

Associated Bacteriological and Chemical Changes in Meat Curing Brines

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INTRODUCTION

The curing of meats with salt, sugar, and nitrates has long been a part of our gastronomic culture and certainly antedates modern technology. The process consists briefly of two stages: first, treating the meat with a mixture of salt, sugar, and sodium or potassium nitrate, either dry or in solution; and second, smoking the meat by suspending it for a time in a warm smoky atmosphere. This process has the effect of rendering the meat less susceptible to spoilage and also confers on it a characteristic and desirable colour and flavour. Like many other ancient arts, meat curing has been re-examined by modern science and has consequently been improved, and its applications and techniques multiplied to conform to the many requirements of our complex civilization. Tanner (1944) and Jensen (1954) have reviewed much of the collected knowledge of the chemistry and bacteriology involved in meat curing. Dunker & Hankins (1951*a*, 1951*b*) and Fields & Dunker (1952) have reviewed and summarized the many diverse types of cures employed in farm, locker plant, and commercial practice in the United States.

Of the various curing methods in use, the so-called long brine cures, because of their similarity to other food fermentations, are of particular interest to the microbiologist. Also this is one of the curing methods recommended by the United States Department of Agriculture for use by farmers (Warner, 1949). It consists of the immersion of meat in a vat or barrel of cover pickle. The meat is left in this solution and held at a temperature of approximately 3° for various lengths of time depending on the particular cut being cured. The usual recommendation for hams has been 4 days per pound (Warner, 1949). During this time the meat must absorb from the brine enough salt to be bacteriostatic during later storage, and take on the characteristic flavour and colour associated with cured meat. The chemistry of colour fixation has been well reviewed (Jensen, 1954), and is understood to begin with the reduction by microorganisms of the nitrate ion, originally present in the brine, to the nitrite form.

This paper is a report of investigations that were undertaken at Beltsville in the hope of arriving at a better understanding of the changes occurring in the brine during long cures. This work is of a somewhat preliminary nature and it is hoped that it will be continued and eventually throw some light on such obscure problems as flavour development. Work reported here deals with changes in pH, acidity, salt and nitrite content, and microbial flora of curing brines.

METHODS

Curing brines were prepared with the 8-2-2 formula recommended for farm cures (Warner, 1949). The brines were prepared 24 hours before use, adjusted to the proper salinometer reading, and left in the curing room to allow for temperature adjustment. In preparing the brines, commercial brown sugar, C.P. potassium nitrate, and fine common salt (Federal Specification No. SS-S-31, Type A) were employed. Curing was carried out in wooden barrels that had been previously used for meat curing and, in one case, in a large wood vat that had been in use for many years. In each instance the wooden containers were scrubbed with hot soap solution, rinsed in hot water, and left to soak in cold water for a few days prior to use. This was not done because of any preconceived idea that it might influence microbial flora but merely because it is standard practice in our laboratory. It is noted, however, so that any effect it may have had on the original microbial inoculation should not be lost sight of.

Since completing the studies reported in this paper, two stainless steel curing vats have been put into use in our laboratory. Such vats are now coming into rather general use in American packing houses and represent an interesting departure from the wooden vats formerly used. The steel vats will carry over only a small microbial load from one cure to another; and the effect of this new practice on chemical changes in the brine and the microbial ecology will be interesting to observe.

The meat used in all instances came from animals of known breeding and feeding history that were slaughtered in connection with other work of the Meat Section.

Samples were collected at various intervals by pipetting about 100 ml of brine from the curing vat into a sterile flask. An effort was made to obtain a sample composed of portions of brine collected equally from the surface and centre of the curing vat. As soon as the sample reached the laboratory—about 10 minutes from the time of collection—the necessary portion for bacteriological plating was removed, dilutions were prepared, and plates were poured. Nitrites, pH, and titratable acidity were determined immediately. About 25 ml of the sample were placed in a 50 ml stoppered Erlenmeyer flask with 0.5 ml of 5% phenol as a preservative, and retained for the determination of sodium chloride.

Nitrites were determined by the method of White (1939). Chlorides were determined by the titrimetric method of Clark (1950*a*, 1950*b*), except that the mercuric nitrate solution was prepared so as to have an NaCl titre of 2.5 mg/ml. Acidity was determined by titrating with 0.1N NaOH solution and phenolphthalein indicator, and pH was measured with a glass electrode in a Beckman pH meter.

