OBSERVATIONS ON THE BACTERIAL FLORA OF SOME SLAUGHTERHOUSES.

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(With 3 Figures in the Text.)

The present work is an attempt to gauge the type and magnitude of the superficial bacterial contamination of carcasses during operations on the killing floor. Since the storage life of meat, especially of lean meat hung in the small refrigerator commonly installed in the butcher's shop, is directly determined by the concomitant micro-flora, it is of obvious importance to investigate how far this flora may be controlled. This paper presents a complementary study to that of the following one, in which the growth of the organisms during storage of the meat is followed.

Possible sources of infection may be by the settling of organisms from the air into which they have been disseminated in the process of skinning and gutting the animal, by contact with blood or faeces on the wall or floors, and from the water and swabs used to wash down the carcass. An attempt was therefore made to estimate the relative importance of infection from these sources, and to identify the chief types of organism concerned. The experiments are essentially of a preliminary nature: the field is enormous and the flora so complex that it is possible to deal only with the main types. No attention has for the present been paid to the anaerobes: we are here concerned with contamination of the surface, and the peculiar problems presented by anaerobic growth require separate study.

The observations were made in slaughterhouses which can be grouped under two types. "Type I" is the small privately-owned erection of which there are many in this country. It is generally a barn or portion of some similar outbuilding, and as a rule little regard is paid to elementary principles of hygiene. "Type II" is typical of the large modern abattoir erected in a big city. A general examination of the conditions in the two types is given first, followed by a comparison of such factors as are directly comparable.

"Type I" slaughterhouse.

The particular slaughterhouse in which most of the experiments were carried out was a rectangular building resembling a barn, some 50 ft. long by 20 ft. broad by 20 ft. high, having a stone floor and roughly plastered

walls, five windows (unglazed or with glass broken) and one door. Pens were located just outside the door into which animals could be driven: sheep were often stuck just outside the door, pigs sometimes shot in the pens, but cutting and dressing were always carried out inside. Larger animals were tethered to a post inside before being killed with a humane killer of the captive-bolt type. The floor resembled a cobblestone street, and in the interstices dried blood and faeces provided a probable breeding ground for micro-organisms. The rough plaster walls were often covered for stretches of several square yards with fairly thick mould growth, and it was not uncommon in hoisting up a dressed carcass for it to brush along the wall and so become inoculated with mould. Frequently part of a carcass was dragged through blood or faeces on the floor, and the washing down of the carcass was performed with a single bucket of water and a piece of cloth. As many as ten carcasses were washed with one bucket of water: by the time the last ones were reached the surface was merely being heavily inoculated instead of being cleansed, as is shown by the bacterial counts given later. Also, carcasses were hung in that atmosphere above the killing floor overnight to "cool out," thereby ensuring the deposition of a large number of organisms on their surface from the air.

Micro-organisms in the air.

From time to time various methods of estimating the number of micro-organisms in the air have been propounded. That of exposing plates gives an idea of the rate of deposition, but for estimation of the number per given volume recourse must be had to filtration. Sterile sand or powdered glass-wool were found to be fairly satisfactory provided the number of organisms was not too great when they tend to obscure the counting if expelled into the Petri dish, while washing may not remove all of them. Filtration through suitable liquids obviated these difficulties, but it was very slow if adequate time were allowed for the bubbles to come through sufficiently slowly to retain the organisms. Preliminary experiments were carried out with a slight modification of Graham-Smith's method (1903). A piece of glass tubing 0·5 cm. bore had another piece of slightly less diameter ground into it, the two then being cut and constricted as shown in Fig. 1. A wad of glass-wool previously ground up was packed tightly in at *A*, another at *C*, and a guard plug at *D*. By this means, on attaching the end *D* to suction, air could be drawn through *A*. Most of the organisms were filtered out here: some escaped to *C*. At the close of the experiment the tube was disconnected at the ground joint *B* and the plug *A* pushed out with a stout platinum wire into a Petri dish. Similarly plug *C* could be shaken out. Before use the end *A* was plugged with cotton-wool and the whole enclosed in a test-tube and sterilised. By this means the same tube could be used repeatedly without having to cut it open as in the Graham-Smith method. This apparatus was found to yield good results so long as the number of organisms was not too great, but for the filtration of say 5 litres of air containing 50–500 organisms per litre, liquid filtration by

