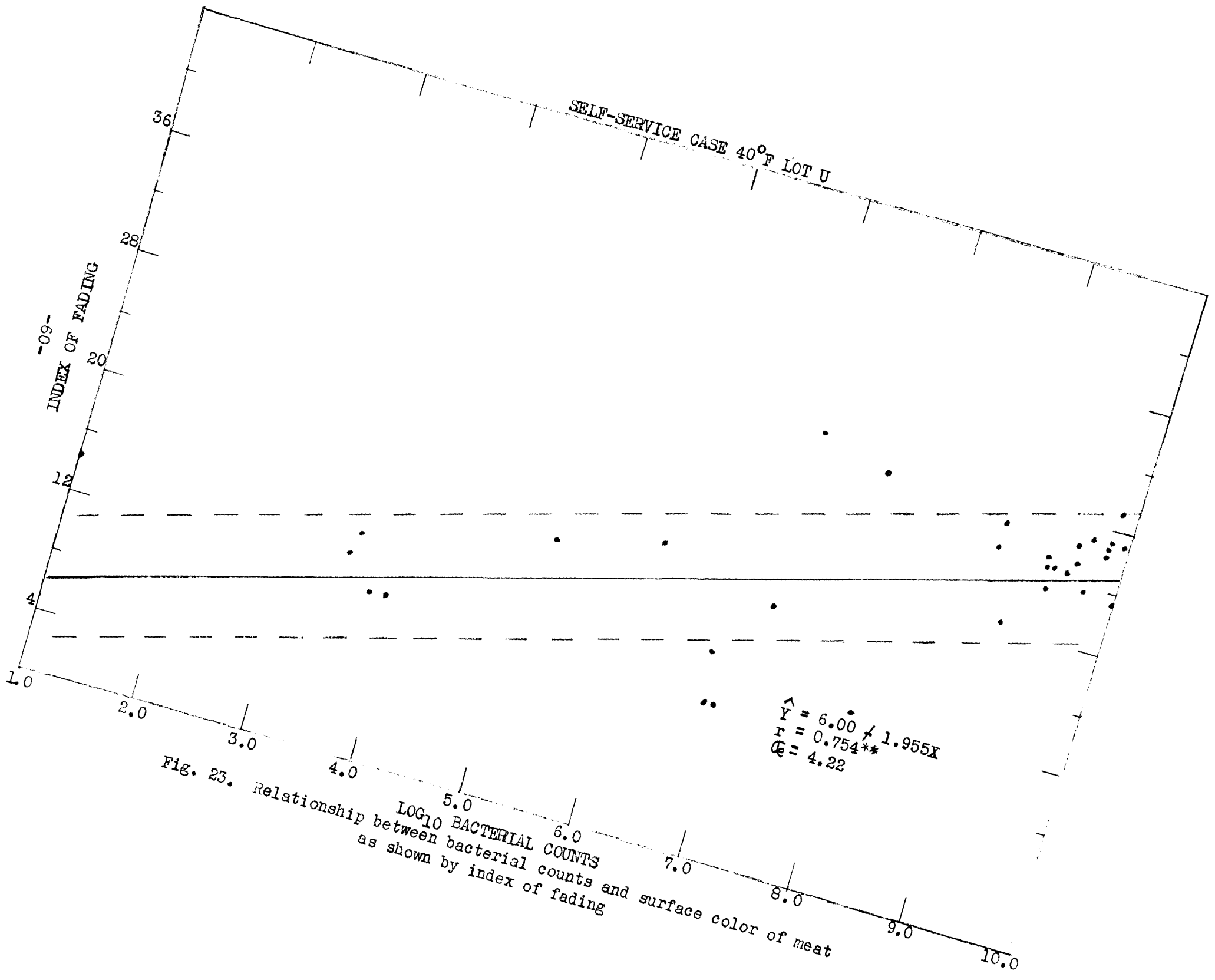

Fig. 23. Relationship between bacterial counts and surface color of meat as shown by index of fading

