INTERNAL BACTERIAL TAINTS ('BONE TAINT' OR 'SOURING') OF CURED PORK LEGS

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(1) INTRODUCTION

The bacteriology of ham souring has been comprehensively reviewed, in relation to American packing-house practice, by Jensen (1944, 1945). Observations accumulated over many years at the Low Temperature Station suggest that Jensen's conclusions may not be wholly applicable in Britain and, though covering an amount of material much smaller than that available to the American workers, they suffice to suggest some new points of view. There is, in fact, still very little published information about the bacteria normally found in such products, a situation recently deplored both by Mundt & Kitchen (1951) and by Ulrich & Halvorson (1951). Moreover, in the past few years, British work has revealed influences (reviewed briefly by Ingram, 1948) which Jensen did not consider. Hence it seemed worth while to give a connected account of our observations in this paper, which surveys the species we have isolated.

A distinction will be made between 'bacon gammons' and 'hams': the former term being used for legs of pork into which considerable proportions (5 % or more by weight) of brine have been injected during curing (by pumping through a long hollow needle); the latter term denoting similar material which has been injected only lightly, or not at all. It is characteristic of present-day tank-cured bacon that it is always thoroughly injected with brine to hasten the cure; whereas in the traditional British cure of hams, still used in home-curing (Ministry of Agriculture and Fisheries, 1945) and approached in some commercial cures, salts were applied only to the outside and nothing was injected into the interior, and the same procedure is still used in the Southern 'country-style' hams of the U.S.A. (Mundt & Kitchen, 1951). Distinction on this basis is admittedly rather arbitrary, in that some hams are nowadays injected while an inefficiently injected 'gammon' would approach the state of a 'ham', and there are additional minor differences between the curing of hams and that of gammons. Nevertheless, the choice of terms has a reasonable basis, and it will be useful in emphasizing the point of difference which is fundamental to the present theme.

(2) METHODS

The problems of obtaining deep bacteriological samples from leg joints, without introducing unwanted contamination from surface organisms, were discussed by Haines (1941), and similar procedures were used in this work. A flat surface for searing was prepared, by cutting through the leg with roughly sterile instruments, after the gross bacterial load on the external surfaces had been removed by washing

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and disinfection with Lysol. The cut passed perpendicular to the femur, just above the stifle-joint, so as to expose the marrow of the femur and the head of the gastrocnemius muscle, from which 5 g. samples were dissected aseptically, after searing. This muscle was chosen because it has a relatively high pH (Table 1), and it always becomes involved when tainting of the flesh occurs.

Table 1. pH *of several different muscles in sound hams*

Ham	Gastrocnemius	Semitendinosus	Sartorius	Gracilis
1	6·16	6·01	5·92	5·58
2	6·16	5·95	6·18	5·85
3	6·64	6·33	6·50	6·26
4	6·23	6·23	6·20	5·85
5	6·06	6·33	6·02	5·75
6	6·13	6·02	5·99	5·77
Average	6·23	6·15	6·13	5·84

After removal of the bacteriological sample, further portions of the muscle were taken for the following analyses:

(i) The salt content was determined by electrometric titration of the chloride in a hot aqueous extract (Ingram & Hawthorne, 1945); but, as the effective factor is the concentration of NaCl in aqueous solution in the meat (Oxhøj, 1943; Hankins, Sulzbacher, Kauffman & Mayo, 1950), it is expressed as the percentage of the water content.

(ii) The water content of meat was determined by drying a minced sample for 96 hr. at 103° C. (values ranged from 65 to 75 % of fresh weight).

(iii) The pH was found by snipping about 10 g. of muscle into 20 c.c. water, adding 0·5 ml. of $CHCl_3$, allowing to stand more than 2 hr. and measuring with a glass electrode at 15° C.: quantities and times were not critical. We have found (with psoas major muscles) that the gross pH of such samples is practically the same for different points in the muscle.

(3) OBSERVATIONS

(*a*) *The flora of hams*

Sound hams are apparently nearly sterile internally. Examination of ten examples failed to yield any viable bacteria either in the bone marrow or the muscle; after which it seemed unnecessary to continue.

In a tainted ham, in marked contrast, the spoiled regions contain enormous numbers of bacteria. Examination has shown that the typical flora consists of Clostridia and/or faecal Streptococci. Frequently this may be indicated simply by making a direct smear of the spoiled flesh and Gram staining, which usually reveals large Gram-positive rods and/or large ovoid Gram-positive diplococci. Gram-negative rods may be present, but they rarely predominate. Typical results of such direct examinations are given in Table 2. (Compare also Jensen, 1951.)

