NITRITES AND NITROSAMINES IN PROCESSED MEATS

SESSION E: MEDICAL ASPECTS

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Ten years ago the word nitrosamines meant little or nothing to us employed in the meat industry or in meat research groups associated with government or universities. Today, nitrosamines is a household term; nitrosamines represent the central issue of a problem that is (has been) the most critical ever faced by the industry.

The industry I speak of is enormous. In the U.S.A. the production of cured meats was valued at 8 billion dollars in 1972. A figure easier to remember though, in regard to the question about using nitrite for meat curing, is that about 70% of our pork production is cured.

Curing is not a modern technological invention but rather is a process steeped in tradition and traceable to earliest history. Curing is defined generally as the treatment of meat with salt, nitrite and/or nitrate and possibly other ingredients such as sugar, spices, phosphates and ascorbates. The purpose is for preservation and flavoring, and cured meat is easily recognized by the characteristic pink, heat-stable color.

Nitrosamines are a broad class of compounds formed from the reaction of nitrite with secondary amines. In view of the widespread publicity about nitrite and nitrosamines you all recognize the potential areas of concern. First, that nitrosamines may be formed in the cured meat since there are amines present and nitrite is added

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s a constituent of the curing formulation. Second, that cured meats represent a ource of residual nitrite which when consumed by humans may react in the gastric nvironment with ingested amines to form nitrosamines.

The hazard of nitrosamines has been known for about 20 years. Dimethylnitrosamine s an extremely potent carcinogen and over 65 different nitrosamines have been shown o be carcinogenic. Analysis of food products for nitrosamines was undertaken in e late 1960's and early 1970's. Some positive results for cured meats were pub- ished. I need say little more about historical aspects from that time onward.

There is no doubt about the danger of certain nitroso compounds. The problem is t one of establishing the danger of nitrosamines but rather it is one of relating itrosamines to food hazards. The problem is a very complex one because of extremely w concentrations, a long laborous procedure for analysis, differences in toxicity, d the need to estabiish a risk-benefit ratio for nitrosamine precursors such as trite.

There are questions still to be answered, but I'm pleased to tell you that much reful diligent research, designed to establish basic principles has already been mpleted. Such will be the topic of the papers contained throughout todays session nitrites and nitrosamines in processed meats.

My objective is to discuss the two papers submitted under session E entitled edical Aspects". I am fortunate because the 2 papers in this session represent gh quality and timely work. A clear point is made by each and I believe I can late this information to some pertinent work in the U.S.A. which will, I hope, be interest to you. Finally as time permits I will present some work conducted in r laboratory. It may relate more to Mr. Rankin's session but I would like to make available for discussion.

Let us turn our attention then to the paper entitled "Some Chemical Studies on e Nitrosamine Problem With Respect to Bacon Production" by Patterson and Mottram the British Meat Research Institute. This paper attempts to answer 2 very impor-

tant questions. First, the amount of nitrite added in curing is known but since the second required component for formation of nitrosamines is amines, then which amines are present in meat and in what quantities. Second, a most frustrating problem has been the observation that raw bacon does not contain nitrosamines but after cooking substantial quantities of nitrosamines - specifically nitrosopyrrolidine - are present.

The authors analyzed for endogenous amines in 10 carcasses at (1) within 1 hr post-slaughter, (2) after 76 hrs of commercial chill, (3) following curing and maturation and (4) following vacuum storage for a number of days. Methylamine, dimethylamine, trimethylamine, ethylamine, diethylamine, n-propylamine and isopropylamine were detected in the fresh meat. The concentrations detected ranged from 10 to 1900 mg/kg. I believe it is of particular interest to see the continual increase in dimethylamine from time of slaughter through to the end of storage. A general conclusion was that the levels of amine detected were not sufficient to result in the formation of detectable levels of nitrosamine. This conclusion was borne out by the fact that the same samples were analyzed for steam volatile nitrosamines and no positive confirmations made.

I will now comment briefly on some work from the U.S.A. and contrast it with foregoing results. This is work of Lakritz, Spinelli and Wasserman of the U.S.D.A. Eastern Regional Lab (Lakritz, et al. 1973) and concerns their determination of amines in fresh and processed pork. They analyzed fresh, cooked and putrified meat for spermine, spermidine, putrescine, cadaverine, histamine, tyramine, tryptamine and ethanolamine. Concentrations found ranged from 0.5 mg/100 g for tyramine to 189 mg/100g for putrescine. Significant increases in spermine, spermidine, putrescine and cadaverine occurred during putrefaction. Cooking at 71°C decreased the concentration of amines. In fresh and processed pork bellies the concentration per 100g of tissue ranged from 0.03 mg for cadaverine to 8.1 mg for spermine. Processing did not significantly alter the levels of the free amines.