aspiration through 1 per cent. cane sugar in the apparatus shown in Fig. 2 was better. Experiments on the rate of filtration showed that, with the glass-wool filter, it should not be greater than 1 litre per 5 min. With the liquid filter the bubbles must just break separately from the end of the fine jet, as long as 15–30 min. per litre of air being required. At the close of an experiment with the liquid filter, the ends *A* and *B* were plugged with cotton-wool, and the liquid gently aspirated up the inner tubes and allowed to fall again several times, the contents of the first and second tubes being then well shaken and plated in 1 c.c. quantities. Organisms seldom passed beyond the first tube. In sterilising the apparatus before use it was preferable to introduce the 10 c.c. of sterile sugar aspectically rather than to autoclave complete, owing to distillation from one tube to the other. Counts were made on nutrient gelatin and agar *p*H 7·4 adjusted colorimetrically. A selection of the results obtained is given in Table I.

Table I.

No.	Time	Type of filter	Place	Mean No. organisms per litre: Agar 37°	Gelatin 20°	Remarks
1	January, morning	Glass-wool	Laboratory	1·8	—	These figures are for comparison and may be taken as typical of good indoor air
2	,,	,,	,,	1·4	—	
3	Afternoon	Glass-wool	,,	4·3	—	
		Liquid	,,	4·5	—	
4	February, afternoon	Glass-wool	"Type I" slaughterhouse	33	—	
5	,,	,,	,,	42	—	
6	,,	Liquid	,,	133	502	Number of animals killed and dressed
7	June, afternoon	,,	,,	35	438	
8	August, afternoon	,,	,,	20	126	Still afternoon in summer and only few sheep and pigs dressed
9	October, morning	,,	"Type II" slaughterhouse	30	72	Centre of room under duct — Large number of animals handled
10	,,	,,	,,	47	65	Corner of room — Large number of animals handled

The significance of these figures is discussed later.

In order to estimate whether or no organisms capable of anaerobic growth were scattered into the air a number of plates were made from one filtration in "Type I" slaughterhouse with Veillon agar incubated at 37° C. in a MacIntosh-Fildes jar containing methylene blue. A mean figure of 27 organisms per litre of air was obtained.

Rate of deposition of bacteria from the air.

The rate of deposition of the air-borne flora on to exposed objects was estimated by exposing agar plates of approximately known diameter in a horizontal position for 15 min. some 3 ft. from the floor:

(*a*) A cold day in February, with the only door of the slaughterhouse closed. Several sheep and pigs and two bullocks killed and dressed. A mean figure of 12 organisms per sq. cm. per 15 min. exposure was obtained.

Fig. 1.

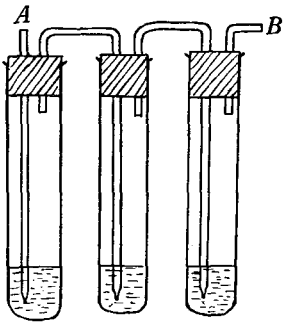

Fig. 2.

Figs. 1 and 2. Apparatus for air-filtration experiments. See text for explanation.

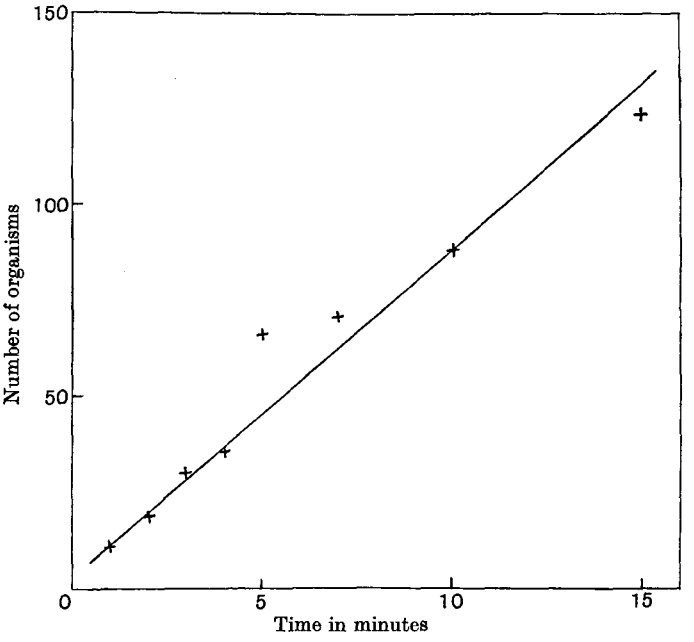

Fig. 3. Rate of settling of organisms from the air on to exposed plates.