The types of bacteria isolated from tainted hams when examined, on various

occasions and from different sources, are listed in Table 3.* As a group, these bacteria possessed the following characters:

(*a*) They were predominantly mesophilic and did not grow well at low temperatures. More than 80 % of them had an optimum temperature of 37° C.

(*b*) They were highly susceptible to salt. Less than 10 % of the isolates withstood 10 % NaCl, and nearly 50 % of them (though growing well at lower concentrations) failed to tolerate even 5 % NaCl in peptone water.

(*c*) They were unable to reduce nitrate. Several of the species indeed, can be inhibited readily by nitrate (cf. Jensen, 1945, p. 14 *et seq.*).

(*d*) They were unfavourably affected by moderately acid conditions (pH 5·5). This topic has been discussed elsewhere (Ingram, 1948).

(*e*) There was a high proportion of probably faecal types. Nearly half the isolations were plainly in this category and, of the remainder, a considerable number might have been so.

Table 2. *Approximate numbers of bacteria per g. in tainted ham, estimated from direct microscopic examination*

Example no.	Gram-positive rods		Gram-positive cocci			Gram-negative rods	Miscellaneous
	Spores	No spores	Ovoid diplococci	Streptococci	Micrococci		
1	10^8	–	10^7	–	–	10^5	–
2	–	–	10^7	10^8	–	–	–
3	–	–	10^6	10^9	–	–	–
4	–	–	10^6	–	–	–	–
5	–	–	–	10^9	–	10^6	–
6	10^8	.	10^8	–	–	+	–
	.	10^6	10^7	.	.	.	.
7	–	–	10^6	–	10^7	–	Yeast 10^7
8	–	10^8	10^8	–	–	–	–
9	–	10^7	10^8	+	–	+	–
	–	10^6	.	.	.	.	.
10	–	–	10^7	–	10^6	10^6	.

Accordingly, one would expect tainting by the above bacteria to develop only where there is a relatively low concentration of curing salts, and where the muscles are less acid. The data included in Table 3 appear to confirm this in several cases. Unfortunately, however, the exact significance of such data is open to doubt. The salt concentrations are difficult to interpret because in some cases the ham was not available for some weeks after curing, by which time there had been ample opportunity for equalization of the salt concentrations by diffusion from the more readily penetrable portions of the leg. The values quoted in Table 3 may thus be considerably higher than those obtaining during the critical initial stages of the cure, as indeed seems very probable in a case like ham B 2, where three organisms

* In this paper, the names are mostly those given in the 5th edition of *Bergey's Manual of Determinative Bacteriology* for species with the characters of the organism in question. In the later (6th) edition some of the groups, e.g. the micrococci, have been over-simplified for the purposes of this discussion.

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Table 3. *Species isolated from femur marrow and gastrocnemius muscle of tainted hams*

(Notes. (i) ^{m} indicates an isolation from marrow. (ii) A, B, etc. indicate different manufacturers. (iii) The order in which the isolations from each ham are listed is that of their numerical preponderance at isolation.)

Hams				Isolated organisms		Optimum temperature	Growth in peptone-water with NaCl	
Source	No.	pH	Percentage NaCl (water basis)	Origin probably faecal	Origin doubtful	(° C.)	5%	10%
A	1	6·68	5·3	*Bact. paracolon*^{m}	.	37	+	−
				.	*Lactobacillus* sp.^{m}	37	+	−
	2	6·68	6·8	.	*Cl. butyricum*	37	+	−
				.	*Kurthia* sp.	25	+	−
	3	6·20	4·2	*Strep. faecalis*	.	37	+	−
				.	*Achromob. candidans*	37	−	−
	4	7·04	5·0	.	*Pseudom. putida*	25	−	−
B	1	6·58	9·4	*Cl. putrificum*	.	37	+	−
				.	*B. megatherium*	30	+	−
				.	*Lactobacillus* sp.^{m}	37	+	−
				.	*Debaryomyces* sp.	25	−	
	2	6·52	7·5	*Cl. fissum*^{m}	.	37	−	−
				.	*Kurthia* sp.	30	−	−
				.	*Pseudom. ureae*	25	−	−
	3	6·78	8·1	*Strep. faecalis*^{m}	.	37	+	−
				Bact. paracolon^{m}	.	37	−	−
				.	*Pseudomonas* sp.	37	−	−
	4	7·31	7·6	*Cl. tertium*^{m}	.	37	−	−
				Strep. faecalis	.	37	+	−
				.	*Kurthia* sp.	37	+	−
				.	*Achromob. candicans*	37	−	−
				.	*Microc. ridleyi*	37	+	+

