equipment is used as standard equipment in quality control laboratories, the initial cost is not such an important factor, especially if the equipment is of rugged design so that it will give many years of service.

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Received for review November 23, 1962. Accepted April 29, 1963. Division of Agricultural and Food Chemistry, 142nd Meeting, ACS, Atlantic City, N. J., September 1962.

CURED MEAT PIGMENTS

Studies of the Photooxidation of Nitrosomyoglobin

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The effects of ascorbic acid, isoascorbic acid, nicotinamide, niacin, orthophosphate, and reduced diphosphopyridine nucleotide on the formation and light stability of denatured globin nitric oxide hemochromes and nitrosomyoglobin were studied. Cured meat pigments of hams were more stable to light irradiation in the presence of nicotinamide and sodium ascorbate than were those treated with ascorbate alone or those treated with a mixture of ascorbate and niacin. The color of ham slices treated with orthophosphate buffer at pH 6.8 was more desirable during storage under light than that of slices from hams treated with pH 6.2 orthophosphate buffer. Nitrosomyoglobin was formed more readily from metmyoglobin in the presence of nicotinamide than in its absence. It was more stable to light irradiation at pH 6.8 than at pH 6.2.

THE HEME PIGMENT responsible for the desirable color of freshly cured meat and its instability to light irradiation has been the object of considerable study. Many adjuncts are currently being used by industry to improve color stability of cured meat products during storage, but the practical solution of the problem has not been forthcoming.

Past work concerning use of various additives on the stability of nitrosomyoglobin can be divided into three general areas of study: the effect of ascorbates and other reducing agents (*1, 3, 12, 13, 18, 24, 25*); the influence of phosphates or other materials which affect hydrogen ion concentration (*2, 4, 10, 17, 20, 21*); and the reactivity of pyridine compounds and meat pigments to form hemachromes (*5, 6, 22*).

The objective of studies reported herein was to determine the influence of nitrogen-containing compounds such as niacin, nicotinamide, and reduced diphosphopyridine nucleotide; ascorbates; and pH on the stability of cured meat pigments.

Methods and Materials

Meat Cuts. Seventy-six hams and 12 picnics were studied in these experiments. These consisted of 32 hams purchased from a commercial source and 44 hams and 12 picnics obtained from animals slaughtered at the University of Missouri.

Curing Procedure. All hams were pumped with a 70° pickle consisting of 0.825% sodium nitrite, 0.54% sodium nitrate, 15.0% sugar, and 83.6% salt and certain other chemical additives. Sodium ascorbate was used at the level of 0.03*M* and the nitrogen ring compounds at 0.01*M*. Sodium orthophosphate (0.05*M*) was used to adjust the brine pH. Control samples included hams pumped with brine containing all constituents except the nitrogen ring compounds or samples pumped with brine containing all constituents except the nitrogen ring compounds and ascorbate. The ascorbates and nitrogen ring compounds were added to the commercial curing pickle just prior to pumping of the hams and picnics. Hams were artery pumped and the picnics stitch pumped to 10% of their trimmed weight, and one-third ounce per pound dry cover cure containing the same proportion of curing ingredients as above was applied. The meat samples were cured at 38° ± 2° F. for 6 days.

Cooking and Smoking Procedures. Following curing for 6 days, the samples were washed with running tap water for 30 minutes to remove excess cover cure. They were then placed in stockenettes and dried for 12 hours at 120° F. in an electric smoke house. Smoke was applied after drying, and the temperature raised 10° F. each hour until it reached 150° F. The cuts were processed to an internal temperature of 142° F. After smoking and cooking, the cuts were cooled to room temperature and stored at 38° ± 2° F. until used for display and analysis purposes.

Preparation of Samples. Five center slices were removed from each of the hams and picnics. Two slices were wrapped individually with a laminated

paper, labeled, and frozen for later chemical analyses. The other slices were processed in a dimly lighted room. They were scraped free of saw smear, trimmed, and placed on backing boards. They were labeled and wrapped with 70-gauge Cryovac film and heat-sealed.