(*b*) Still day in June. Sheep only were dressed. The mean count was 2 organisms per sq. cm. per 15 min. exposure.

In order to gain further insight into the rate of fall, a series of plates were exposed at given intervals, starting with one plate and uncovering others side

by side progressively with the time. This can be done only over a short period, as the congestion soon renders counting impossible, and the results are only approximate because of the disturbing air currents. Several plates were exposed for each time interval and the mean taken. The results are shown graphically in Fig. 3. Apparently during the early stages of exposure, the number of organisms deposited is roughly proportional to the time of exposure.

The walls of the slaughterhouse.

In this particular case the walls were covered with a rough plaster which readily retained and absorbed material splashed on to it. It has already been pointed out that considerable areas were often covered with mould growth, and that carcasses were frequently brushed along the wall in hoisting them up.

Several areas of approximately 1 sq. cm. each were marked on the walls and the superficial layer of plaster scraped off into a sterile tube with a sterile scalpel. The scrapings were well washed with 10 c.c. of sterile saline and plates poured. The mean count was 103 organisms per sq. cm.

The floor of the slaughterhouse.

In the act of gutting the animal the internal organs were all thrown down on to the floor into blood and faeces. Frequently the paunch was cut open disgorging a large quantity of partially digested grass, etc. It was not uncommon for part of the carcass to be dragged through this on hoisting it up. In order to form an estimate of the numbers of organisms which might be picked up by light contact with the floor, sterile rubber bungs of approximately known area were lightly pressed on to the floor and removed. These were subsequently washed with sterile water and suitable dilutions plated:

(*a*) On part of the floor where killing was not in actual progress, but dried blood and faeces were evident, 290 organisms were picked up per sq. cm.

(*b*) Where killing had taken place but the floor had been swilled and brushed, 546 organisms were picked up per sq. cm.

(*c*) Where blood, paunch contents, etc., were lying on the floor, 265,000 organisms were picked up per sq. cm.

The liquid on the floor after it had been largely diluted with water preparatory to brushing over gave counts of 850,000–920,000 organisms per c.c., with an anaerobic count of 55,300 organisms per c.c.

The water used in swabbing the carcass.

The common practice in slaughterhouses of this type is to have a bucket or tub of water in the middle of the floor in which a piece of cloth is soaked and used to rub down carcass after carcass, without change of either cloth or water. Far from having any cleansing value, except in so far as any extraneous blood or faeces on the surface is removed which might be offensive to the eye

of the purchaser, the carcass is merely being heavily inoculated with bacteria. This procedure is reflected in the following counts:

	Organisms per c.c.
(*a*) Winter. Agar at 37° C. (10 sheep washed in this water)	1,500,000
(*b*) Summer. Agar at 37° C.	24,800,000
Total gelatin count	130,000,000
Liquefiers (6 sheep washed in this water)	10,000,000

Dilutions of washing water were inoculated into tubes of MacKonkey broth, and neutral red glucose broth, and those tubes yielding acid and gas subcultured on to MacKonkey agar. Pink colonies were picked off and studied. The count of coliform organisms was 500 per c.c. The significance of this figure may be appreciated by comparison with the standard for ordinary drinking water, namely, the absence of *coli* from 10 c.c.

"Type II" slaughterhouse.

The observations were made in the killing booth of a large modern slaughterhouse built to supply a big city. A number of such booths are arranged parallel to each other, with smooth concrete floors and white tiled walls. These walls do not rise directly to the roof, so that air currents can pass from one booth to another. Purified ozonised air is pumped into each booth from a duct some 15 ft. above the floor, at such a speed as to cause appreciable draughts through the centre portion of the booth. A big improvement is effected by the provision of ducts and drains to carry away offal and dung. The paunch was in no case cut open on the killing floor but travelled down a chute to rooms below. There are four chutes "General," "Paunch," "Guts" and one for hides, and two drains, a general drain and a "Blood" drain. During killing the wall and floors were frequently washed down with a hose. Two tanks are provided on the centre of the floor containing a supply of clean hot water for washing the carcasses, this being changed at frequent intervals. The size of the killing booth is such that often four sheep and perhaps five or six bullocks are being dressed simultaneously, so that the decrease in actual numbers of bacteria which may be assured by the cleaner conditions may be largely counteracted by the increased number of sources of infection. But probably the more noxious type of organism is more largely eliminated by dealing with the intestine, etc., in a separate room, as the results obtained go to show. Two obvious improvements are the provision of clean water for swabbing and the removal of the meat from the killing floor immediately after dressing for hanging in separate chambers to cool. Even so, it might perhaps be possible to accelerate the process of dressing and thus avoid much of the bacterial infection which is heaviest at this point. One case witnessed was that of a bullock which was bled 2½ min. after killing. Skinning commenced 15 min. after killing, but 66 min. elapsed before the removal of the carcass to the hanging room.