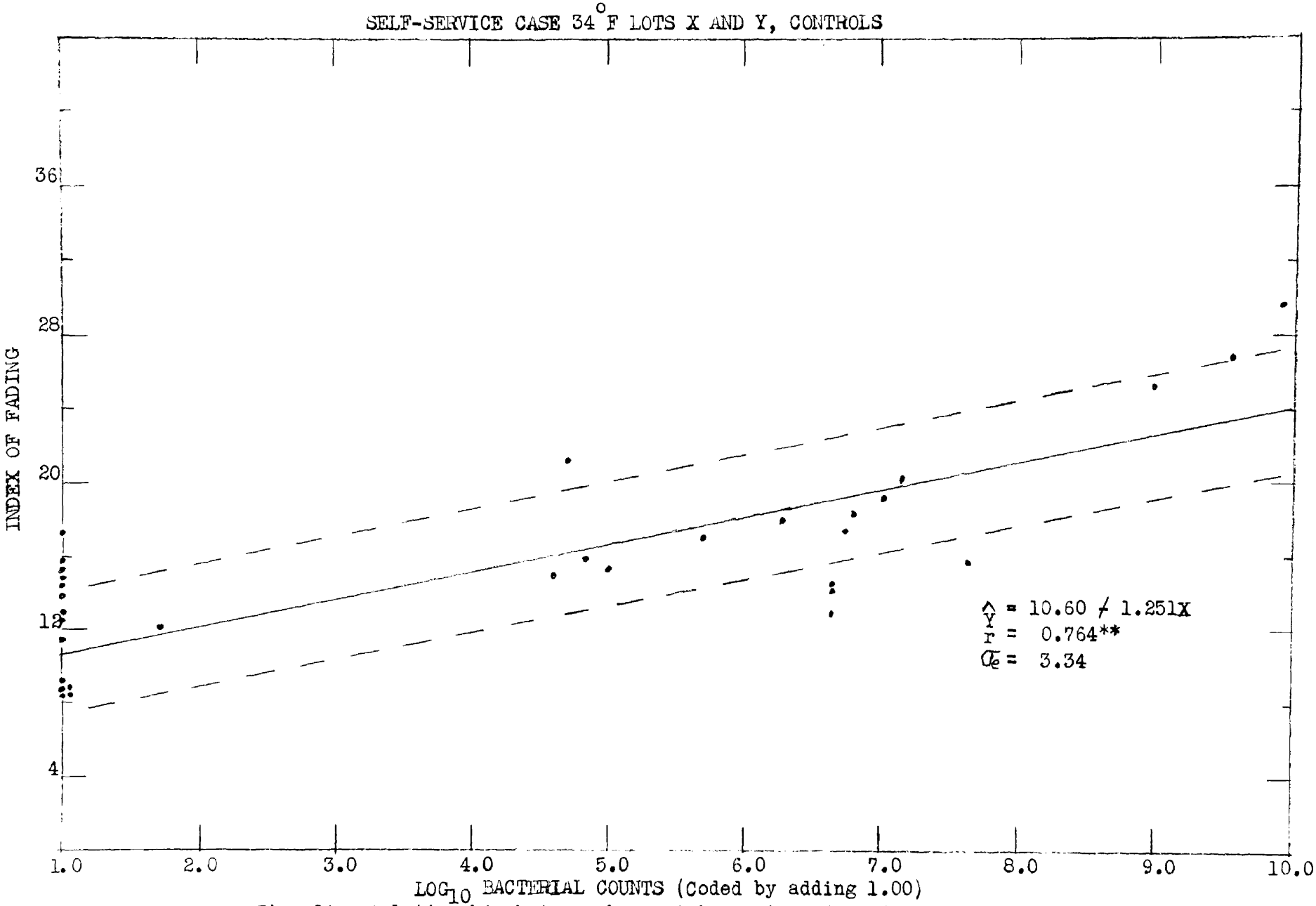

Fig. 24. Relationship between bacterial counts and surface color of meat as shown by index of fading

