# THE BACTERIAL FLORA DEVELOPING ON STORED LEAN MEAT, ESPECIALLY WITH REGARD TO "SLIMY" MEAT.

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(With 1 Figure in the Text.)

It has been shown in the previous paper that when cattle are slaughtered large numbers of bacteria may reach the surface of the carcass either by settling out from the air, or by contact with splashed contaminated blood, faeces, or dirty swabbing water. A preliminary study of the growth of micro-organisms on chilled and frozen meat, stored in carcass form, has already been carried out (Haines, 1931 *a*). If the meat is stored as an uncut carcass, or in quarters, the greater part of its surface is covered with a layer of fat and connective tissue upon which little or no bacterial growth occurs during normal hanging (*op. cit.*). It is largely upon this fact that the storage life of chilled beef depends. The point is well illustrated by the experimental observations that chilled meat in carcass form may be stored for 60 days at 0° C. (Lea, 1931), whereas it will be shown in the course of the present work that small pieces of *lean* meat consistently developed "slimes" in from 8 to 18 days at 0° C., even when the surrounding (uncirculated) air had a comparatively low humidity.

## Composition of the "slime."

When small joints having a large area of exposed lean are stored for periods varying from 8 to 18 days at approximately 0° C. either in the small refrigerator of the butcher's shop or under laboratory conditions, a characteristic "slime" appears all over the lean surface. It commences with the appearance of small glistening brown droplets which eventually coalesce to form the uniform brownish "slime." At the same time a characteristic odour, variously described as "sour meat," "tainted," or "cold-store taint," appears. Altogether, about a dozen samples of meat from different butchers in various parts of England have been subjected to bacteriological analysis, and in every case the "slime" has been found to consist almost entirely of short, Gram-negative rods, apparently belonging to the *Achromobacter* group, all of which give rise to the characteristic odour when first sown into artificial media. Two interesting examples are given in Table I. A small platinum loop, of area approximately known, was lightly pressed in the slime, the material

https://doi.org/10.1017/S0022172400018490 Published online by Cambridge University Press

removed emulsified in saline and suitable dilutions plated. Plates were incubated at 37, 20, 4 and 0° C. In addition all possible colonies from a suitable plate incubated at each temperature (20–30 colonies per plate) were picked off and subcultured in order to find the chief types present.

Table I. *Analysis of "slime."*

"Slime" on meat killed in a poor "Type I" slaughterhouse and stored on the premises for about 1 week in the range 5–0° C.

Temperature of incubation of plates

| 37° C. | 20° C. | 4° C. |
|---|---|---|
| 14,400,000 organisms per sq. cm. | 27,800,000,000 organisms per sq. cm. | 10,000,000,000 organisms per sq. cm. |
| Streptococci 5 % | *Actinomyces* 7 % | *Pseudomonas* 4 % |
| *Azotobacter* type 5 % | *Pseudomonas* 11 % | *Achromobacter* 96 % |
| Staphylococci 20 % | *Proteus* 11 % | |
| Micrococci 20 % | *Achromobacter* 71 % | |
| *Proteus* group 45 % | | |

*"Slime" on meat killed in a slaughterhouse of modern construction, but not so large as "Type II," and stored in laboratory store for 10 days at 0° C.*

Temperature of incubation of plates

| 37° C. | 20° C. | 4° C. | 0° C. |
|---|---|---|---|
| 12,500 organisms per sq. cm. | 14,300,000,000 organisms per sq. cm. | 5,000,000,000 organisms per sq. cm. | 4,000,000,000 organisms per sq. cm. |
| *Achromobacter* 10 % | *Achromobacter* 100 % | *Achromobacter* 100 % | *Achromobacter* 100 % |
| Micrococci 40 % | | | |
| *Proteus* group 50 % | | | |

It will be seen that the proportion of organisms having their optimum at 37° C. is insignificant, less than 0·1 per cent. as compared with those having optima at 20° C. Assuming that the figure obtained from plates incubated at 20° C. represents the total number of organisms, in both cases about a third of the organisms present are capable of growth at + 4 and 0° C. An interesting point is, however, that the proportion of 37° C. organisms is very much higher on meat from the poor than the better slaughterhouse. The butcher at the poorer premises was complaining of his meat developing "slime" in a very few days, while meat was being stored for the normal period with success in the other case. While it is impossible to generalise from a few cases in work of this type, the results suggest that, in accord with the views advanced in the previous paper, a high "intestinal" count is indicative of unsatisfactory working conditions. It must be clearly realised that the organisms having their optimum at 37° C. are not causal agents in the production of "slime": they are rapidly outgrown by other types at the low temperature of storage, but they are useful indicators of the cleanliness of handling to which the meat has been subjected.