Subjective Color Evaluation. After wrapping, the three slices for color evaluation were displayed under 50 foot-candles of fluorescent light at 38° ± 2° F. and immediately scored by five panel members for color desirability. The samples were subsequently scored following 4 hours of irradiation and then every 24 hours for 7 days or less. An hedonic scale ranging from 6 (very desirable) to 1 (very undesirable) was used to rate color desirability. Slices were arranged in sets of three or sets of four and the judges asked to rate each for its color desirability and for its preference compared to the other slices in the group. Between judgings, the slices were randomly rearranged within groups to eliminate bias.

In certain cases, stability of nitrosomyoglobin as indicated by subjective color desirability scores was analyzed by a mathematical procedure to determine the rate of fading of this pigment. The method used was described by Hornsey (*14*) for the determination of nitroso pigment stability of cooked cured pork.

Color panel evaluation data were analyzed by Student's *t* test (*19*) to determine if there were significant differences between samples with various treatments.

Objective Procedures for Measuring Pigment Concentration. The rate of nitrosomyoglobin formation was studied in model systems of purified metmyoglobin preparations containing ascorbates, phosphate buffers pH 6.2 and 6.8, nitrite, and nitrogen ring compounds such as niacin, nicotinamide, and reduced diphosphopyridine nucleotide.

A spectrophotometric method similar to that of Ginger and Schweigert (*10*) was used to determine the concentrations of metmyoglobin, myoglobin, and nitrosomyoglobin in these studies. The conversion of metmyoglobin to myoglobin in the presence of $2.8 \times 10^{-2}M$ ascorbic acid and $8.2 \times 10^{-3}M$ niacin or nicotinamide was carried out in the dark at room temperature (77° ± 5° F.).

Three-tenths milliliter of a solution containing 14 mg. per ml. of beef heart myoglobin, purified by the method of Lewis and Schweigert (*15*), and 11.5 ml. of phosphate buffer at pH 6.2 or 6.8 was centrifuged at 10,000 G for 20 minutes to eliminate turbidity. The supernatant liquid was poured into clean test tubes and 0.1 ml. of $1.2 \times 10^{-1}M$ niacin or nicotin amide added to each tube along with 0.1 ml. of $2.8 \times 10^{-2}M$ ascorbic acid previously adjusted to pH 5.5 with 10% sodium hydroxide. The tubes were placed in the dark and the absorbances at 555 and 635 mμ [absorption maximum

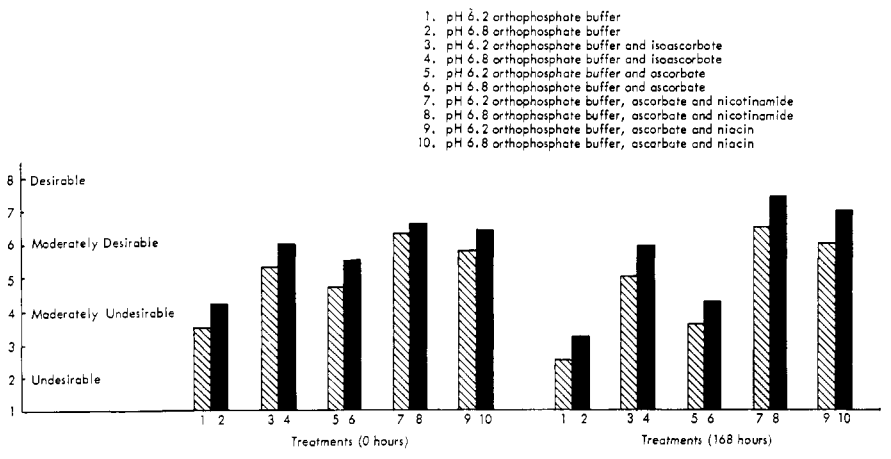

Figure 1. Effect of chemical additives on color desirability scores of ham slices after 0 and 168 hours of storage under 50 foot-candles of fluorescent light at 40° ± 2° F.