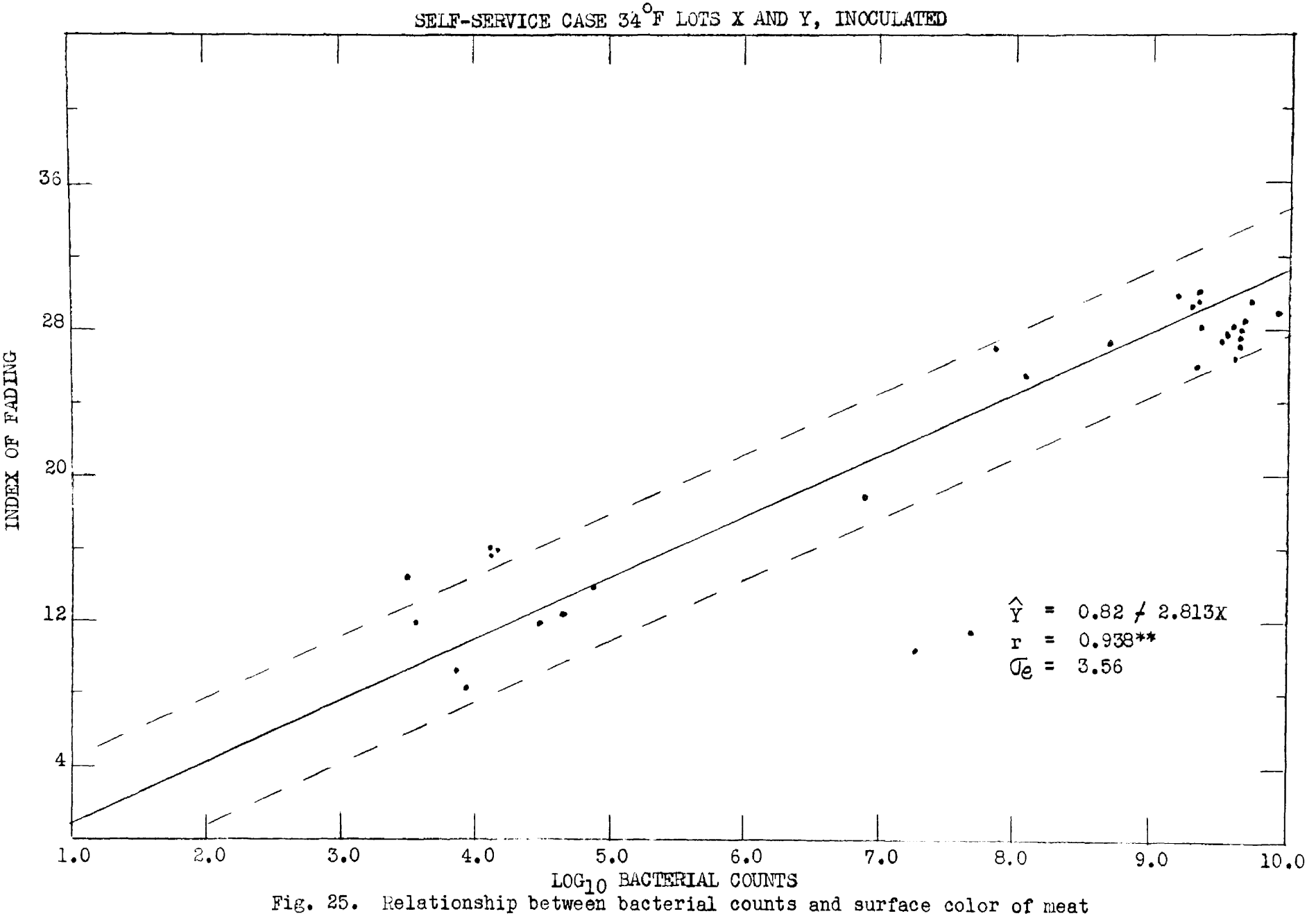

Fig. 25. Relationship between bacterial counts and surface color of meat as shown by index of fading

showed a slight increase with time of storage, due probably to drying.

Oxygen relationships were believed to be responsible for the pigment changes. Earlier reference was made to reports which showed that metmyoglobin was not formed in the absence of oxygen, and that the rate of formation was greatest at low oxygen concentrations.

The cellophane wrapper had a low oxygen permeability according to Allen (1949), so the oxygen of the air admitted to the package was limited. The great requirement of aerobic bacteria for oxygen during the logarithmic growth phase is well known. During that stage of growth metmyoglobin was formed at the greatest rate. It seems logical to conclude that the main effect of the bacteria was to reduce the oxygen supply, which caused a large increase in the rate of metmyoglobin formation when the oxygen pressure reached critically low values.

V. SUMMARY AND CONCLUSIONS

1. During the period of increasing formation of metmyoglobin, there was a high correlation between the index of fading calculated from Munsell hue, value, and chroma determined by disk colorimetry, and the percent metmyoglobin determined spectrophotometrically.
2. Bacteria commonly found on meat cuts caused discoloration. The main effect was to increase the rate of metmyoglobin formation. This effect was greatest during the logarithmic growth phase.
3. The color of freshly cut beef was not significantly correlated with the percent metmyoglobin.
4. Desirable color was maintained longer at 34°F than at 40°F in a self-service case. A temperature of 32°F in a cooler was more effective than a rack level temperature of 34°F in the self-service case.

APPENDIX A
Table 1
DATA COLLECTED ON UNSTORED SAMPLES
FROM SIXTEEN GOOD AND CHOICE GRADE STEERS LOT P

Steer Number	Hue	Value	Chroma	Index of fading**	Percent met-myoglobin	Concentration of myoglobin %	pH
692	9.9R*	3.9	6.1	13.4	7	0.37	5.5
693	9.6R	3.9	6.4	12.1	7	0.38	5.4
694	10.0R	3.7	7.0	13.2	7	0.42	5.4
695	9.4R	4.0	7.8	8.1	7	0.37	5.4
696	10.0R	4.0	7.2	11.0	7	0.46	5.5
697	1.2YR*	4.1	6.2	16.4	7	0.32	5.5
698	10.0R	3.8	6.6	13.3	7	0.44	5.4
699	0.3YR	3.9	6.9	13.0	5	0.40	5.4
701	9.9R	4.2	6.4	13.4	6	0.35	5.6
702	9.6R	3.9	6.4	12.1	7	0.44	5.4
703	9.4R	3.6	6.4	13.3	7	0.45	5.4
704	0.1YR	3.7	6.5	14.4	7	0.37	5.4
705	10.0R	3.4	6.5	15.9	6	0.48	5.4
707	0.1YR	3.8	6.6	13.6	6	0.42	5.4
708	9.7R	4.0	6.0	12.5	6	0.38	5.4
709	9.1R	4.3	6.0	12.8	4	0.38	5.4