C	1	6·90	8·6	*Strep. faecalis*	.	37	+	−	−
				.	*Pseudom. putida*	37	−	−	−
	2	6·34	8·9	.	*Aerob. aerogenes*	37	−	−	−
	3	.	.	*Strep. faecalis*	.	37	+	−	−
				.	*Pseudom. fluorescens*	25	−	−	−
	4	.	.	*Esch. coli*	.	37	−	−	−
				.	*Microc. ochraceus*	25	+	+	+
D	1	6·35	6·2	*Cl. welchii*[m]	.	37	−	−	−
				.	*Prot. morgani*[m]	37	+	−	−
	2	6·45	6·6	*Cl. sporogenes*	.	37	+	−	−
				Strep. faecalis	.	37	+	−	−
				.	*Streptococcus* sp.	37	+	−	−
				.	*Micrococcus* sp.	25	+	+	+
E	1	6·55	3·5	*Cl. welchii*	.	37	+	−	−
	2	6·20	3·3	*Cl. oedematiens*	.	37	−	−	−
				.	*Streptococcus* sp.	37	−	−	−
F	1	6·16	3·5	*Cl. welchii*	.	37	+	−	−
				Cl. welchii	.	37	−	−	−
				Strep. faecalis	.	37	+	−	−
				.	*Strep. faecalis* atypical	37	+	−	−
				.	*Micrococcus* sp.	37	+	−	−
	2	6·30	5·3	.	*Cl. fallax*	37	−	−	−
				Strep. faecalis[m]	.	37	+	−	−
				.	*Streptococcus* sp.	37	−	−	−
	3	5·75	3·4	*Cl. welchii*	.	37	−	−	−
				Cl. welchii atypical	.	37	+	−	−
				Strep. faecalis	.	37	+	−	−
				.	*Strep. faecalis* atypical	37	+	−	−
	4	5·81	3·1	*Esch. coli*	.	37	−	−	−
				Strep. faecalis	.	37	+	−	−
				.	*Strep. faecalis* atypical	37	+	−	−
				.	*Klebsiella* sp.	37	−	−	−

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unable to resist even 5 % NaCl had grown in a muscle which contained 7·5 % NaCl when observed. This may account for the fact that the development of taint often seems to be arrested in a ham, because it is rare to find the muscles completely broken down, although strongly proteolytic species may be present. The meaning of some of the pH values is similarly questionable because, where taint is well advanced, the pH of the flesh may have been changed by the metabolic activity of the bacteria themselves. Most of the values, however, are consistent with those for unspoiled material given in Table 1, and the clear relations between acidity of the fillet (psoas) muscle and freedom from taint in the cured legs (Callow, 1937) suggest that low acidity must be a predisposing cause of taint.

In some of the cases set out in Table 3, bacteria were isolated from the femur marrow and not from the flesh. Although more observations would be desirable, there is no evidence yet of any systematic difference between the floras of the two sites. This is interesting in relation to the distinction made by Jensen & Hess (1941). Of the several types of ham taint they list (which, in fact, apparently differ more in location in the ham than in the nature of their floras) only a 'puffer' has been recognized. This contained numerous cells of *Clostridium welchii*, which produced gas, giving a spongy texture to the muscles analogous to that seen in putrefying whalemeat (Ingram & Hauge, 1949).

There are several point of interest about the species recorded in Table 3:

(1) The nature of the Clostridia, etc. Several species isolated from hams have been regarded as specific causative agents of taints: *Bacillus foedans* (Klein, 1908), regarded by Jensen as perhaps identical with *B.* (*Bacteroides*) *putidus* Weinberg, *Cl. putrefaciens* (McBryde, 1911), *Cl. putrificum* (Tucker, 1929), *Cl. sporogenes* (Moran, 1929). However, Boyer (1926) found *Cl. histolyticum*, *Cl. novyi*, *Cl. putrefaciens*, *Cl. sporogenes* and *Cl. tertium*; and Jensen & Hess (1941) likewise found a diversity of types, although they observed *Cl. putrefaciens* to be the most common. A variety of species, including some of the above, has since been found by Mundt & Kitchen (1951) in uninjected country-style hams in the U.S.A.: *Cl. bifermentans*, *Cl. mucosum*, *Cl. parabifermentans*, *Cl. paraputrificum*, *Cl. putrefaciens*, *Cl. septicum* and *Cl. sporogenes*. Meyer & Gunnison (1929) mention two occurrences of *Cl. botulinum*. Together with Table 3, this suggests the broadest view, for it seems that any of a wide range of faecal Clostridia may be involved. The range of species corresponds indeed quite well with that recorded from the gut of sheep (Watts, 1938)—there seem to be no comparable records for the pig: *Cl. bifermentans*, *Cl. multifermentans*, *Cl. putrificum*, *Cl. sporogenes*, *Cl. tetanomorphum*, *Cl. tertium* and *Cl. welchii* occurred frequently; and in addition *Cl. histolyticum*, *Cl. novyi*, *Cl. septicum*, *Cl. sphenoides*, and *Cl. tetani* were found occasionally. Our observations indicate that one of the frequently occurring species in hams here is *Cl. butyricum*, a view strengthened by the experience that this has been the only anaerobe isolated regularly from pasteurized (170–190° F.) canned hams (private communication, Mrs R. Mullaney, Food Control Laboratory, Royal Veterinary College of Ireland, Dublin), and it is noteworthy that this species has been found in abundance in the caecum of pigs fed on starchy diets (Baker, Nasr, Morrice & Bruce, 1950).