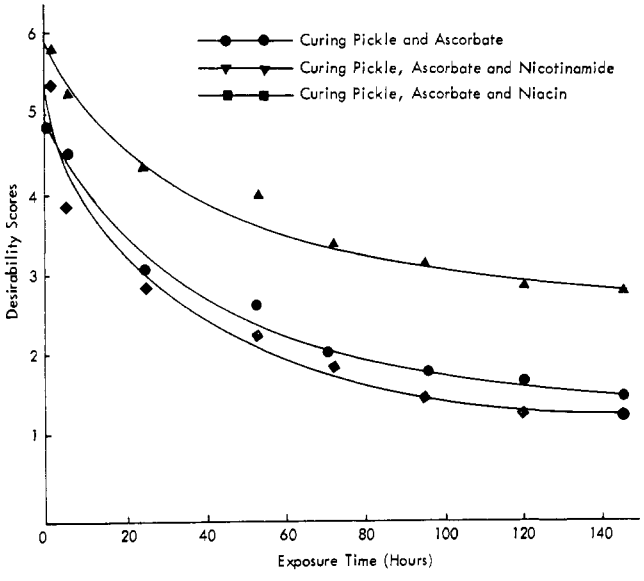

Figure 2. Effect of 50 foot-candles of fluorescent light on color desirability of ham slices

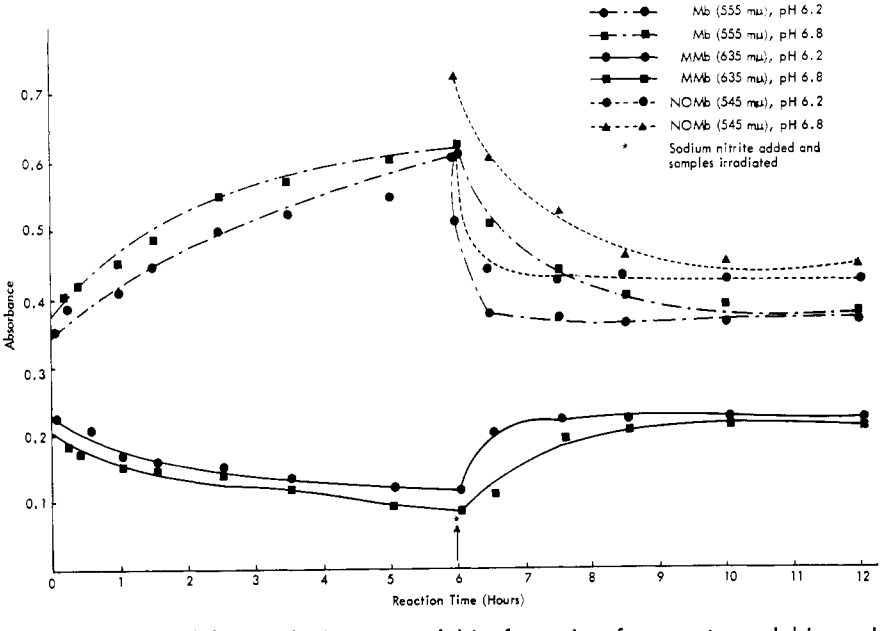

Figure 3. Myoglobin and nitrosomyoglobin formation from metmyoglobin and the stability of nitrosomyoglobin to light irradiation at pH 6.2 and pH 6.8

Table I. Desirability Preferences of Ham Slices Treated with Vitamins and Orthophosphate Buffers

Comparison	Observations	Treatment Preferred	Percentage for Preferred Treatment
pH 6.8 and pH 6.2	195	pH 6.8	81.03
Niacin or nicotinamide and control samples	160	Pyridine compounds	90.00
Ascorbates and control samples	40	Ascorbate	95.00

Table II. Fading Rate and Half Life of Nitrosomyoglobin in Ham Slices Treated with Vitamins and Exposed to 50 Foot-Candles of Fluorescent Light

Treatment	Rate Constant, $Hour^{-1}$		Half life, Hours	
	0–24 Hours	24–120 Hours	0–24 Hours	24–120 Hours
Nicotinamide-ascorbate	0.127	0.003	5.42	231.0
Ascorbate	0.184	0.005	3.72	138.6
Niacin-ascorbate	0.316	0.009	2.19	77.0

for myoglobin and metmyoglobin, respectively, (*8*)] read periodically using a Bausch & Lomb Model 20 spectrophotometer. Although the resolution of this instrument in the identification of the myoglobins is not as great as that obtained with the Beckman DU, results obtained with the two instruments at the wavelengths and dilutions employed are quite parallel and, in some cases, almost identical (unpublished results).