*R = Red, YR = Yellow Red

**Standard = Hue 7.0 Red, Value 4.0, Chroma 8.0

APPENDIX A
Table 2
DATA FROM LOT H SAMPLES STORED IN COOLER AT 32°F

Controls steer number	Days storage	Hue	Value	Chroma	Index of fading**	Percent met-myoglobin	Concentration of myoglobin %	pH	Weight into storage grams	Weight out of storage grams
702C	6	9.4R	4.2	5.6	13.8	24	0.42	5.5	121	-
701C	10	9.9R	3.9	6.1	13.4	12	0.44	5.6	104	103
696C	10	9.3R	3.7	5.9	13.5	17	0.48	5.4	102	102
698C	10	0.3YR	3.6	5.2	17.7	12	0.47	5.5	101	99
704C	13	0.6YR	4.2	5.6	16.5	19	0.42	5.3	95	93
709C	13	0.2YR	3.6	5.2	17.5	32	0.41	5.3	113	111
693C	13	9.7R	3.9	7.1	11.0	15	0.54	5.3	95	93
695C	14	9.0R	4.0	7.0	8.6	18	0.44	5.8	135	132
708C	14	9.6R	3.7	5.8	14.4	24	0.44	5.8	106	104
694C	14	1.9YR	3.5	5.4	21.4	30	0.47	5.9	123	120
697C	17	10.0R	3.8	6.0	14.4	23	0.34	5.9	177	174
699C	17	1.4YR	3.9	5.2	18.2	36	0.45	5.9	108	104
705C	17	1.4YR	3.3	4.8	22.2	37	0.48	5.8	123	119
Inoc.										
702I	6	0.8YR***	4.0	5.6	15.7	25	0.44	5.5	107	-
701I	10	3.5YR	3.5	3.9	25.4	38	0.44	5.6	105	104
696I	10	9.9R	3.9	3.9	17.4	49	0.51	5.6	101	99
698I	10	0.8YR	3.6	4.4	19.9	52	0.50	5.5	97	96
704I	13	5.5YR	3.3	3.3	29.5	53	0.41	5.5	100	98
709I	13	6.1YR	3.6	3.3	28.5	67	0.41	5.5	122	117
693I	13	3.1YR	3.3	3.5	26.2	48	0.50	5.6	98	96
695I	14	4.4YR	3.4	3.6	27.5	47	0.42	5.9	145	143
708I	14	4.6YR	3.2	3.1	28.9	56	0.42	5.9	112	110
694I	14	5.1YR	3.4	3.4	28.4	38	0.43	6.0	131	128
697I	17	5.0YR	3.5	3.1	27.6	41	0.37	6.0	154	150
699I	17	6.1YR	3.6	3.3	28.5	49	0.42	5.9	115	112
705I	17	5.7YR	3.4	2.9	29.0	60	0.46	5.8	127	123
693I,,,	28	3.0YR	2.9	3.2	28.7	18*	0.48	6.3	92	87
704I,,,	28	1.9YR	3.6	3.5	22.8	2*	0.42	6.2	100	96
709I,,	28	1.1YR	3.3	3.3	23.7	4*	0.45	6.3	122	117

*Reduced stage following oxidation
**Standard = Hue 7.0 Red, Value 4.0, Chroma 8.0
***YR = Yellow Red
Cooler relative humidity at 32°F, approx. 90%

APPENDIX A

Table 3

DATA FROM LOT T SAMPLES STORED IN SELF-SERVICE CASE AT 40°F

Controls sample number	Days storage	Hue	Value	Chroma	Index of fading***	Percent met-myoglobin	Concentration of myoglobin %	pH	Log_{10} bacty count	Weight into storage grams	Weight out of storage grams
13	2	9.5R	3.6	6.3	13.8	10	0.46	5.4	1.00	105	103
2	4	0.1YR	3.2	4.7	20.5	26	0.47	5.5	5.34	154	150
3	4	0.6YR	3.5	4.9	19.4	29	0.45	5.5	3.70	153	151
6	4	0.1YR	3.7	4.9	17.2	23	0.46	5.5	5.02	125	123
1	7	0.5YR	3.7	4.6	18.4	34	0.44	5.5	7.06	149	145
4	7	0.9YR	3.6	4.2	20.4	37	0.41	5.5	7.66	135	131
7	7	2.8YR	3.6	3.9	23.7	34	0.46	5.5	6.62	107	104
11	9	4.1YR	3.4	2.9	27.1	67	0.47	5.5	8.24	113	110
14	9	5.7YR	3.6	2.7	27.7	65	0.44	6.0	9.16	111	106
8	12	1.0YR	3.0	2.3	26.8	32	0.55	6.1	9.39	124	117
9	12	4.9YR	3.3	2.7	28.6	55	0.46	5.7	9.15	140	132
5**	16	9.8R	3.5	5.9	15.9	22	0.47	5.5	1.00	-	-
12**	16	9.5R	3.6	6.2	14.0	22	0.45	5.5	7.44	-	-
Inoc.											
13I	2	9.0R	3.8	6.4	11.1	10	0.45	5.4	4.60	112	111
2I	4	2.3YR	2.7	4.7	27.7	38	0.47	5.5	8.51	154	148
3I	4	0.5YR	3.4	5.4	19.0	27	0.45	5.5	8.42	148	145
6I	4	4.8YR	3.5	2.5	27.3	40	0.46	5.6	8.74	109	107
1I	7	-	-	-	-	23*	0.41	5.8	9.12	151	145
4I	7	2.4YR	2.9	3.4	27.7	17*	0.47	6.0	9.55	114	108
7I	7	3.5YR	2.9	3.1	29.4	5*	0.57	6.3	9.68	112	107
11I	9	9.6R	2.7	3.2	25.5	2*	0.49	6.0	9.60	103	99
14I	9	0.1YR	2.9	3.0	25.3	7*	0.53	6.2	9.83	108	104
8I	12	0.2YR	3.1	2.6	24.9	9*	0.53	6.0	9.46	121	114
9I	12	5.0YR	2.8	2.6	31.7	7*	0.53	6.0	9.46	126	115
5I**	16	5.7YR	3.4	3.2	29.1	58	0.44	6.0	9.07	-	-
12I**	16	7.1YR	3.7	3.0	16.9	62	0.44	5.8	8.71	-	-