Total aerobic counts were made by diluting the brine in standard dilution bottles using sterile 5% NaCl solution as a diluent and then plating in the usual manner. The plating medium was prepared by suspending the specified quantity of Difco veal infusion medium in 5% NaCl solution and adding 1.5% agar as a solidifying agent. Plates were incubated at 20°.

In pure culture studies on brine organisms, which would frequently not grow on the usual laboratory media of low salt content, the following medium, referred to as synthetic brine, was used:

NaCl	100 g
Commercial brown sugar	25 g
KNO ₃	3 g
Beef extract	3 g
Peptone	3 g
MgSO ₄	0.1 g
CaCl ₂	0.1 g
Soluble starch	0.1 g
Water to make	1 litre
pH adjusted to 6.8-6.9	

RESULTS AND DISCUSSION

The results of analytical studies on brine used to cure a batch of hams weighing 419 pounds are shown graphically in Fig. 1. The initially high pH of the brine, 8.2, was due to carbonates added to the salt as drying agents. This rapidly fell to below 7 and then changed only slightly, staying at a level of about 6.7 until the 25th day in cure and thereafter at about 6.5 until after the 53rd day. The titratable acidity followed a course quite similar to the pH but, in general, showed a gradual increase in acidity during the periods when the pH did not change. This probably indicates that there was enough dissolved protein to exert a buffering action on the brine. The sodium chloride content decreased during the time in cure and the rate of decrease is a good measure of salt absorption by the hams. The very rapid absorption during the first 20 days and the slow absorption thereafter are noteworthy.

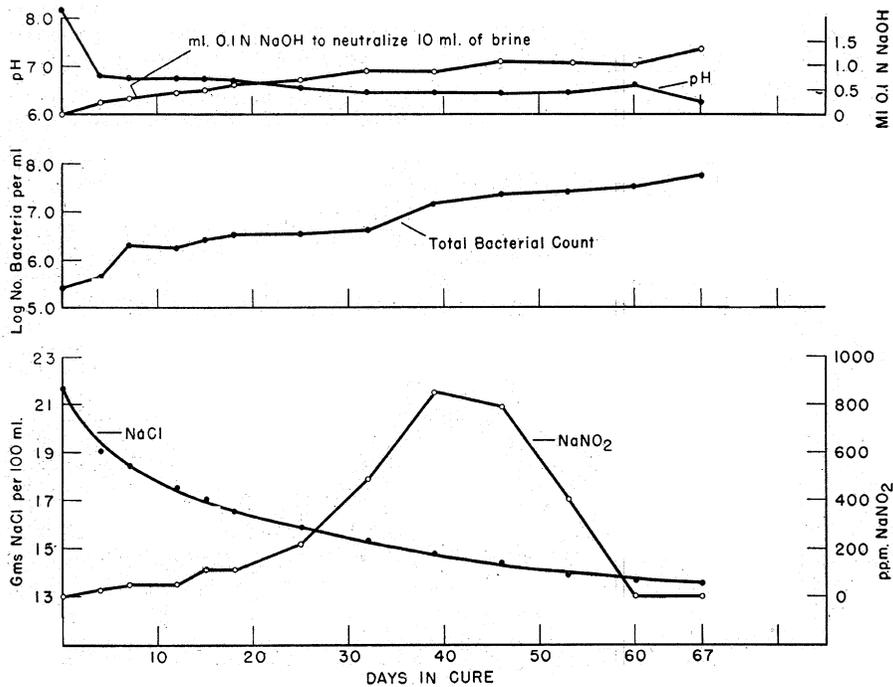


FIG. 1 Analysis of ham curing brine cured 3.5 days per lb. 8-2-2 brine

The total bacterial count as determined on salt agar rose continually but somewhat erratically throughout the curing period. Counts also were made of acid-forming organisms on nutritive caseinate agar, but results obtained seemed completely unrelated to time in cure, pH, total acidity, or total count; an exception is that a high count ($2.5 \times 10^5/\text{ml}$) was observed on the 53rd and 60th day in cure. Counts on acidified dextrose agar were low, from 10^3 to $6 \times 10^3/\text{ml}$, and varied within that range from sample to sample, but the variations bore no relationship to other factors such as time in cure or acidity. The author assumes that in this case the yeast and mould flora remained constant, varying only with irregularities of sampling.