### Characteristics of the *Achromobacter* organisms.

With the exception of a certain number of organisms of the *Pseudomonas* group, and a few *Proteus*, the bacteria growing on lean meat stored in the range 4–0° C. almost all belong to the *Achromobacter* group. About 120 strains

in all have been studied. They are small Gram-negative rods, some strains are motile, and most strains liquefy gelatin and almost all strains produce acid in dextrose. Great difficulty has been experienced in attempting any grouping owing to the fact that these organisms have so few well-marked characteristics, morphological or biochemical. An added difficulty is that many strains do not produce acid in a given carbohydrate until after perhaps 14 days' incubation, so that in all cases the time of incubation of the tests is given. Moreover, ability to produce acid appears to vary within a single strain: for instance, organism 1 (Table III) when first isolated effected no noticeable change to Andrade's indicator in maltose-peptone until after 10 days' incubation, but after being kept in artificial culture for 2 months a deep red was obtained after 2 days' incubation. The organisms were first grouped according to the scheme shown in Table II, using their biochemical characters as a basis, after which a representative of each strain was re-plated and studied in detail. The detailed characteristics of the type organisms are given in full in Table III. Bergey's *Manual* (3rd edition, 1930) has been used as the standard work of reference, but it is impossible to identify with certainty any of the strains studied owing to the inadequate descriptions available in the literature. For example, organisms 1 and 4 (Table III) apparently agree fairly well with the published descriptions of *Achromobacter multistriatum* so far as these are given, but no carbohydrate reactions have been found in the literature for this organism. Strain 1 produces acid in levulose and strain 4 does not. In all cases the type organisms have been under observation for 1 or 2 months, and all significant biochemical tests carried out at least in duplicate. Cultures of the strains studied in detail have been deposited with the Lister Institute, London.

Table II. *Grouping of* Achromobacter *organisms.*

GROUP I. Gelatin liquefied rapidly (1–2 days).

| (*a*) | (*b*) | (*c*) | (*d*) |
|---|---|---|---|
| Dextrose, maltose, levulose, acid | Dextrose acid. Maltose and levulose not acid | Dextrose and maltose acid, levulose not acid | Dextrose not acid |
| 6 organisms | 46 organisms | 61 organisms | 2 organisms |

GROUP II. Gelatin not or very slowly liquefied (5 days or greater).

| (*e*) | (*f*) |
|---|---|
| Dextrose acid, maltose and levulose not acid | Dextrose not acid |
| 13 organisms | 4 organisms |

RATE OF GROWTH OF *ACHROMOBACTERIA* ON LEAN MEAT AT 0° C.

Small pieces of lean beef (1–2 lb.) were hung in large glass jars at $0 \pm 0{\cdot}1°$ C. having a shallow layer of water on the bottom to maintain a saturated atmosphere. A nearly constant area was excised from each piece from time to time with a sterile cork-borer, the area of which was approximately known, and the tissue cut off to a depth of 2–3 mm. The material so obtained was thoroughly shaken for 5 min. with glass beads and 10 c.c. of sterile saline, and suitable dilutions plated on nutrient agar *p*H 7·4. The plates were incubated

Table III. *Detailed characteristics of typical* Achromobacteria.