Micro-organisms in the air.

Filtration was carried out with a liquid filter, one experiment being carried out beneath the air duct and another in the corner of the booth. The results are given in Table I.

Rate of fall.

(*a*) A plate exposed for 15 min. in the centre of the booth gave a result of 5 organisms per sq. cm. per 15 min.

(*b*) 30 min. exposure in the centre of the booth gave a figure of 13 organisms per sq. cm. per 30 min.

(*c*) Results obtained in a corner of the booth:

5	min.	2	organisms	per sq. cm.
10	,,	3	,,	,,
15	,,	8	,,	,,

The water used in swabbing the carcass.

A count on water from the tank in the centre of the floor gave a figure of 18,900 *organisms per c.c.* (agar at 37° C.).

The count of coliform organisms was 5 per 10 c.c. of water.

Identification of organisms.

In order to obtain some idea of the composition of the flora encountered, certain of the air filtration and water experiments were sampled by picking off as many as possible of the colonies on one or two representative plates, subculturing and examining their cultural and morphological characteristics. In all well over a hundred organisms have been studied and placed as far as possible in their respective groups. Very many types of organism occurring in smaller numbers must inevitably be overlooked, since the samples represent so small a fraction of the total, but the labour of identification makes it impossible to deal with larger samples. No search has been made for particular pathogenic or food-poisoning organisms, and the approach has been throughout cultural and morphological rather than serological, since it is ultimately with the biochemical effects of these organisms that food storage is concerned. A brief classification of the organisms studied is given in Table IV. In every instance the organism has been under survey over a number of generations, so that doubtful cases of gas production, etc., could be checked. In grouping the bacteria, most reliance has been placed on Gram-staining (Burke's modification, 1922) nitrate reduction, gelatin liquefaction, litmus milk, and potato, with some fermentation tests. Bergey's *Manual* and the terminology of the American Society of Bacteriologists have been adhered to throughout.

Discussion of results.

It is difficult to draw a comparison between the two types of slaughterhouse on the bacteriological evidence because of the entirely different conditions. For example, whereas in the small "Type I" example perhaps a dozen cattle may have been dealt with, in the larger abattoir possibly of the order of 50–100 may have been killed and dressed in a similar period. If we regard each animal as a potential source of a given load of bacteria which will be scattered during handling, the gross infection at the source is obviously much greater in the larger abattoir. Since the greater degree of infection is not reflected in the content of bacteria in the air, as shown by air filtration, it follows that the system of ventilation is better in "Type II" slaughterhouse. For a rough comparison of working conditions, filtrations 7 and 9 (Table I) may perhaps be taken as representative. That is to say, despite the far greater number of animals handled, the air on the whole carries a smaller load of organisms in the large abattoir than in the small slaughterhouse.

Evidence based on the type of flora encountered is more conclusive. An analysis of the bacteria found in the air illustrates this (Table II).

Table II.

"Type I" slaughterhouse.

Cocci 46 %

Staphylococci 11 % — Micrococci 35 %

Rods 54 %

"Soil" 35 %	"Intestinal" 19 %
Subtilis type	*Proteus*
Fluorescent (*Pseudomonas*)	Coliform
Achromobacter	*Aerobacter cloacae*
Actinomyces	
Flavobacteria	
Azotobacter type	
Moulds	

"Type II" slaughterhouse.

Cocci 47 %

Staphylococci 6 % — Micrococci 41 %

Rods 53 %

"Soil" 44 %	"Intestinal" 9 %
Subtilis type	Coliform
Fluorescent (*Pseudomonas*)	*Aerobacter cloacae*
Azotobacter type	
Moulds	

Thus, the precautions adopted in "Type II" slaughterhouse in the disposal of offal are reflected in the 9 per cent. of "intestinal" organisms as against the 19 per cent. of the "Type I" slaughterhouse. The most striking difference is found, however, in the degree of infection of the water used for swabbing the carcasses (Table III).