The nitrite content of the brine depicted in Fig. 1 showed only a very small increase until after the 25th day in cure, when it rose rapidly to its maximum on the 39th day, then decreased even more rapidly than it had increased. Thus there was only a comparatively short period of the long curing time when nitrite ion was actually available for colour fixation in the meat. Furthermore, the reduction of nitrates to nitrites was greatest after the rate of salt absorption began to slow down. It is possible that there may have been such rapid absorption and fixation of nitrites during the first part of the cure that the nitrite content of the brine was kept low. This possibility was not investigated chemically in the course of the studies reported here, but appears unlikely because most of the active nitrate-reducing bacteria found in the brine were isolated from samples that showed a high nitrite content.

Results discussed above and the indications of other studies carried on concurrently with these but not reported here suggested that during the latter part of the curing period little actual change took place in the meat. Another batch of hams was cured in the same manner as those just discussed, except that they were left in cure for a time equal to three days per pound of meat. The hams averaged 15 pounds and were left in cure for 46 days. The results are shown in Fig. 2, which is generally similar to Fig. 1. The explanation for the higher-than-usual initial pH is not known. There appears to have been a rather marked increase in bacterial count that began just before the increase in nitrite content; it is interesting to conjecture that this might have been responsible for a somewhat earlier maximum nitrite content than occurred in the batch represented on Fig. 1. The hams cured in the brine summarized by Fig. 2, when evaluated organoleptically, were judged to have desirable aroma and flavour of both lean and fat; they were given an over-all rating of good by the taste panel. The colour fixation was normal—a point of interest, since the hams were removed from cure before the nitrite content had fallen off completely. The success of this and subsequent batches of ham indicates that three days per pound is an ample time for ham curing by this method.

Many other meat products beside hams are, of course, cured. Our laboratory has been particularly interested in the development of cured lamb legs and, since these are cured in a manner similar to hams but in somewhat milder brine, a comparison with ham curing is interesting. Fig. 3 shows a summary of the analytical results of a study of brine used to cure a batch of lamb legs. The general picture is quite similar to that of the two foregoing figures. The more regular increase seen in the bacterial counts is no doubt due to the longer interval between samplings rather than to a closer approximation to true logarithmic growth. The rise in pH during the latter part of the curing period was accompanied by a heavy mould growth on the surface of the curing brine and probably indicates a change in the acids present.

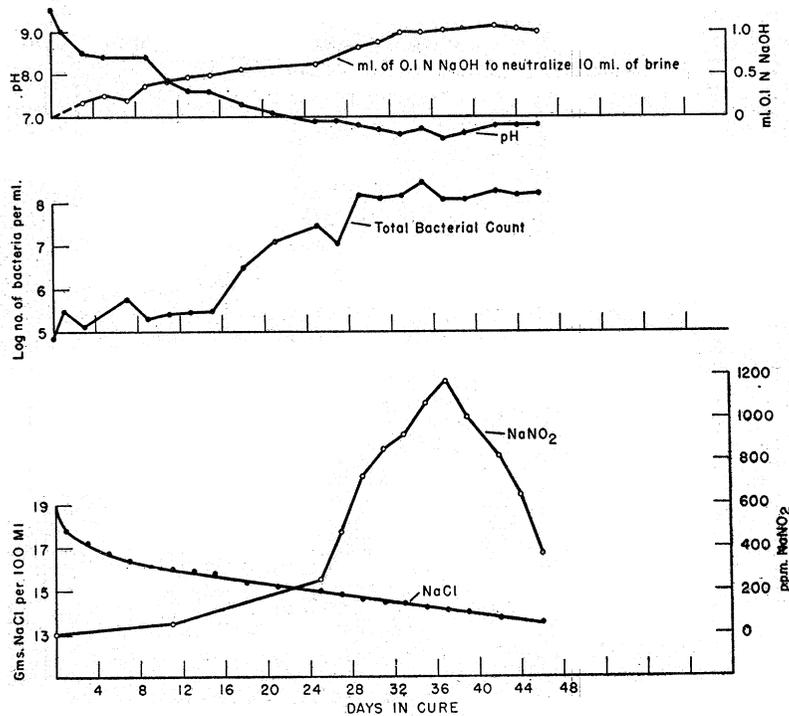


FIG. 2 Analysis of ham curing brine cured 3 days per lb. 8-2-2 brine

Figs 1, 2 and 3, discussed above, were chosen as typical examples of the changes occurring and represent, in each case, results obtained from a single curing run. Data from other runs were in all cases similar and would present the same general picture.