| Organism | Agar colony | Agar slant | Gelatin | Potato | Litmus milk | Dextrose | Lactose | Sucrose |
|---|---|---|---|---|---|---|---|---|
| 1 | Convex, circular, white, glistening | Thick white, shining | Crater liquefaction 2, becoming stratiform | Thick white | Litmus decolorised, slight acidity and coagulation | A 2 | N 10 | N 10 |
| 2 | Thick white | Thick white | Ditto | Ditto | Unchanged or faintly alkaline | A 2 | N 15 | N 15 |
| 3 | Thick, creamish white, lacerate edge | Thick creamish | Crater liquefaction 1, becoming stratiform | Ditto | Slight acidity and slight coagulation | A 2 | N 8 | N 8 |
| 4 | Thick flat white | Glistening white, flat | Ditto | Ditto | Sometimes faintly acid, and some coagulation | A 2 | N 10 | N 10 |
| 5 | Thick, creamish | Thick, creamish | Ditto | Ditto | Slightly alkaline | N 21 | N 5 | N 5 |
| 6 | Thick white | Thick white | Not liquefied 5 days | Ditto | Faint acidity | N 21 | N 7 | N 7 |
| 7 | Creamish, lacerate edge | Thick, creamish | Slight crater 5 days | Ditto | Faint acidity, litmus reduced | A 2 | N 7 | N 7 |
| 8 | Globular, glass-like | Thick, smooth, glossy | Not liquefied 5 days | Thick, glossy | Faintly alkaline | N 21 | N 7 | N 7 |

| Organism | Maltose | Levulose | Galactose | Xylose | Arabinose | Mannitol | Dulcitol | Sorbitol | Salicin | Nitrates | Indole | Acetyl methyl carbinol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A 2 | A 2 | A 4 | A 4 | A 4 | N 17 | N 8 | N 21 | A 2 | Negative | Negative | Negative | Gram-negative rod, $1{\cdot}0\mu \times 0{\cdot}5\mu$, actively motile, flagella monotrichous |
| 2 | N 15 | N 15 | A 4 | A 4 | A 4 | N 21 | | | N 18 | ,, | ,, | ,, | Gram-negative rod, not motile |
| 3 | A 5 | N 11 | A 2 | A 4 | A 4 | N 8 | N 8 | N 8 | N 8 | ,, | ,, | ,, | Gram-negative rod, not motile |
| 4 | A 3 | N 11 | A 2 | A 4 | A 2 | N 21 | N 21 | | N 21 | ,, | ,, | ,, | Gram-negative rod, $1{\cdot}0$–$1{\cdot}6\mu \times 0{\cdot}4\mu$, actively motile, flagella monotrichous |
| 5 | N 11 | N 11 | | N 21 | N 21 | N 21 | N 21 | | N 16 | ,, | ,, | ,, | Gram-negative rod, not motile |
| 6 | N 7 | N 7 | | N 21 | N 21 | | N 21 | | N 16 | ,, | ,, | ,, | Gram-negative rod, not motile |
| 7 | N 7 | N 11 | A 2 | A 3 | A 4 | N 21 | N 21 | | N 5 | ,, | ,, | ,, | Gram-negative, rod actively motile, flagella monotrichous |
| 8 | N 7 | N 7 | | N 7 | N 7 | N 11 | N 21 | | N 10 | ,, | ,, | ,, | Gram-negative rod, $2\mu \times 1\mu$, actively motile, flagella monotrichous |

A = Acid; N = Not acid. The numbers refer to the time in days in which the reaction was obtained.

for 48 hours at 20° C. and counted. In addition, the surface of the meat was carefully watched and the time of appearance of "slime" correlated with the bacterial count. Some typical counts are given in Table IV.

Table IV. *Rate of growth of* Achromobacteria *at* 0° *C.*
*Organisms per sq. cm. of surface.*

| Time in days | Sample number 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 0 | 40,000 | 17,300 | 2,700 | 270* | 43 |
| 4 | 875,000 | 35,000 | 5,600 | 3,000 | <200 |
| 8 | 56,000,000 | 6,500,000 | 980,000 | 26,000 | 59,000 |
| 11 | 1,650,000,000 | 70,000,000 | — | — | 595,000 |
| 15 | 20,000,000,000 | 2,000,000,000 | 11,300,000,000 | 12,100,000 | 45,000,000 |
| Time of appearance of "slime" | 8 days | 10 days | 11 days | 16 days | 18 days |

* Stored at relative humidity 70 per cent.