Table III.

"Type I" slaughterhouse.

Total counts: 2,000,000–25,000,000 organisms per c.c.

Coli: 5000 per 10 c.c.

Chief types:

Aerobacter cloacae	*Proteus vulgaris*
Staphylococcus epidermis	*Micrococcus luteus*
Micrococcus candicans	*Pseudomonas*
Escherichia communior	*Micrococcus perflavus*

"Type II" slaughterhouse.

Total counts: 15,000 organisms per c.c.

Coli: 5 per 10 c.c.

Chief types:

Sarcina lutea	*Flavobacterium*
Escherichia communior	*Achromobacter*

Table IV. *Organisms isolated and studied in detail.*

The nomenclature of Bergey's *Manual* (3rd edition) has been used, and where an organism is named it was identical, as far as could be ascertained, with Bergey's description, except for the differences noted. Any additional cultural characters to those given in the *Manual* are also listed.

Organism	Where found
Staphylococcus albus	Air T_1, air T_2
S. citreus	Air T_1
S. epidermis	Water T_2, air T_2
Micrococcus conglomeratus	Air T_1
M. percitreus. Acid in dextrose, sucrose, maltose but not lactose	Air T_1
M. candicans. Acid in dextrose and sucrose	Air T_1, floor T_1, water T_1, air T_2
M. aurantiacus	Air T_1, walls T_1, air T_2
M. perflavus	Air T_1, water T_1, air T_2
M. cereus. Acid in dextrose and sucrose	Air T_1, floor T_1
M. varians?	Air T_1, floor T_1
M. freudenreichii. Acid in lactose	Air T_1
M. ochraceus	Walls T_1
M. subflavescens. Acid in lactose	Floor T_1
M. subgranulatus	Floor T_1
M. luteus	Water T_1, air T_2
M. saccatus?	Air T_2
Rhodococcus rosaceus	Walls T_1
R. cinnebareus	Air T_2
Sarcina flava	Air T_2
S. lutea	Water T_2
Achromobacter fermentationis?	Air T_1
Achromobacter. Not grouped	Air T_1
Achromobacter. Not grouped	Water T_2
Aerobacter cloacae	Air T_1, water T_1, air T_2
Actinomyces. Not grouped	Air T_1, air T_2
Escherichia communior	Air T_1, water T_1, air T_2, water T_2
E. coli	Air T_1, air T_2
Flavobacterium sulphureum	Air T_1
F. diffusum	Air T_1
F. rigensis	Air T_1
F. denitrificans	Floor T_1
Flavobacterium. Not grouped	Air T_2
Flavobacterium. Not grouped	Water T_2
Flavobacterium. Not grouped	Water T_2
Pseudomonas viscosa. No acid in sucrose, dextrose or lactose	Air T_1, water T_1
Ps. fluorescens	Floor T_1
Pseudomonas. Not grouped	Air T_2
Proteus vulgaris	Air T_1, water T_1

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Table IV (*continued*).

Organism	Where found
Bacillus subtilis. Acid in dextrose, sucrose, maltose but not lactose and raffinose	Air T_1
B. subtilis. Acid in dextrose, sucrose mannitol, but not lactose or maltose	Air T_2
B. subtilis. Acid in dextrose, sucrose, lactose and raffinose	Air T_2
B. subtilis. Acid in sucrose and raffinose but not dextrose or lactose	Air T_2
B. subtilis. Acid in dextrose, sucrose, lactose and maltose	Air T_2
B. subtilis. Acid in dextrose and mannitol but not lactose	Air T_2
B. albolactis? Acid in dextrose, sucrose and lactose	Air T_1

Certain unidentified large rods resembling *Azotobacter*, some thermophiles, unidentified micrococci and diplococci were also found.

T_1 = "Type I" slaughterhouse; T_2 = "Type II" slaughterhouse.

Summary.

1. A preliminary survey of the bacterial flora of some different types of slaughterhouse indicates the sources from which heaviest contamination with bacteria is likely to occur.

2. Cleaner working conditions are shown to lead to a diminution in the numbers of organisms present in the air, swabbing water, etc., and in a reduction in the proportion of certain types of organism.

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