Little is to be found in the literature on the type of micro-organisms that might play a part in the chemical changes indicated above. Sturges & Heideman (1924) proposed a separation of meat-curing brine flora based on sodium chloride tolerance. Tanner & Evans (1933) and Evans & Tanner (1934) studied the effect of curing brines on anaerobes of public health significance. In the course of our study, organisms occurring in predominant numbers on the plates were transferred to the synthetic brine medium described above. This medium permitted a rapid separation of the organisms according to four categories; that is, those that did or did not produce acid in brine and those that did or did not reduce nitrates to nitrites.

As might be expected from the curing conditions and techniques of isolation, all the organisms reserved for study were salt-tolerant and psychrophilic. Only 5% of the bacterial isolates were cocci and, since we are particularly interested in Gram-negative psychrophiles in our laboratory, additional study of cultural characteristics has been restricted to the Gram-negatives. Later studies will be directed to both Gram-negative and Gram-positive types.

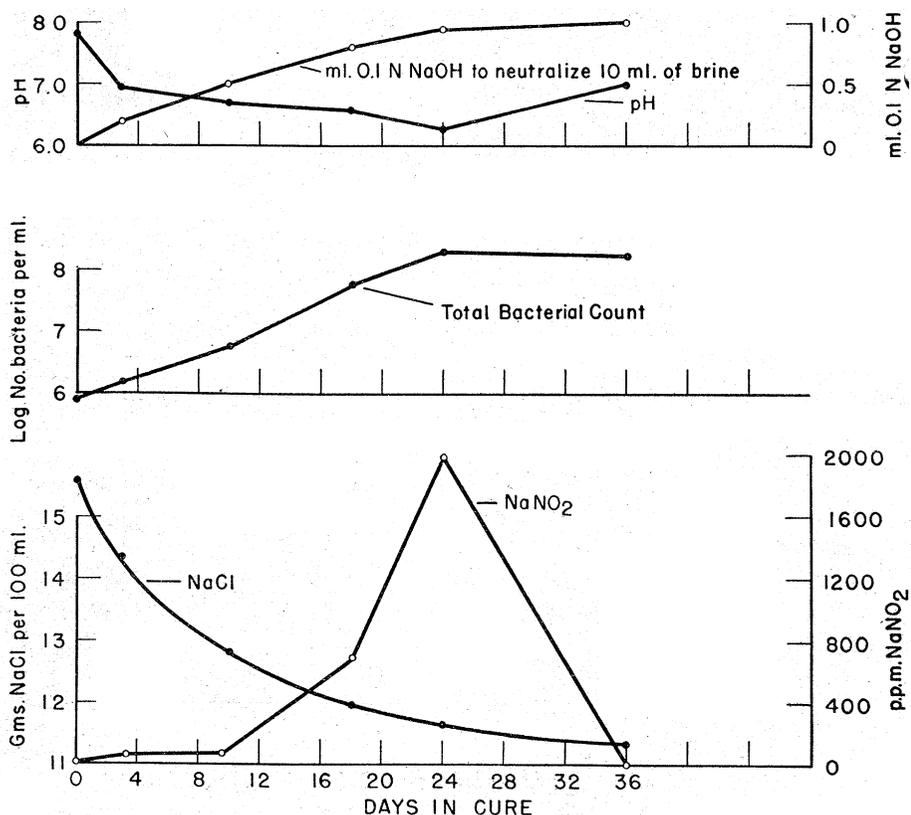


FIG. 3 Analysis of lamb curing brine

When freshly isolated, and while grown on media containing 5% NaCl, all but a very few (about 1%) of the Gram-negative organisms were motile. When grown with 0.5% NaCl, however, only 41% of the isolates studied retained their motility. Similarly, of 106 isolates which reduced nitrates in the presence of 5% NaCl, only 25% retained this important faculty in its absence.

On the basis of gelatin liquefaction and action on carbohydrates, the Gram-negative, motile, nitrate-reducing brine isolates were divided into the following groups:

Group I. Gelatin liquefied, acid produced from dextrose, sucrose, glycerol, and mannitol, and sometimes from maltose. Do not attack lactose, salicin, or arabinose. Frequently form soluble brown or black pigment on old salt agar slants. (About 60% of our isolates fall into this group.)