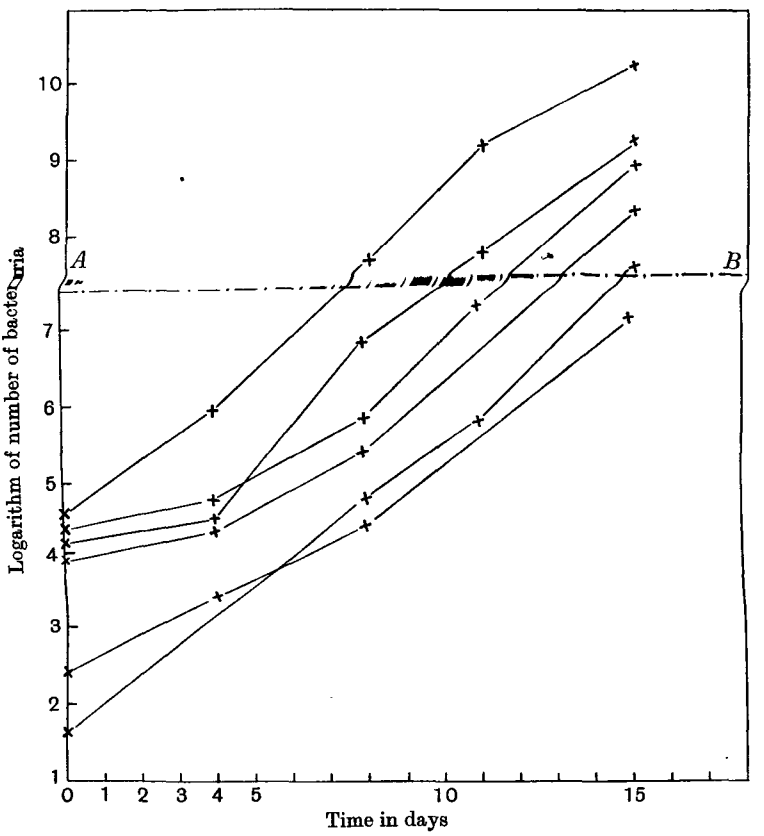


Fig. 1. Rate of growth of bacteria on lean meat at 0° C. Six samples of meat with varying initial loads of bacteria. The point where the line $AB$ cuts the curves of growth represents the onset of "slime," as explained in the text.

It will be seen that there is a relation between the initial bacterial load carried by the meat and the time of appearance of "slime". This is most easily apprehended from Fig. 1, in which the logarithms of the bacterial numbers

https://doi.org/10.1017/S0022172400018490 Published online by Cambridge University Press

are plotted against time. "Slime" apparently becomes noticeable in every case when the bacterial count is of the order of $10^{7\cdot5}$ organisms per sq. cm. approximately. In other words, the point at which the line $AB$ cuts the line representing the number of bacteria present on the meat gives approximately the limit of the "storage life" of the meat. Meat being put into storage with an initial load of 40,000 organisms per sq. cm. is practically inedible after 8 days' storage, whilst meat giving a first count of 43 could be kept for 18 days. There is therefore a clear-cut case for exercising every care to lower the bacterial contamination of the meat during killing and handling.

### Rate of growth of a pure strain of *Achromobacter*.

The foregoing counts refer to the mixed flora on the meat, including several strains of *Achromobacter* and any other organisms present capable of growth at 0° C. Large errors, too, are inherent in work of this type owing to difficulties of adequate sampling and emulsification of the superficial flora. More accurate estimates of the rate of growth of a pure strain of *Achromobacter*, belonging to group (*b*) of Table III, have been made by growing this organism in broth. A culture vessel was used such that 200 c.c. of nutrient broth *p*H 7·4 could be spread as a shallow layer on the bottom, and samples withdrawn by siphon as required. The apparatus was continuously rocked by a mechanical shaker, and sterile air passed over throughout the experiment. Counts were carried out at 20, and + 4 and 0° C. respectively. The results are given in Table V. The generation time has been calculated in each instance over the period of most rapid growth.

Table V. *Rate of growth of* Achromobacter *in nutrient broth* p*H* 7·4.