Jensen (1945, pp. 130–1) suggests that *B. megatherium* and *B. mycoides* are characteristic of taints of stifle-joint and tibia-marrow respectively. These species and similar types have been isolated here, from the flesh, and there was no indication that they had any special significance.

(2) The proportion of Streptococci was remarkably high—about one-third of all the strains isolated. Roughly half of them were of strictly faecal type (implying the possession of all the following characters: ovoid cells occurring in pairs, heat-resistance, no haemolysis, resistance to pH 4·2, to bile and to 6·5% NaCl, ability to hydrolyse aesculin, and to grow at 10 and 45° C.). Of the remainder, most possessed some, often several, of these properties, together with others held to be characteristic of enterococci (cf. Table 4), and it is difficult to draw a distinct line between the faecal and non-faecal types. The lack of ability to ferment raffinose argues against salivary origin. A few of the strains were rather strictly anaerobic.

It is interesting that we have so far not encountered the H_2S-producing streptobacilli, described by Jensen & Hess as characteristic of flesh sours, which possessed some of the characters of *Streptococcus viridans*. A *Lactobacillus* was isolated, similar to those reported by Niven, Castellani & Allanson (1949) from discoloured sausages.

(3) Gram-positive non-sporing rods occurred with considerable frequency, comprising about 10% of the strains represented in Table 3. They possessed the general characters of the genera *Achromobacter* or *Pseudomonas* but were obstinately Gram-positive. Sulzbacher & McLean (1951) have also found that Gram-positive rods compose about 15% of the flora of pork sausages. Unlike the Gram-negative rods, such bacteria receive scant attention in current bacteriology, though their archetype *Kurthia zopfii* has been known for many years. It seems possible that Gram-positive non-sporing rods may be relatively common outside pathology, for recently it has also been found that they sometimes dominate the flora of soil (after steaming and exposure to field conditions—private communication, Mr Ernest Gray, N.A.A.S., Trumpington).

A characteristic of the bacterial flora of tainted hams is its limited distribution, which is frequently obvious macroscopically from the colour, texture, smell, and taste of the flesh at different places. This is confirmed by bacteriological examination. The bacteria are never present throughout the ham; usually they are numerous only in a few muscles near the bone. This may be related to the relatively low acidity of these muscles (cf. Table 1) which (Callow, 1936), with their deep-seated position, makes penetration of salt slow, and to the fact that they remain warm longest. Also it seems possible that infection may occur from the bone outwards, because bacteria were isolated only from the marrow in some of the hams examined, as indicated in Table 3; and observations in the Westfield Freezing Works (Auckland, New Zealand) have suggested that the analogous bone taints in beef begin in the blood vessels of the femur marrow, along which they can extend into adjacent muscles (private communication, Mr P. Parr).

Table 4. *Characters of Streptococci isolated from tainted hams*

(*Notes.* (i) u = not determined. (ii) O indicates no reaction with Burroughs Wellcome sera A, B, C, D, E, F, G and H.)