Group II. Gelatin liquefied, do not produce acid from carbohydrates, pigment not formed.

Group III. Gelatin not liquefied, acid produced from dextrose; may or may not ferment sucrose, maltose, glycerol, lactose, mannitol, and arabinose with production of acid. Some members of this group produce soluble pigment on old salt agar slants.

Group IV. Gelatin not liquefied, do not produce acid from carbohydrates, pigment not formed.

All members of these groups were indole, methyl red, and Voges-Proskauer negative, and did not grow on Koser's citrate. None produced urease.

The taxonomic position of these organisms is uncertain. The marked pigment production of many members of Groups I and III, together with their action on carbohydrates, seems to suggest that they may be members of the *Enterobacteriaceae*. However, they hardly conform to any existing genus and are probably transitional between the *Achromobacteriaceae* and the *Enterobacteriaceae*. Groups II and IV are probably composed of species of *Achromobacter*, although no described species agree with their characteristics. For the time being questions of nomenclature must be postponed until they may be resolved by further study.

Organisms that were repeatedly observed in heavy concentration during the latter stages of curing are particularly interesting. They produced an alkaline reaction and gas in synthetic brine and did not hydrolyze carbohydrates. Most of them did not give a positive test for nitrites but further investigation showed that the gas produced was nitrogen from the complete reduction of nitrates. These organisms, which utilize nitrates quite rapidly, are no doubt responsible for the disappearance of nitrites from the brine because they remove the nitrate substrate that is slowly reduced by other organisms. In the synthetic medium a ham-like aroma was produced. These organisms fall in Group IV above and probably belong to the genus *Achromobacter*. They differed from previously described forms such as *Bacillus halobicus* Horowitz-Wlassowa and others (Tanner, 1944) in that the Beltsville isolates are capable of growing in media containing only 0.5% NaCl. Also McLean (1956) observed that the Beltsville isolates are osmophiles rather than halophiles since they grew well in high glucose concentrations without salt.

The marked salt tolerance of these organisms makes it most unlikely that they are normal inhabitants of swine skin, although other types of *Achromobacter* have been shown to be prevalent on fresh pork trimmings (Sulzbacher & McLean, 1951). The salt used in these cures had extremely low counts, and can almost be considered sterile. The wooden curing vats, on the other hand, carry a large load of micro-organisms from cure to cure and are no doubt the immediate source of the organisms described above.

CONCLUSIONS

Analyses of chemical changes occurring in the brine during long cures indicated that hams could be removed from the pickle after a period equal to three days per pound of meat in each ham.

The motile Gram-negative nitrate-reducing bacteria of brine can be divided into four groups on the basis of gelatin liquefaction and carbohydrate fermentation.

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REFERENCES

- CLARKE, F. E. (1950a). *Analyt. Chem.*, **22**, 553.
CLARKE, F. E. (1950b). *Analyt. Chem.*, **22**, 1458.
DUNKER, C. F. & HANKINS, O. G. (1951a). *A survey of farm meat curing methods*. Circular No. 894, United States Department of Agriculture.
DUNKER, C. F. & HANKINS, O. G. (1951b). *Food Tech., Champaign*, **5**, 293.
EVANS, F. L. & TANNER, F. W. (1934). *Zbl. Bakt.*, **91**, 135.
FIELDS, M. D. & DUNKER, C. F. (1952). *Food Tech., Champaign*, **6**, 329.
JENSEN, L. B. (1954). *Microbiology of meats*. 3rd Ed. Garrard Press, Champaign, Ill.
MCLEAN, R. A. (1956). United States Department of Agriculture unpublished report.
STURGES, W. S. & HEIDEMAN, A. G. (1924). *Abstr. Bact.*, **7**, 14.
SULZBACHER, W. L. & MCLEAN, R. A. (1951). *Food Tech., Champaign*, **5**, 7.
TANNER, F. W. & EVANS, F. L. (1933). *Zbl. Bakt.*, **88**, 44.
TANNER, F. W. (1944). *The microbiology of foods*. 2nd Ed. Garrard Press, Champaign, Ill.
WARNER, K. F. (1949). *Pork on the farm*. Farmers' Bulletin No. 1186, United States Department of Agriculture.
WHITE, W. H. (1939). *Canad. J. Res.*, **D17**, 125.