*Organisms per c.c.*

| 20·2 ±0·2° C. | 4·3 ±0·3° C. | 0·1 ±0·1° C. |
|---|---|---|
| 0 hr. 12,000 | 0 hr. 138,000 | 0 hr. 57,000 |
| 11 ,, 184,000 | 24 ,, 206,000 | 24 ,, 75,000 |
| 24 ,, $200 \times 10^6$ | 96 ,, $398 \times 10^6$ | 96 ,, 290,000 |
| 32 ,, $4{,}500 \times 10^6$ | 144 ,, $11{,}100 \times 10^6$ | 168 ,, $15{\cdot}6 \times 10^6$ |
| 49 ,, $11{,}600 \times 10^6$ | 312 ,, $16{,}000 \times 10^6$ | 264 ,, $1{,}680 \times 10^6$ |
| 73 ,, $9{,}600 \times 10^6$ | 384 ,, $19{,}800 \times 10^6$ | 336 ,, $6{,}900 \times 10^6$ |
| 96 ,, $8{,}400 \times 10^6$ | 552 ,, $31{,}200 \times 10^6$ | 432 ,, $9{,}000 \times 10^6$ |
| Generation time 1·3 hr. | 6·6 hr. | 9·1 hr. |

A similar calculation for the growth of the mixed flora on the meat gives a value of 12·8 hours for the "average" generation time at 0° C. or about a third longer than for the pure strain in broth. It must be remembered, however, that the experimental errors are much greater in sampling the meat than in sampling the broth.

### Discussion.

It will be seen that the predominating flora on lean meat stored in the range 4–0° C. is one composed almost entirely of *Achromobacteria*. At lower temperatures, *i.e.* when the meat is frozen, a different flora predominates, consisting chiefly of yeasts and moulds (Haines, 1931 *a*). The temperature-

growth relationships of the latter as so far studied suggest that they cease growth at about − 7 to − 10° C. (Haines, 1931 *b*), so that continued storage below that temperature should lead to another characteristic flora depending upon which organisms were most resistant to prolonged cold. The predominance of *Achromobacteria* in the range 4–0° C. is probably explained by their more rapid growth at that temperature as compared with that of organisms having a higher optimum temperature. They are possibly identical with the "Aromabakterien" of Glage (1901), but no evidence has been obtained for his stated optima at 10–15° C., the optima of these organisms apparently being at 20–25° C.: that the temperature effect is not the sole reason for their predominance on meat is shown by the fact that they still far outnumber other types if the meat is incubated at 20° C. Nor can it be explained altogether by their greater occurrence at the outset, for although they are widely distributed in air, water and soil (Bergey), according to the results obtained in the foregoing paper, there are large numbers of other organisms present. It is interesting to speculate whether the freshly killed tissues may not have some resistance to the common "body" organisms with which they may often have been in contact before, with little towards the comparatively foreign *Achromobacteria*, but only a statistical examination of a large number of cases could show whether such a speculation is idle. These bacteria in fresh artificial culture, and on meat, produce a characteristic "taint" odour and a definite "cold-store taint" flavour in the meat even when cooked. Some of these organisms grown on agar containing emulsified fat (Turner, 1929) gave a blue zone, indicating liberation of free fatty acid. All attempts to isolate an extracellular lipase by centrifuging off the organisms after growth and adding the centrifugate to emulsified ethyl butyrate or castor oil in phosphate buffer according to the method of Avery and Cullen (1920) have so far failed. If this can be substantiated by further experiment it follows that the lipase of these organisms is entirely endogenous and there can be no question of its diffusion from the lean on to the fat and so causing high free acidity. If these bacteria are responsible for any increase in free acidity of the fat it must be by actual multiplication on the fatty tissue, and evidence for this is wanting except during certain abnormal cases of storage. The fat may, however, readily acquire a "tainted" flavour by diffusion from the lean, as it is very sensitive in this respect (Haines, 1932).

## SUMMARY.

1. Examination of the "slime" obtained on lean beef stored at temperatures just above zero Centigrade has shown it to be composed chiefly of organisms of the *Achromobacter* group.

2. A study of about 120 strains of these organisms has been carried out and the characteristics of typical strains are given in detail.

3. Data of the rate of growth of these organisms on meat and in broth are given.

4. The dependence of the "storage life" of the meat on the initial bacterial load is shown.

The author is indebted to Dr G. S. Graham-Smith for his interest in this work, and to Mr J. Barlow for experimental assistance.

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(*MS. received for publication* 24. XI. 1932.—Ed.)