	Enterococcal form		Growth in presence of					Fermentation of						Lance-field sero-logical		Growth at	
Strain	Oval	Pairs	Bile salts	Methy-lene blue 0·1 %	pH 4·2	pH 9·5	NaCl 6 %	Raffinose (i)	Aesculin	Mannitol	Sorbitol	Glycerol (i)	Reaction to blood	group (i), (ii)	Resists 30 min. at 60° C.	10° C.	45° C.
1	+	−	−	−	+	−	−	u	+	−	−	u	γ	u	+	+	−
2	+	+	+	+	−	+	+	u	+	−	−	u	γ	u	+	+	+
3	+	+	+	−	+	−	−	u	+	−	+	u	γ	u	+	+	−
4	+	−	+	+	+	+	+	u	+	−	+	u	γ	u	+	+	−
5	+	−	+	+	+	−	−	u	+	−	+	u	γ	u	+	+	−
6	+	+	+	+	+	−	+	u	+	−	−	u	γ	u	+	+	−
7	+	+	+	+	+	+	+	u	−	−	−	u	γ	u	+	+	+
8	+	+	+	−	−	−	−	u	−	−	−	u	γ	u	+	+	−
9	+	+	+	+	+	u	−	−	+	+	−	−	γ	D	+	+	+
10	−	+	+	−	+	u	+	−	+	+	−	−	γ	O	+	+	+
11	+	+	+	−	+	u	+	−	+	+	−	−	γ	O	+	+	+
12	+	+	+	−	+	u	+	−	+	+	+	+	γ	D	+	+	+
13	+	+	+	−	+	u	+	−	+	+	−	+	γ	D	+	+	+
14	+	+	+	+	+	u	−	−	+	−	−	−	γ	O	−	+	+
15	+	+	+	+	+	u	+	−	+	+	+	−	γ	O	+	+	+
16	+	+	u	u	+	+	+	u	u	+	u	+	γ	u	+	+	u
17	+	+	u	u	−	+	+	u	u	+	+	+	γ	u	−	−	+
18	+	+	u	u	−	+	+	u	u	−	u	+	γ	u	−	+	+
19	+	+	u	u	−	+	+	u	u	u	u	+	γ	u	−	−	+

(*b*) *The flora of injected material* ('*bacon gammons*')

In typical cases the flora of gammons differs markedly from that of hams, as is evident from Table 5, because of the predominance of a group of bacteria with the following characteristics:

(1) Optimum temperature of 25° instead of 37° C.

(2) They tolerate relatively high concentrations of NaCl, at least considerably greater than that likely to be found in the bacon itself.

(3) They reduce nitrate.

(4) They are mainly Micrococci.

(5) Several species may occur together.

(6) Many do not correspond closely with well-recognized species.

The presence of this group of bacteria gives a salt-tolerant psychrophilic character to the flora of gammons, which is generally absent from that of tainted hams. The following additional points of interest may be noted:

(i) There were several isolates which, while possessing characters of species typical of quite different habitats (e.g. Streptococci, *Alkaligenes bookeri*, faeces; *Pseudomonas* spp., soil and water; *Achromobacter iophagum*, soil), nevertheless exhibited salt-tolerance characteristic of brine species. It has often been suggested that halophilic bacteria are merely saprophytes which have adapted themselves to tolerate salt (Hof, 1935; Le Fevre & Round, 1919).

(ii) Salt-tolerant strains were isolated, belonging to two species of *Pseudomonas* which are capable, given appropriate conditions, of producing blue colours. They may perhaps be related to the 'marking-ink' discolorations which are not uncommon on cured meats where considerable numbers of bacteria have been allowed to develop (compare also Hof, 1935).

(iii) Only one halophilic *Vibrio*, not very similar to those which caused rib-taints of bacon in Australia (Smith, 1938), has ever been noted. Such forms are certainly not common in material like that considered here.

(iv) Several strains, allocated to particular species on the basis of other characters, and which would correspondingly not be expected to reduce nitrate, nevertheless did so as indicated in Table 5. This was true for Sarcinae and species of *Pseudomonas*, as well as Micrococci. Such behaviour has previously been noted here in strains isolated from curing brines. It remains to be decided whether strains of this kind are distinct varieties or species, or whether (as seems possible, cf. Pollock, 1946) the ability to reduce nitrate may be acquired by adaptation.

(v) Representatives of an element of the flora similar to that dominant in hams were isolated, besides the halophiles, in the majority of cases: among the faecal group were Clostridia, faecal Streptococci, and coliforms; and among the isolations of unknown origin also, there were types analogous to those found in hams: e.g. non-faecal Streptococci, a Gram-positive non-sporing rod, and a species of *Bacteroides* of interest for its resemblance to *Bacillus putidus* Weinberg. This element plays an insignificant part when curing has been properly carried out, because rapid cooling, thorough penetration of salt, and smoking (Jensen, 1951)

Table 5. *Species isolated from tainted bacon gammons*

(Notes. (i) The examples have been collected into a more typical (*a*) and a less typical group (*b*); (*c*) exhibits the characters of both. Either group includes samples from different sources examined at different times. (ii) The order in which the isolations from each gammon are listed is that of their numerical preponderance on isolation. (iii) The symbol (−) indicates a strain which, according to the rest of its characters, ought not to reduce nitrate.)