*Reduced stage following oxidation

**Cooler storage

***Standard = Hue 7.0 Red, Value 4.0, Chroma 8.0

Relative humidity at 40°F approx. 55%

APPENDIX A

Table 4

DATA FROM LOT U SAMPLES STORED IN SELF-SERVICE CASE AT 40°F

Controls sample number	Days storage	Hue	Value	Chroma	Index of fading**	Percent met-myoglobin	Concentration of myoglobin %	pH	Log_{10} bacty count	Weight into storage grams	Weight out of storage grams
26	0	8.9R	3.9	6.1	10.9	1	0.61	5.7	3.74	-	-
28	1	9.6R	3.7	5.8	14.4	8	0.43	5.6	1.00	134	134
34	2	8.7R	3.6	5.8	12.9	12	0.64	5.7	3.48	84	83
24	3	9.5R	3.6	6.5	13.4	12	0.57	5.6	6.78	103	102
27	3	8.9R	3.8	6.3	11.1	12	0.60	5.7	6.84	82	81
20	4	8.9R	3.8	6.3	11.1	9	0.56	5.6	6.92	123	121
31	5	0.2YR	3.5	5.6	17.4	8	0.54	5.7	7.18	110	108
22	6	9.2R	3.8	6.2	12.1	19	0.51	5.6	8.18	108	105
21	7	0.5YR	3.3	4.9	20.4	32	0.56	5.7	9.11	101	98
33	8	3.1YR	3.2	4.0	26.6	30	0.55	5.8	9.56	94	92
30	9	0.9YR	3.2	3.8	23.3	39	0.53	6.0	9.40	136	133
23	10	1.5YR	3.2	3.4	24.7	24	0.55	6.9	9.41	101	97
29	11	1.6YR	3.3	3.1	24.6	11*	0.51	6.0	9.38	142	138
25	12	2.2YR	3.0	3.0	27.2	12*	0.64	5.8	9.84	85	80
32	13	1.5YR	3.1	2.7	26.2	33	0.62	6.2	9.81	100	96
35	14	2.3YR	2.7	2.9	29.2	47	0.68	6.4	9.85	68	62
Inoc.											
26I	0	8.9R	3.9	6.1	10.9	2	0.57	5.7	3.88	-	-
28I	1	9.0R	3.4	5.9	14.6	7	0.42	5.6	3.54	137	136
34I	2	0.3YR	3.5	5.6	17.6	14	0.55	5.7	5.20	95	94
24I	3	0.6YR	3.4	5.3	19.3	20	0.51	5.6	6.11	100	99
27I	3	4.7YR	3.2	3.0	29.0	38	0.59	5.8	7.18	84	83
20I	4	3.4YR	3.2	2.5	27.7	28	0.55	5.7	7.80	121	119
31I	5	10.0R	2.8	2.5	26.7	5*	0.55	5.9	8.93	126	123
22I	6	0.1YR	2.9	3.0	25.3	16*	0.61	5.8	8.91	110	107
21I	7	9.3R	2.8	2.9	25.2	2*	0.60	5.9	9.34	113	109
33I	8	0.2YR	2.9	3.0	25.4	5*	0.64	6.1	9.58	91	87
30I	9	9.9R	2.8	2.4	26.8	4*	0.59	6.1	9.82	142	137
23I	10	9.5R	3.0	2.7	24.6	5*	0.54	5.9	9.53	101	97
29I	11	8.7R	3.0	2.7	23.7	6*	0.59	6.3	9.72	128	122
25I	12	1.6YR	2.9	2.9	27.2	5*	0.66	5.8	9.68	90	85
32I	13	1.6YR	2.9	2.9	27.2	13*	0.65	6.4	9.96	117	112
35I	14	0.2YR	3.2	3.2	23.3	?	?	6.8	10.00	86	78