An increase in absorbance at 555 mμ and a decrease at 635 mμ were the criteria used for the formation of myoglobin and the disappearance of metmyoglobin.

After the metmyoglobin had been reduced to myoglobin (maximum absorbance at 555 mμ), nitrosomyoglobin formation and its stability in the presence of $8.2 \times 10^{-4}M$ sodium nitrite was studied. One-tenth milliliter of $6 \times 10^{-2}M$ sodium nitrite was added to the myoglobin solution, and absorbances were immediately determined at 555, 635, 545, and 575 mμ.

Absorbances at 545 and 575 mμ were used to measure nitrosomyoglobin concentration (*8*). Colorimeter tubes containing the samples were then irradiated with 200 foot-candles of fluorescent light and the changes in the concentration of metmyoglobin, myoglobin, and nitrosomyoglobin were determined by absorbance readings.

Increase in absorbance at 545 and 575 mμ and decrease at 555 mμ were used as criteria for the formulation of nitrosomyoglobin and the disappearance of myoglobin.

pH Determinations. Hydrogen ion concentration was determined with a Beckman Model G pH meter. One-half-inch cores taken from the quadraceps femoris, biceps femoris, and adductor muscles of center ham slices were blended with equal weights of distilled water in an Omnimixer for 1 minute. The resulting slurry was then used for pH determination. The average pH value of samples from these muscles was recorded as the pH of the ham slice.

Results and Discussion

Preliminary Study. A preliminary study was made of two groups of hams containing five hams in each group. Both groups of hams were treated with $0.05M$ orthophosphate buffer, one at pH 6.2 and the other at pH 6.8. Sodium ascorbate ($0.03M$) or sodium erythrobate ($0.03M$) were used as reducing agents, either in the presence or absence of $0.01M$ niacin or $0.01M$ nicotinamide.

The phosphates at pH 6.8 and pH 6.2 did not buffer the ham tissues at these hydrogen ion concentrations, but at higher values—approximately pH 6.2 to 6.4 and pH 5.7 to 6.0, respectively. These values are in the ranges reported by other investigators (*9*, *20*, *23*) for maximum reactivity between nitrite and myoglobin in the presence of ascorbates.

Figure 1 is a histogram used to compare the desirability of ham color at 0 storage and after 168 hours of storage following various chemical treatments. These results indicate that niacin or nicotinamide in the presence of ascorbates preserved desirable cured meat hemochromes better than other treatments did. Samples treated with orthophosphate buffer at pH 6.8 were more stable to light irradiation than samples treated with orthophosphate buffer at pH 6.2. Slices treated with ascorbates or with a combination of ascorbate and niacin or nicotinamide were more desirable during the storage period than samples not treated with these additives.

Results from the preference studies (Table I) indicate that pH 6.8–treated samples were preferred to treatments with pH 6.2 buffer. When the control samples were compared with samples treated with niacin or nicotinamide, the niacin- and nicotinamide-treated samples were more desirable. The ascorbate-treated samples were more desirable than samples not treated with this vitamin.

Figure 2 shows the effect of 50 foot-candles of fluorescent light at 40° ± 2° F. on the color desirability of ham slices from 10 other hams treated and analyzed by the methods used in the preliminary experiment. Desirability scores are plotted against exposure time in hours, and each point on the curve represents 30 observations by panel members. In this study, nicotinamide-treated samples were more desirable and niacin-treated samples less desirable than the controls throughout the storage period. Both the control samples and those treated with niacin were undesirable after 48 hours of exposure to 50 foot-candles of light, but samples treated with nicotinamide were not judged undesirable until they had been displayed under these conditions for over 100 hours.

Results concerning the rate of fading and half life of nitrosomyoglobin (*14*) when color desirability was used as a measure of its concentration are presented in Table II. The rate constants for fading and the half lives for nitrosomyoglobin are given for the first 24 hours exposure time and for the exposure period between 24 and 100 hours. The half lives were computed from the first-order rate constants by the method described by Daniels (*7*). These results indicate that nicotinamide-treated samples containing ascorbate were more stable during storage than samples treated with ascorbate alone and that the ascorbate-treated samples were more stable than the niacin-ascorbate–treated samples.