Gammons			Organisms isolated			Optimum temperature	Growth in peptone-water with NaCl		Nitrate reduction
No. (i)	pH (ii)	NaCl (%) water	Origin probably faecal	Origin doubtful	Origin perhaps curing brine etc.	(° C.)	5 %	10 %	(iii)
a 1	6·15	10·1	.	.	*Microc. perflavus*	25	+	+	+
			.	.	*Microc. epidermidis*	25	+	+	+
			.	.	*Microc. varians*	25	+	+	+
			.	.	*Sarcina* sp.	25	+	+	+
a 2	.	.	.	.	*Microc. freudenreichii*	25	+	+	+(−)
			.	.	*Microc. candidus*	37	+	+	+(−)
			.	.	*Microc. luteus*	25	+	+	+(−)
			.	.	*Pseudom. syncyanea*	25	+	+	+(−)
			.	.	*Pseudom. striata*	25	+	+	+(−)
			.	.	*Sarc. lutea*	25	+	+	+
			.	.	*Sarcina* sp.	25	+	+	+
			Strep. faecalis	.	.	37	+	−	−
a 3	6·02	9·1	.	.	*Sarc. hamaguchii*	25	+	+	+(−)
			.	.	*Microc. nitrificans*	25	+	+	+
			.	.	*Sarc. lutea*	25	+	+	+
			.	*Streptococcus* sp.	.	37	+	−	−
a 4	6·15	7·3	.	.	*Microc. ureae*	25	+	+	+(−)
			.	.	*Sarc. lutea*	25	+	+	+
			Strep. faecalis	.	.	37	+	−	−

a 5	.	.	.	.	*Microc. epidermidis*	25	+	+	+
			Strep. faecalis	.	.	37	+	−	−
			.	.	*Pseudomonas* sp.? (Gram-positive)	25	+	+	+
			.	*Streptococcus* sp.	.	37	+	+	−
a 6	6·23	6·3	.	.	*Microc. flavescens*	25	+	+	+(−)
			Strep. faecalis	.	.	37	+	−	−
			.	*Streptococcus* sp.	.	25	+	+	−
b 1	5·96	5·9	.	*Kurthia* sp.	.	37	−	−	+
			.	.	*Pseudom. putida*	25	+	+	+
			.	.	*Achromob. iophagum*	25	+	+	+
b 2	6·35	3·1	*Alcal. faecalis*	.	.	37	−	−	+
			.	.	*Vibrio* sp.?	25	+	+	+
b 3	6·58	6·8	.	*Alcal. bookeri*?	.	25	+	+	+
			.	*Bacteroid. fragilis*	. .	37	−	−	−
			.	.	*Microc. aurantiacus*	25	+	+	+(−)
b 4	.	.	.	*Alcal. bookeri*?	.	25	+	+	+
			Alcal. faecalis	.	.	37	−	−	+
b 5	.	.	.	*B. subtilis*	.	30	+	−	+
			Alcal. metalcaligenes	.	.	25	−	−	+
b 6	.	.	*Cl. bifermentans*	.	.	37	−	−	−
			.	*B. cohaerens*	.	30	+	+	−
b 7	6·70	4·2	*B. tritus*	.	.	37	+	−	−
c	6·19	3·4	.	*B. subtilis*	.	30	+	−	+
			Cl. bifermentans	.	.	37	−	−	−
			.	*Streptococcus* sp.	.	37	−	−	−
			.	*Rhodotorula* sp.	.	25	−	−	−
c	(Gracilis muscle)		.	.	*Microc. epimetheus*	25	+	+	+
	5·64	8·3	.	.	*Microc. varians*	25	+	+	+

are unfavourable to such bacteria: of these bacteria, faecal Streptococci seem to be most likely to survive curing.

(vi) The spores of Clostridia and Bacilli are often present in small numbers in curing brines. Some of those isolated here might conceivably have been introduced with the injected brine to which, in all these examples, a proportion of curing brine had been added. Comparison with the occurrence of these types in Table 3, where this factor did not operate, does not suggest that any new situation arose from this cause, except perhaps in the case of *B. cohaerens*, recorded previously from hams by Jensen, Wood & Jansen (1934). This organism, salt-tolerant and able to grow better at moderate temperatures than the mesophilic *Bacilli*, represented a type which might well have been derived from the curing brine. Bacilli like *B. cohaerens* may be important in 'pasteurized' (170–190° F.) canned gammons, because their spores survive processing and they are able to grow readily in the salt-concentration often prevailing in such products (see also Verhoeven, 1950).