*Reduced stage following oxidation

**Standard = Hue 7.0 Red, Value 4.0, Chroma 8.0

? - Solution too cloudy for spectrophotometric determination

APPENDIX A
Table 5
DATA FROM LOTS X AND Y STORED IN SELF-SERVICE CASE AT 34°F

Controls sample number	Days storage	Hue	Value	Chroma	Index of fading*	Percent met-myoglobin	Concentration of myoglobin %	pH	Log_{10} bacty count	Weight into storage grams	Weight out of storage grams
X2	0	9.1R	3.3	5.7	15.9	6	0.47	5.4	0	-	-
Y2	0	9.3R	3.4	5.7	15.7	6	0.53	5.4	0	-	-
X10	1	8.3R	3.7	6.7	9.2	7	0.52	5.7	0	134	134
Y10	1	8.7R	3.5	6.3	12.4	6	0.49	5.8	0	167	166
X6	2	8.3R	3.6	6.0	11.5	10	0.45	5.5	0	128	127
Y6	2	8.5R	4.0	6.5	8.4	9	0.52	5.7	0	76	76
X12	3	8.5R	4.0	6.5	8.4	9	0.45	5.7	0	122	120
Y12	3	8.1R	3.7	6.6	8.9	12	0.48	5.8	0	120	119
X3	4	9.6R	3.7	6.6	12.9	11	0.47	5.7	0	144	142
Y3	4	9.1R	3.3	6.4	14.4	14	0.55	5.7	0	117	114
X1	5	7.9R	3.8	6.2	8.8	14	0.48	5.4	0	130	126
Y1	5	9.4R	3.2	6.3	15.9	17	0.54	5.4	3.78	106	102
X4	6	9.6R	3.6	6.4	13.9	17	0.49	5.4	0	148	143
Y4	6	9.6R	3.7	5.6	14.8	19	0.49	5.5	0	115	111
X8	7	9.0R	3.5	5.5	14.9	20	0.53	5.4	3.18	90	87
Y8	7	9.6R	3.2	5.8	17.4	20	0.57	5.4	0	90	86
X9	8	9.0R	3.5	5.3	15.3	22	0.56	5.4	4.00	58	54
Y9	8	9.6R	3.2	6.0	17.0	18	0.54	5.4	4.70	124	118
X7	9	8.7R	3.5	6.1	12.8	24	0.54	5.4	5.64	109	103
Y7	9	9.6R	3.2	5.8	17.4	25	0.54	5.5	5.47	75	71
X5	10	9.2R	3.7	6.4	12.2	23	0.49	5.4	0.70	148	142
Y5	10	9.0R	3.4	5.9	14.6	25	0.52	5.5	5.63	90	85
X15	11	5.2YR	3.2	2.9	29.6	76	0.53	5.4	8.90	101	93
Y15	11	0.4YR	3.5	5.5	18.0	23	0.54	5.4	5.27	101	96
X14	12	0.4YR	3.8	5.8	15.7	26	0.45	5.4	6.64	123	116
Y14	12	0.4YR	3.3	6.0	18.4	25	0.55	5.5	5.80	102	95
X13	13	9.1R	3.3	6.5	14.2	19	0.50	5.4	5.63	125	117
Y13	13	0.6YR	3.3	5.8	19.2	23	0.54	5.4	6.04	113	104
X11	14	0.3YR	3.0	5.2	21.3	26	0.55	5.4	3.70	138	124
Y11	14	1.0YR	2.8	4.4	25.2	44	0.52	5.5	7.98	134	124
X16	18	2.3YR	3.0	3.5	26.9	53	0.57	5.4	8.54	93	81
X17	18	9.9R	3.3	4.4	20.1	35	0.58	5.4	6.15	86	78

*Standard = Hue 7.0 Red, Value 4.0, Chroma 8.0
Relative humidity in self-service case at 34°F, approx. 45%