Results from further study of the photooxidation of ham nitrosomyoglobin in the presence of ascorbates, nicotinamide, and niacin are presented in Table III. These data show the differences between preference ratings of ham slices treated with nicotinamide and niacin compared to control samples. Observations were made by five panel members during 50 foot-candle light irradiation of slices from 60 hams for 96 hours at 40° ± 2° F. These studies revealed that the nicotinamide-ascorbate treatment was preferred to the ascorbate treatment alone or the niacin-ascorbate treatment, and the ascorbate treatment was preferred to treatment with niacin and ascorbate.

Results of the *t* analysis of data obtained from study of 22 hams and 12 picnics are presented in Table IV and show the effects of pH on desirability of slices during storage under 50 foot-candles of fluorescent light at 40° ± 2° F. These results were analyzed without regard to treatment other than that which affected pH, and included only those samples which were compared directly for pH effect. Treatments in all cases were with $0.05M$ orthophosphate buffer at pH 6.2 or pH 6.8. At time periods subsequent to 24 hours, there was a significant preference for samples receiving treatment with pH 6.8 buffer. When the results from all time periods were compared, pH 6.8 treatment was

Table III. Differences between Preference Ratings of Ham Slices Exposed to Fluorescent Light for 96 Hours

Treatments Compared	Degrees of Freedom	Treatment Preference	"t" Value
Nicotinamide and control	285	Nicotinamide	4.88[a]
Nicotinamide and niacin	109	Nicotinamide	4.20[a]
Control and niacin	233	Control	5.89[a]

[a] Significant at 0.01 level.

Table IV. Effect of pH on Desirability of Slices from 34 Hams during Storage under 50 Foot-Candles of Fluorescent Light at 40° ± 2° F.

Irradiation Time (Hours)	Degrees of Freedom	pH Treatment Preferred	"t" Value
0	31	none	0.655
24	31	none	0.522
48	31	pH 6.8	2.753[a]
72	31	pH 6.8	2.364[b]
120	31	pH 6.8	2.659[b]
0–120	159	pH 6.8	3.150[a]

[a] Significant at 0.01 level.
[b] Significant at 0.05 level.

significantly ($P < 0.01$) more desirable than treatment with pH 6.2 buffer.

The more desirable effect of pH 6.8 treatment compared to pH 6.2 treatment substantiates the belief that the pH optimum for nitrosomyoglobin formation and stability in ham is in the range pH 6.2 to pH 6.4 rather than lower ranges reported by some investigators (*9*, *23*). The mechanism for these desirable effects at lower hydrogen ion concentrations is apparently quite complex. Nitrite in the curing mixture is readily depleted, and since quantities of this substance are restricted, any step which will preserve this constituent will probably prolong the storage life of desirable cured meat pigments irradiated by light. Nitrite is easily reduced to nitric oxide at low pH and even though the formation of nitric oxide is desirable, if formed too rapidly, it would be dissipated as a gas. Slow production of nitric oxide from nitrite is desirable for efficient formation of nitrosomyoglobin in hams. Nitric oxide may be depleted at lower pH because of its association with water and nitric acid to produce nitrous acid. This association is more rapid at low pH (5.5) than at higher pH values. Nitrous acid concentration can be decreased by its participation in many biochemical reactions, including the Van Slyke reaction with protein and amino acids.