In occurrence and distribution, too, the flora of bacon gammons differs from that of hams:

(i) Bacteria are generally present in all gammons, and throughout the tissues of each, in numbers up to 100/g. or more, even when the gammon shows no sign of any taint. Of those in Table 5, only numbers *b* 2, *b* 7, and *c* showed taint at the end of the curing process. The remainder developed internal off-odours in cool storage at 15° C. only after several weeks. The gammons which showed early taint were those with little salt.

(ii) The bacteriological picture may be different in different gammons or at different places in the gammon. Where relatively little salt has been injected and conditions approximate to those in hams, the flora may be mainly 'faecal'; but in typical cases, where the injected brine has thoroughly penetrated the tissues, the flora is almost wholly halophilic. If the injected brine is badly distributed both conditions may exist in the same gammon. This happened for example in gammon *c*, where the outer muscles had a relatively high salt content and a relatively sparse halophilic flora while the inner had little salt and exhibited a severe bone taint comparable with that in hams.

(4) DISCUSSION

It seems probable that the difference between the floras of 'hams' and 'gammons' arises because the former consists of intrinsic bacteria which occur naturally within the body of the animal and are largely mesophilic (i.e. 37° C. optimum, usually with poor growth below 15° C.), while the latter consists largely of extrinsic bacteria introduced from external sources, many of which are psychrophilic (optimum 15–25° C., growing down to freezing-point) (compare Ingram, 1949*a*).

Clostridia are the most important representatives of the intrinsic flora, faecal or probably faecal species having been observed many times within the tissues of mammals, sometimes even before death. Streptococci have been recorded much less frequently, and their occurrence in association with Clostridia seems to have attracted attention only on a few occasions: from beef in a case of 'bone-taint' (Haines & Scott, 1940), from the flesh of whales (Ingram & Hauge, 1949), and here

from the pig. From experience in this laboratory, however, it is believed that Streptococci may be more common than this would suggest, and that the association of faecal Streptococci with the Clostridia is characteristic.

The general nature of this flora suggests strongly that it is mainly recruited, directly or indirectly, from the gut of the animal. There is plenty of evidence that micro-organisms can pass directly from the gut into the flesh of mammals: besides that reviewed by Jensen (1945), and Haines (1937), Tanner & Ruyle (1932) made the elegant demonstration that various yeasts fed to rabbits could be recovered from the blood of the living animals. Indirectly, faecal bacteria might be introduced into the body by the use of a contaminated sticking knife when bleeding the carcass, as Jensen (1945, p. 96) has ingeniously shown with *Serratia*: it is difficult to estimate what proportion of the faecal types found in meat might be introduced by this indirect route.

The small number of typical coliform bacilli isolated, though they are very common in the gut and are sometimes present in the flesh of pigs after death (Reith, 1926), may be due to several causes. They are especially readily inhibited by the bacteriostatic mechanisms of the blood (cf. Reith, 1926; Jensen, 1945, p. 117 *et seq.*). They possess too, in unusual degree, those properties of the intrinsic bacteria which make them unable to grow in properly handled and cured meat, namely, inability to proliferate at low temperatures and susceptibility to acidity and salt. Hence, the relative scarcity of such bacteria in the intrinsic flora of the meat is no indication that this flora does not originate in the gut.

Although enterococci have long been recognized to be associated with faecal contamination (cf. Savage & Wood, 1917), even to the extent that their use as an index of faecal contamination in foods has been suggested (Ostrolenk, Kramer & Cleverdon, 1947), it is noteworthy that hitherto no special significance seems to have been attached to the occurrence of Streptococci in meats, the more so as faecal strains are sometimes very numerous in cured pork products. Vienna sausage and ham Bologna are cited by Dack, Niven, Kirsner & Marshall, 1949; and similar occurrences in Britain, not involving food-poisoning outbreaks, have been reported privately. Some of the characters of the faecal Streptococci, resistance to acidity and moderate salt-concentrations, and ability to grow at low temperatures, are rare among faecal bacteria, and are such as make their possessors particularly likely to survive the controlling conditions imposed during a curing process. In addition, the heat-tolerance of faecal *Streptococci* makes them likely to survive in lightly processed canned hams, where we have in fact sometimes found them in considerable number and variety, an observation recently confirmed by Buttiaux (1951).