APPENDIX A
Table 6
DATA FROM LOTS X AND Y STORED IN SELF-SERVICE CASE AT 34°F

Inoc. sample number	Days storage	Hue	Value	Chroma	Index of fading**	Percent met-myoglobin	Concentration of myoglobin %	pH	Log_{10} bacty count	Weight into storage grams	Weight out of storage grams
X2I	0	9.1R	3.3	5.7	15.9	8	0.43	5.4	4.15	-	-
Y2I	0	9.3R	3.4	5.7	15.7	6	0.47	5.4	4.12	-	-
X10I	1	8.3R	3.7	6.7	9.2	7	0.51	5.7	3.85	118	117
Y10I	1	8.7R	3.5	6.3	12.4	6	0.50	5.8	4.64	135	134
X6I	2	9.0R	3.4	6.0	14.4	9	0.47	5.6	3.48	141	139
Y6I	2	8.5R	4.0	6.5	8.4	9	0.52	5.8	3.93	71	70
X12I	3	9.3R	3.7	6.7	11.9	9	0.49	5.7	3.54	131	128
Y12I	3	9.3R	3.7	6.7	11.9	10	0.50	5.6	4.48	125	123
X3I	4	9.6R	3.6	6.4	13.9	13	0.49	5.5	4.89	145	140
Y3I	4	9.4R	3.2	6.3	15.9	14	0.54	5.6	4.08	120	116
X11	5	8.5R	3.5	6.5	11.4	25	0.48	5.4	7.69	117	113
Y1I	5	9.9R	3.1	5.7	18.9	22	0.58	5.4	6.89	108	104
X4I	6	8.8R	3.8	6.5	10.4	23	0.53	5.4	7.26	148	141
Y4I	6	3.8YR	3.3	4.1	27.1	38	0.53	5.5	7.85	93	88
X8I	7	4.4YR	3.1	2.3	29.3	53	0.57	5.7	9.30	111	104
Y8I	7	3.3YR	3.1	2.8	28.1	59	0.56	5.7	9.36	82	77
X9I	8	4.7YR	3.0	2.4	30.2	35	0.62	5.8	9.34	80	74
Y9I	8	2.3YR	3.3	3.0	25.6	53	0.55	5.5	8.18	139	132
X7I	9	4.0YR	3.0	2.5	29.5	40	0.58	5.8	9.33	126	118
Y7I	9	3.9YR	3.2	2.3	28.2	31	0.56	5.8	9.60	83	78
X5I	10	2.7YR	3.1	2.9	27.3	46	0.54	5.6	8.70	155	144
Y5I	10	1.8YR	2.5	2.9	29.9	16*	0.56	5.6	9.20	80	74
X15I	11	1.8YR	2.9	2.8	27.6	21*	0.53	5.7	9.65	103	94
Y15I	11	2.6YR	3.0	2.8	27.9	13*	0.55	5.9	9.57	91	83
X14I	12	2.7YR	3.1	2.5	27.6	15*	0.55	5.8	9.56	110	98
Y14I	12	2.0YR	3.1	2.3	27.1	14*	0.61	6.0	9.85	89	80
X13I	13	1.2YR	3.0	3.0	26.0	16*	0.50	5.7	9.32	117	105
Y13I	13	2.5YR	2.9	2.6	28.5	12*	0.60	5.9	9.68	107	96
X11I	14	1.4YR	2.6	2.8	28.9	8*	0.61	6.0	9.92	135	122
Y11I	14	2.7YR	2.7	2.9	29.7	18*	0.59	6.0	9.74	128	114
X16I	18	9.7R	2.6	3.1	26.4	10*	0.72	5.9	9.62	95	79
X17I	18	0.7YR	2.7	3.1	27.1	10*	0.85	6.3	9.65	73	61

*Reduced stage following oxidation
**Standard = Hue 7.0 Red, Value 4.0, Chroma 8.0
Relative humidity in self-service case at 34°F, approx. 45%