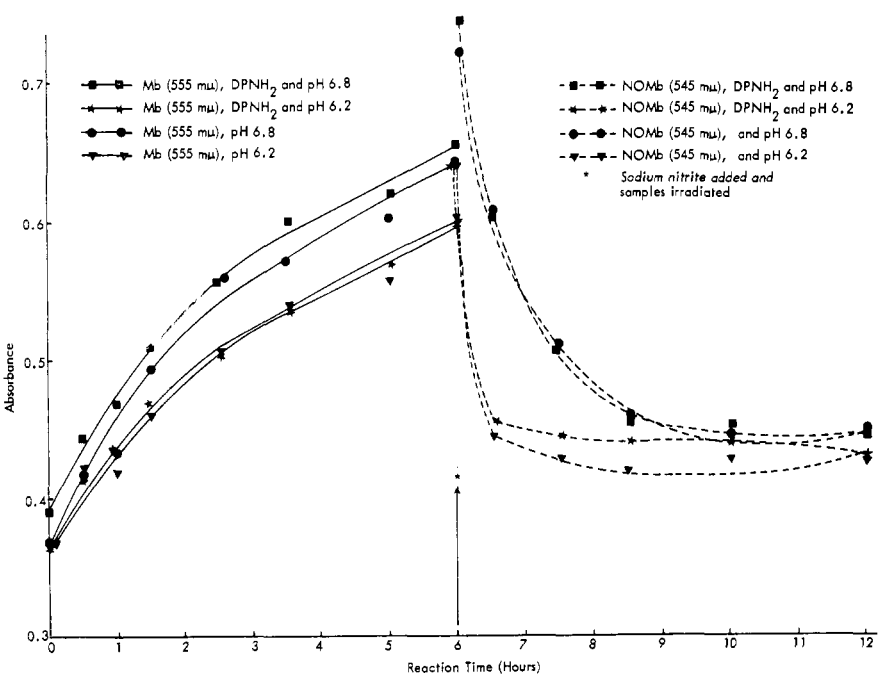

Figure 4. Influence of reduced dyphosphopyridine nucleotide on the formation and stability of nitrosomyoglobin to light irradiation at pH 6.2 and pH 6.8

Use of Purified Systems to Study Formation of Nitrosomyoglobin from Metmyoglobin and the Stability of Nitrosomyoglobin to Photooxidation. A spectrophotometric method similar to that of Ginger and Schweigert (*10*) was used to study the influence of various chemicals and light conditions on the oxidation and complex states of myoglobin following its extraction from beef heart and its purification. These studies were made in an attempt to determine the effects of pH and N-ring compounds on the formation and stability of nitrosomyoglobin. Even though these results cannot be directly translated to effects concerning denatured globin nitric oxide hemochrome of cured meat, the light stabilities of the two components in the presence of ascorbates are possibly quite similar. Stabilities of the denatured pigments are generally difficult to determine.

Results from this experiment are shown in Figure 3. Samples were buffered at pH 6.8 and pH 6.2 to study the effects of hydrogen ion concentration on reduction of metmyoglobin to myoglobin and nitrosomyoglobin formation and stability during lightirradiation at 77° ± 5° F. Myoglobin and nitrosomyoglobin formation was greater and nitrosomyoglobin was more stable at the higher pH. The accuracy in measuring changes in metmyoglobin and myoglobin during this reaction is indicated by the symmetry of the curves.

One possible explanation for the effect of nicotinamide on ham nitrosomyoglobin is the preservation of diphosphopyridine nucleotide (DPN). Mann and Quastel (*16*) studied an enzyme in brain tissue which destroyed diphosphopyridine nucleotide. This was called coenzyme I nucleosidase, and its activity was found to be inhibited by nicotinamide. Further studies of similar enzymes were made by Handler and Klein (*13*), who observed that broken cell preparations of brain, liver, kidneys, and muscles from rats and dogs inactivated both coenzyme I and coenzyme II.

Gutmann *et al.* (*11*) found that nicotinamide in the presence of hexose diphosphate or lactate could serve as a substrate in the reduction of metmyoglobin in hemolysates as well as in intact erythrocytes and that methemoglobin interacted with reduced DPN to yield hemoglobin. Similar reactions could occur with metmyoglobin during the curing reaction. The presence of nicotinamide and coenzyme I in hams should