Pork legs are rather favourable material for the study of the intrinsic flora of meat, because they seem particularly likely to develop bacteria internally, for several reasons. The pig is an unclean feeder. It is easily fatigued, and therefore specially liable to have less acid flesh after death (Bate-Smith, 1948), containing relatively large numbers of bacteria, if we accept the view that fatigue encourages transfer of bacteria from the gut to the blood stream (Haines, 1937, p. 5). The leg includes regions of unusually high pH. The leg, being the largest and most compact

mass of muscular tissue on the carcass, thickly covered with fatty tissue, is most difficult to cool quickly; and it also offers considerable resistance to the penetration of salts, especially as the flesh is likely to be less acid than in other meat animals (Callow, 1936).

The contrasting flora of the injected gammons, though internal, is almost certainly extrinsic in origin and derived from the brines injected into the interior during curing: indeed, it resembles that found on the surface of bacon (slime). Salt-tolerant psychrophilic Micrococci are the commonest viable bacteria in curing brines (Garrard & Lochhead, 1939; Brooks, Haines, Moran & Pace, 1940); I have observed that they form a major part of the flora developing as slime on cured pork; they are universally distributed in curing cellars etc. (Garrard & Lochhead 1939); and for all these reasons they are likely to be introduced when injecting brine into the meat. Above all, it is a common practice to add a proportion of used brine ('tank pickle') to that employed for injection, because the used brine contains nitrite which produces the colour desired in meat curing. The widespread presence of the Micrococci, when the salt is adequately distributed, agrees with the view that they are introduced in this way.

Corresponding to the difference in the floras, the nature of a typical gammon taint is different from that in a ham. It develops relatively gradually, and may not become evident for some time after curing; whereas in a ham, it seems probable that tainting often commences before curing is begun (cf. Mundt & Kitchen, 1951). Further, the off-flavours produced by the halophilic cocci in gammons tend to be cheesy and rancid rather than offensively putrid as in hams. The use of the 'trier' (a metal spike pushed into the leg, and then smelled to detect taint) might be expected to introduce similar bacteria into hams but this usually occurs some time after the completion of the cure.

The differences between the intrinsic and extrinsic floras are of considerable practical interest. The salt-tolerant psychrophilic bacteria introduced into the tissues of injected gammons are able to develop slowly under the conditions there, even if the material is kept cool: consequently, such gammons are perishable. This was not the case with hams traditionally cured, for if, by a proper combination of low initial infection, acid flesh produced by proper handling and slaughter, adequate penetration of salt, and cool conditions, the intrinsic mesophilic flora was suppressed without admitting bacteria from outside, there resulted a ham which was to all intents and purposes sterile internally, and which would consequently keep almost indefinitely if the surface was preserved, as by smoking. When the cure of a ham fails, however, the result is a catastrophe as the ham is rapidly spoiled; whereas, though a gammon is generally perishable, spoilage by its internal bacteria is so slow as to present no problem in normal handling.

If, as is often the case in modern practice, hams are injected to hasten the cure, it would seem on bacteriological grounds to be better not to add tank pickle for injection, but to use instead a sterile brine, adding nitrite to it if necessary. Addition of nitrite, though illegal under the Foods and Drugs Acts, has been permitted since the issue of a Ministry of Health Provisional Regulation (Circular 1892, 25 October, 1939). It may be suggested, too, that the addition of an appro-

priate acid, sufficient at least to bring the pH of the internal muscles below 6, might be beneficial (cf. Gibbons & Rose, 1950; Ingram, 1949*b*).

(5) SUMMARY

1. The kinds of bacteria found inside hams and bacon gammons are listed and discussed.

2. Tainting of lightly- or un-injected hams is caused by a flora composed chiefly of various faecal Clostridia and/or faecal Streptococci, which are probably intrinsic to the animal's body. In bacon gammons which are heavily injected with brine, the characteristic flora consists of psychrophilic halophiles, mostly Micrococci, probably derived mainly from the brine; if injection is only partly effective, the intrinsic flora may develop also, faecal Streptococci being the type most likely to survive.

3. Within sound hams, bacteria are virtually absent; in tainting, the numbers may become enormous even before curing is finished. Injected gammons, on the contrary, generally contain bacteria in moderate numbers, but they multiply relatively slowly.

4. In hams, the taint is always confined within a group of muscles near the bone which are less acid, and initially less salty, than the outer muscles. In gammons, off-flavours caused by bacteria may occasionally develop in the outer layers as well.

5. A ham properly cured without injection, and internally 'sterile', can be made to keep almost indefinitely. The bacteria injected into gammons slowly cause internal off-flavours even in cool storage. For long storage, it would seem wise to sterilize brine used for injection, and the addition of acid might be beneficial.

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