RÉSUMÉ

Ce travail traite des saumurages de longue durée à la température de 3° avec des saumures contenant initialement 20 p.100 de Chlorure de Sodium et 15 p.100 en fin d'opération.

Après une trentaine de jours, le nombre de bactéries revivifiables (cultivées sur gélose à l'infusion de veau contenant 5% de ClNa et incubées à 20°) s'élève à près de 10⁸ par 1 ml et le taux de nitrites atteint à peu près 1000 parties par million. Plus tard on constate une diminution des nitrites; la saumure tend alors à devenir moins acide. Ces modifications surviennent lors de l'apparition de bactéries détruisant les nitrites.

Les bactéries isolées sont toutes halotolérantes et psychrophiles. Les *Micrococci* sont peu abondants. Les bactéries en forme de batonnets se répartissent en 4 groupes: 2 sont vraisemblablement intermédiaires entre les *Enterobacteriaceae* et les *Achromobacteriaceae*; les deux autres sont des espèces probablement nouvelles d'*Achromobacter*. Les bactéries détruisant les nitrites se trouvent parmi les secondés. Le fait que ces bactéries sont halotolérantes indique qu'elles proviennent plutôt du matériel que de l'animal.

L'auteur conclut qu'un saumurage de 4 jours par livre de viande est inutilement long car on atteint ainsi la phase de destruction des nitrites. 3 jours par livre de viande suffisent.

ZUSAMMENFASSUNG

Die Untersucher befassen sich mit Pökellaken mit einem Kochsalzgehalt von anfänglich 20% und einem Endkochsalzgehalt von 15% bei Temperaturen von 3°. In etwa 30 Tagen steigt die Zahl der Bakterien (nachgewiesen auf Kalbfleischagar mit 5% Kochsalz bei 20°) auf etwa 10⁸/ml und der Nitritgehalt auf etwa 0.1%. Später kommt es zu einem erheblichen Verlust von Nitrit und die Pökellake wird weniger sauer. Diese Vorgänge gehen einher mit dem

Auftreten zahlreicher nitritzerstörender Bakterien. Die isolierten Bakterien sind alle salztolerant und psychrophil, Mikrokokken treten in geringer Zahl auf. Die Stäbchen zerfallen in 4 Gruppen, 2 scheinen intermediär zwischen *Enterobacteriaceae* und *Achromobacteriaceae* zu sein, 2 sind wahrscheinlich neuere Arten von *Achromobacter*, wobei die nitritzerstörenden Stämme zu den letzteren zählten. Die Salztoleranz der Bakterien lässt vermuten, dass sie eher aus der Umgebung stammen als von dem Tierkörper. Die Pökeldauer von 4 Tagen pro lb Fleisch ist unnötig lange, da es in der Lake zu einer Nitritzerstörung kommt und 3 Tage ausreichen.

DISCUSSION

MR HODGKISS. There is no mention in this paper of the flagella of the Gram-negative rods. It is obvious that determination of the position of the flagella, together with biochemical characters, would help to determine the true taxonomic position of these organisms.—*Our laboratory data are not complete with regard to flagellation. However, representative organisms of each group which we have stained showed peritrichous flagella. It is for this reason that we lean toward the Achromobacters.*¹

DR LISTON. We would suggest that groups 1 and 2 should be put in the genus *Pseudomonas*. They are almost identical with strains isolated from fresh fish which seem to be similar to *Ps. nigrificiens*.—*We would hesitate to place these organisms in the genus Pseudomonas in view of the yet incomplete data on flagellation.*¹

MR HORNSEY. With reference to the reported occurrence of surface mould on the brine at one stage, we have sometimes observed this to occur on a still (undisturbed) brine, when the atmospheric conditions are warm and humid, and a very thin layer of water is deposited on the cold surface of the brine. Being lighter it remains for a while as a layer, permitting the growth of airborne moulds.—*This is an interesting observation and may be pertinent. Our curing is done in refrigerated rooms and it is quite possible that if the door is left open for a time in warm weather condensation might occur as described on the brine surface. It is also true, however, that mould contaminations are not uncommon in our refrigerators and we are well supplied with salt-tolerant moulds growing on stored cured hams. I doubt if we need postulate any mechanism beyond their prevalence in the atmosphere to account for their occurrence on brines.*¹