The second part of the work by Patterson and Mottram was conducted more as a laboratory model experiment but produced some interesting results about the conversion of amines to nitrosamines under simulated manufacturing conditions. Moreover the role of ascorbate in reducing the formation of nitrosamines was considered. High concentrations of dimethylamine were introduced into pork slices or pork middles, in order to insure a measurable reaction, and curing was conducted at varying pH's and concentrations of ascorbic acid. The pork slices were subsequently heated to 90°C for 1/2 hour and the rashers were fried to a final temperature of 140°C. Three findings were forthcoming. First, cooking increased the concentrations of dimethylamine and high concentrations of dimethylamine were always found in the fried bacon. Concentrations of nitrosamine in the fatty portion were more variable and higher than in the lean. Second, the use of ascorbate suppressed the formation of nitrosamines. Third, and of most interest to me was the figure generated by the authors for conversion of secondary amine to nitrosamine in the absence of ascorbate. The suggested conversion is 0.1% meaning that an extremely small amount of amine is converted to nitrosamine. In fact, with the figures given for endogenous amine in the first part of the paper the 0.1% conversion would not produce a detectable amount of nitrosamine.

To summarize the findings of this paper I would say that endogenous amines are present in pork muscle, but the concentration is not sufficiently high to produce detectable nitrosamines in the cured finished product. Ascorbate suppress the formation of nitrosamines and heating (particularly frying of bacon) promotes their formation.

Before I proceed now to the next paper I want to comment briefly on 2 other considerations. First, is some very recent work by Fiddler et al. (1974) which was presented at the recent IFT meeting. It is about the formation of nitrosoproline in uncooked bacon and its possible role as a precursor to nitrosopyrrolidine in cooked bacon. The possibilities for formation of nitrosopyrrolidine are (1) from the nitrosation of pyrrolidine which had resulted from cyclization of putrescine or decarboxyla-

tion of proline, (2) from the formation of nitrosoproline followed by decarboxylation. Present evidence suggests that nitrosoproline might be the precursor for nitrosopyrrolidine in fried bacon. The authors concluded that putrescine had little role in the formation of nitrosopyrrolidine. They were able to measure nitrosoproline in uncooked bacon at quantities around 1 ppm. Following cooking, nitrosopyrrolidine was present in the ppb (7-17) range. It should also be mentioned that this work represents the first report of a non-volatile nitrosamine in a food product. Additional work by these same authors in which they fried separated lean and fat portions of bacon led to the conclusion that the adipose tissue contains the nitrosopyrrolidine precursor.

One would conclude an involvement of connective tissue in the formation of nitrosopyrrolidine because of its high content of proline and hydroxyproline. However, other products such as ham and cured shoulder do contain substantial quantities of connective tissue but are essentially free of nitrosopyrrolidine. The issue remains a mystery but one which clearly must be resolved.

I believe it is appropriate that I comment also at this time on the very extensive series of studies which have been conducted in the U.S.A. to produce more information about the role of nitrite in inhibiting production of botulinal toxin (see for example Christiansen, et al. 1974). These experiments are classics I believe because they represent an immense cooperative effort by the U.S.D.A., F.D.A., and industry represented by the AMI. Four types of cured meat were examined. (1) cooked sausage (frankfurters), canned cured meat (ham), bacon and fermented sausage (thuringer). The design specified preparation of the product under commercial conditions with a wide range of concentrations of nitrite followed by innoculation with clostudium botulinum. The research was conducted in an effort to answer the following questions: (1) does nitrite at present levels reduce the risk of c. botulinum toxin formation?, (2) are detectable levels of nitrosamines formed when presently permitted nitrite levels are used, (3) can changes in permitted curing agents be made that would reduce

risk of nitrosamine formation? If I can speak in generalities, the results found from the work can be set forth as five statements: (1) the amount of nitrite needed for protection is dependent upon bacterial innoculum level, (2) nitrite is effective in preventing formation of botulinal toxin, (3) nitrate is essentially non-functional as a botulinal inhibitor, (4) residual nitrite decreases rapidly during meat processing and continues to decrease with time, (5) the quantity of nitrite needed for botulinal protection also varies by product type.