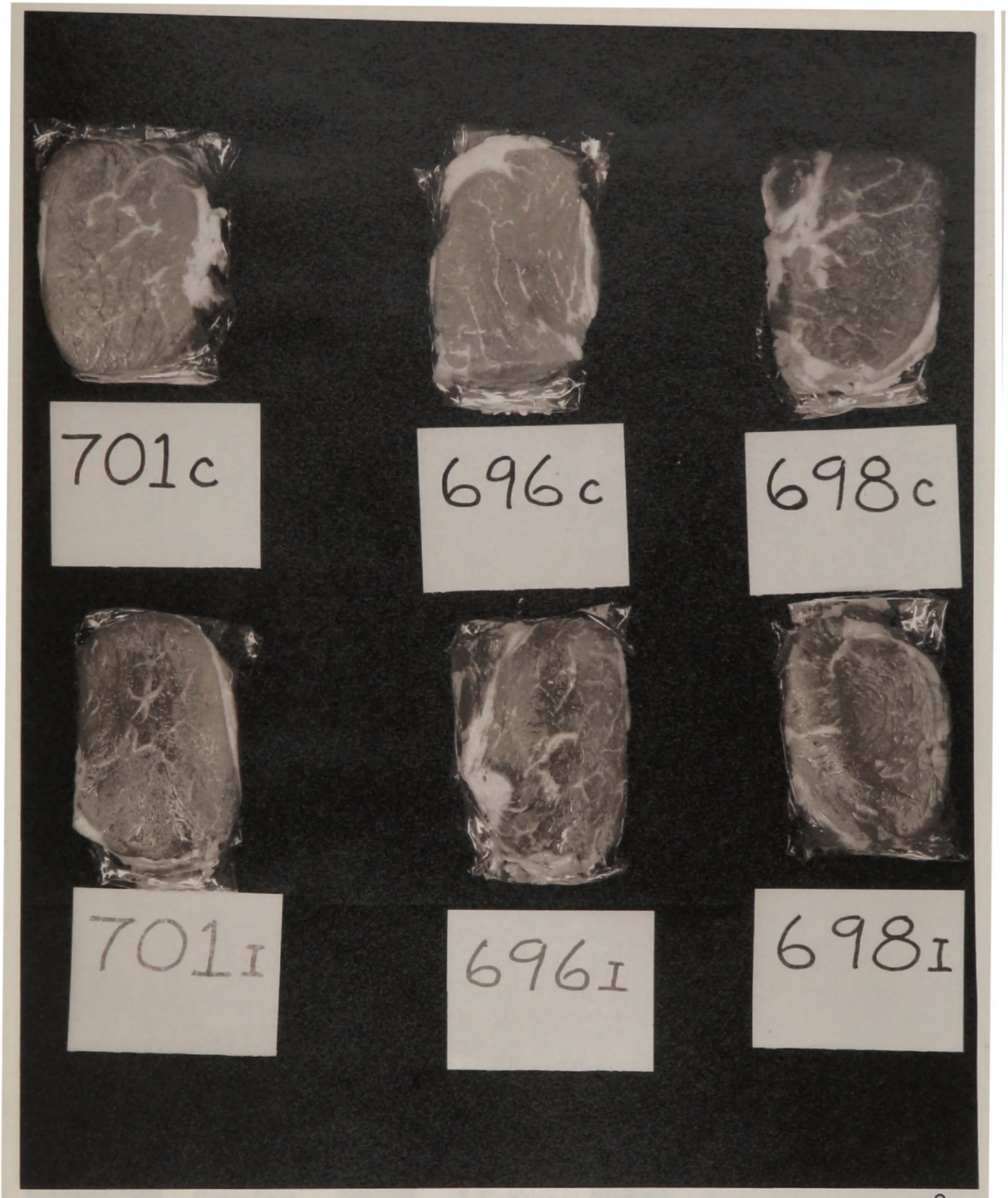

Fig. 26. Steaks from lot H after ten days storage in cooler at 32°F

Note dark streaks down the center of the inoculated steaks 701I, 696I, and 698I, which were inoculated by streaking. In color photographs the streaks showed as dark tan color against the bright red of surrounding muscle.

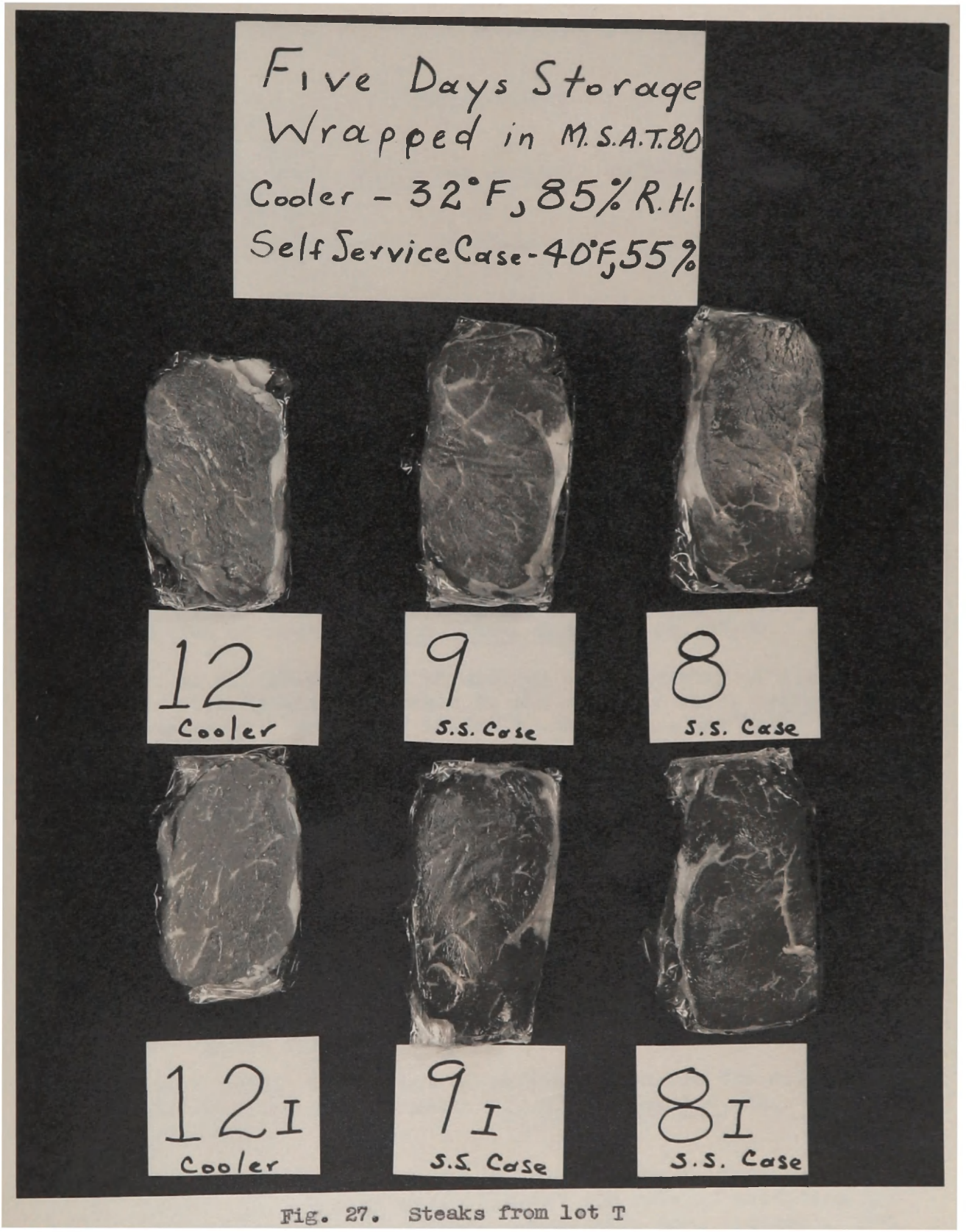

Fig. 27. Steaks from lot T

Note darkness of inoculated steaks 9I and 8I compared to their controls. Although 12I was inoculated, it was stored in the cooler at 32°F, and developed no discoloration during five days of storage.

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