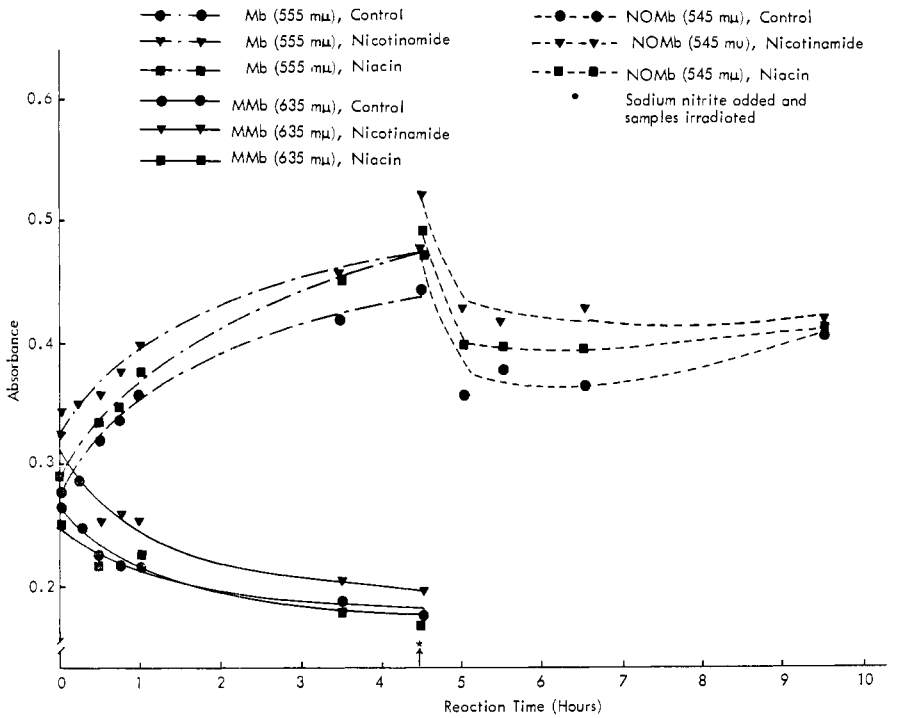

Figure 5. Formation and stability of nitrosomyoglobin to light-irradiation in the presence of nicotinamide or niacin at pH 6.2

augment nicotinamide, and coenzyme I in hams should augment nitrosomyoglobin formation during curing.

The objective method previously described for measuring pigment changes in model systems was used to study the effect of $1.5 \times 10^{-4}M$ reduced DPN on myoglobin and nitrosomyoglobin and the stability of nitrosomyoglobin to light irradiation.

Model systems containing purified myoglobin (*15*) buffered with $0.05M$ orthophosphate buffer and $2.8 \times 10^{-2}M$ ascorbic acid were studied. Sodium nitrite ($6 \times 10^{-2}M$) was added to convert myoglobin to nitrosomyoglobin, and the tubes containing the nitrosomyoglobin were irradiated with 200 foot-candles of fluorescent light. In this procedure, an increase in absorbance at 555 mμ was used to measure myoglobin (Mb) production from metmyoglobin (MMb) and the absorbance of the system at 545 mμ used as a measure of nitrosomyoglobin (NOMb).

Results in Figure 4 indicate that myoglobin was formed from metmyoglobin much faster at pH 6.8 than at pH 6.2 and that the presence of reduced DPN enhanced formation of myoglobin slightly at pH 6.8 and did not effect myoglobin formation at pH 6.2.

Reduced DPN had no apparent effect on the conversion of myoglobin to nitrosomyoglobin in the presence of sodium nitrite either at pH 6.8 or pH 6.2 and was more effective at pH 6.2 than at pH 6.8 in protecting nitrosomyoglobin from light degradation.

If the protection of DPN is the mechanism by which nicotinamide stabilizes nitrosomyoglobin to light irradiation, other redox carriers must be involved in electron transfer in ham tissue which were not included in the model system used.

An experiment similar to the one outlined above was carried out to determine if nicotinamide or niacin were effective in stabilizing nitrosomyoglobin at pH 6.2. Results from this experiment are presented in Figure 5 and indicated that both nicotinamide and niacin enhanced myoglobin and nitrosomyoglobin formation and retarded nitrosomyoglobin degradation during lightirradiation compared to the control sample.

Differences in the reduction of metmyoglobin and the formation and stability of nitrosomyoglobin were not apparent when solutions of metmyoglobin were treated with ascorbates in the presence and absence of nicotinamide or niacin at pH 6.8. Adjustment of pH to 6.8 was more effective than the addition of nicotinamide or niacin for the formation of nitrosomyoglobin from metmyoglobin in the presence of ascorbic acid and sodium nitrite. Since all samples in these model studies were buffered at their respective pH values by the orthophosphate, the differences observed in the presence of nicotinamide relative to control samples were apparently not due to differences in hydrogen ion concentrations.

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Received for review December 19, 1962. Accepted April 1, 1963. Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 2516.