It is interesting that at the presently permitted levels of nitrite, no nitrosamine were found in any product except fried bacon. High levels of ascorbate served to inhibit the formation of nitrosamines in bacon but some results also indicated that ascorbate might reduce the effectiveness of nitrite as a botulinal inhibitor. No samples with botulinal toxin were noted in the fermented sausage study and such sausage with 50 ppm nitrite would produce toxin only if dextrose or sugar was not present to bring about the fermentation and lowering of pH.

This extensive series of experiments forms much of the information base upon which decisions about the continued use of nitrite as a meat curing agent in the U.S.A. will be based. Tentative recommendations (meeting of July 15, 1974 in Washington D.C.) from our Secretary of Agriculture's Expert Panel on Nitrite Nitrosamines calls for (1) elimination of nitrate in cooked sausage, bacon, canned, cured shelf-stable and perishable meat products and pickle-cured products, (2) elimination of nitrate in fermented sausage and dry cured primal cuts in 2 years unless research shows its need, (3) standardization of ingoing nitrite at 156 ppm in all processed meat except bacon and cured primal cuts pending more research and (4) reduction of residual nitrite levels from 200 ppm to: 100 ppm for cooked sausage, 125 ppm for canned, cured, shelf-stable and perishable products and pickle cured items; 50 ppm for cured, canned sterile and retorted products.

Let us now turn our attention to the paper entitled "Reductive dechlorination of DDT during meat cooking and nitrite inhibition of the process". This manuscript was written by the Russian workers Shoumkova, Karpova, Rouzankova and Alexeyeva of

the All Union Meat Research Institute. It presents a very interesting approach to a lingering problem. The lingering problem is that residues of DDT continue to be found, even though admittedly at low levels, in raw tissue of meat animals. Some countries, such as the U.S.A., have suspended the use of DDT except for very isolated and special permission uses. I understand though that some of our manufacturers continue to make it for shipment and use elsewhere. Therefore, even though we have stopped putting it into the environment, that which is already there together with its metabalities continues to linger and residues are found in raw meat products. The purpose of this paper then was to study the influence of heating and of heating in the presence of nitrite on the metabolism of DDT. The results are set forth quite clearly and I would like to review them with you. First though I must say a word about the reduction dechlorination of DDT to DDD. This is an established route of metabolism and involves the replacement of a chlorine with a hydrogen. Such conversion is desirable for 2 reasons. First DDD is about 10 times less toxic than is DDT. Moreover DDD does not accummulate in the organism to the same extent as does DDT. DDT is known to be highly thermoresistant, but it does degrade to DDD when heated in solution in the presence of reducing agents including metal ions. The authors put this information to good use and by apparently utilizing the endogenous reducing system in muscle, they were able to show a marked conversion of DDT to DDD when meat is heated. The data set forth in the manuscript show that a high percentage of conversion took place. The conversion was slower if the fat content of the meat was higher.

The authors then reasoned that since nitrite is reduced in a meat system, it may compete with DDT for the hydrogens. The results leave no doubt that the presence of nitrite inhibits completely the reductive dechlorination of DDT. The authors concluded that if one expects to detoxify DDT residues in meat by heating, such cannot be accomplished in the presence of nitrite.

This work represents an important piece of information - namely that the presence of nitrite blocks the destruction of DDT by heating in meat. This information should

e remembered in the debates about use of nitrite. As a matter of information, I hecked with our National Residue Monitoring Program in Washington D.C. to find ut about levels of DDT found in our meat supply. Our Environmental Protection A- ency has reduced the tolerance level from 7 ppm to 5 ppm. During the period of anuary to March, 1974 they sampled 266 beef carcasses. Thirty nine were negative, 7 had 0.01 - 0.03 ppm, 21 had 0.31 - 1.00 ppm, 3 had 1.01 - 1.50 ppm, 4 had 1.51 - .00 ppm, 1 had 2.01 ppm and 1 had greater than 7.00 ppm. It is obvious that DDT esidues do persist even when the use of DDT is no longer allowed.

That is all I have to say at this time about the 2 manuscripts. I compliment he authors on well designed and clearly reported experiments and I enjoyed reviewing hem.

It is clear that nitrite added to a meat for the purpose of curing is rapidly st. Aside from the amount of nitrite bound to pigment, detectable as nitrosothiols given off as a gas little is known concerning the fate of nitrite. Our own work the University of Wisconsin and conducted by Dr. Sebranek and Miss Kubberød has en directed at trying to collect some quantitative information in this regard by ing nitrite labeled with ^{15}N, the stable isopope of nitrogen. Initial experiments ebranek, et al. 1973) were conducted on a canned cured meat product in which at- pts were made to quantitatively recover all of the ^{15}N by trapping gases evolved ring processing and subsequently analyzing various fractions of the cured meat for N. Total recoveries of 70-85% were realized, but in the course of that work we scovered that the water soluble extract of the meat contained a substantial portion the ^{15}N in addition to that present in the form of residual nitrite and that the ncentration of ^{15}N increased during storage as ^{15}N in the residual nitrite declined. About 25% of the originally added ^{15}N was found in this portion of the meat. I uld, therefore, like to share with you some results (Sebranek, et al. 1974) about rther work on that water soluble fraction. It was subjected to a sephadex G-10

column and to our surprise most of the ^{15}N was concentrated in a single peak. This is illustrated in slide 1 which shows analysis of the fractions for total nitrogen and ^{15}N. There was one large sharp peak of ^{15}N at fraction 80 and a second much smaller peak at fractions 109 and 111. The large peak represents 76% of the ^{15}N in the concentrate applied to the column. The actual percent of ^{15}N of total N in the peak was approximately 5% indicating that the total fraction contained much ^{14}N in addition to ^{15}N from the nitrite. However, approximately 16% of the ^{15}N added originally to the meat product in the form of sodium nitrite is accounted for in the one major peak.

We are currently trying to learn more about the compounds contained in this peak.

Optical density examination of the fractions is shown in slide 2. Since absorbance at 260 mm is taken as evidence for the presence of nucleotides and absorbance at 280 mm is taken as evidence for aromatic amino acid residues it is clear that the peak at fraction 80 did not contain detectable nucleotides or aromatic amino acids. The peak also gave a minimal reaction with ninhydrin indicating few if any free amino groups and it was negative to analysis for nitrite.

Preliminary attempts to determine molecular weight by comparing retention time of the unknown to retention time for known amino acids showed the contents of the peak to be low molecular weight, in the vicinity of 130.

Both of the ^{15}N containing peaks were tested for inhibition of clostridium botulinum and were found to be negative.

I hope that the next time I see the members of this audience I can tell about the components contained in the peak, and that moreover the results will in some way provide us with useful information about the fate of nitrite in cured meat.

I mentioned earlier the fact that nitrite can react with sulfhydryl groups. This has been discussed previously in detail by Mirna and Hofmann (1969). We have done some work using myosin as an experimental system (Kubberød, et al. 1974).

We found that the reaction between nitrite and the sulfhydryl groups of the myo-

sin was strongly dependent upon the following conditions: (1) nitrite concentration, (2) pH and (3) temperature. To summarize, we found that by applying high nitrite concentrations, low pH and high temperature it was possible to have all the sulfhydryl groups reacted within a few minutes.

Under conditions more similar to those found in meat however, the reaction rate was much lower. Some results in this regard are shown in slide 3. In these experiments we had about 10 times as high nitrite concentration as would be found initially in a meat product. The pH was 5.0 which is somewhat lower than the 5.5 - 6.0 range usually found in meat products. The system also underwent an incubation for 1 hr at 100°C which is a strong heat treatment. In this system less than 10% of the sulfhydryl groups reacted during the incubation.

The loss of nitrite in these experiments was nearly equimolar to the sulfhydryl loss when the samples were incubated under anaerobic conditions. We interpret these results as an indication that the main reaction between nitrite and protein is with the sulfhydryl groups, and nitrosothiols are possibly formed.

The presence of any nitrosothiols can be determined using the fact that they are split by heavy metal ions. Nitrite is reformed during the reaction and can be measured before and after splitting of the nitrosothiols with mecuric ions and the difference between the two determinations taken as the nitrosothiols.

By applying this method we found that 20% of the nitrite loss was bound as nitrosothiols. Nitrosothiols are known to be unstable compounds. It is therefore possible that all of the nitrite will bind with the sulfhydryl groups into nitrosothiols initially. The nitrosothiols may then be partly broken down during subsequent heat treatment. Nitrite itself is an oxidizing agent of intermediate strength and whether disulfides or other oxidation products are formed from the breakdown of nitrosothiols in proteins has still to be shown.

From our results we concluded that the rate of reaction between nitrite and sulfhydryl groups in myosin in this model system was low under conditions similar to

those found in a meat product. The sulfhydryl groups of the myosin fraction of meat are therefore assumed to be responsible for only a small proportion of the total nitrite lost in a cured meat product.

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