Effect of Delayed Evisceration on the Microbial Quality of Meat

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The postmortem invasion of muscle and other tissues by bacteria from the intestinal tract was studied with the use of radioactive tracers. The injection of 14C-labeled bacteria or spores into the intestines of guinea pig carcasses within 24 h of death resulted in the rapid spread of 14C throughout the carcasses. When live bacteria were injected along with the labeled cells, it was not possible to isolate viable organisms from the body tissues if the living animal had been exposed to the bacteria. It appears that animals are immune to their normal intestinal flora and that this immunity persists after death; thus passage of these bacteria into the lymphatic system does not necessarily result in the presence of live bacteria in carcass tissues. It therefore seems that a delay of up to 24 h before evisceration would not lead to deep tissue contamination of the carcass by organisms usually present in the intestines. Further evidence for this hypothesis was obtained by showing that muscle and lymph nodes from uneven- cinated lamb carcasses hung for 24 h at 20 C remained sterile.

It has been established, in vivo and in vitro, that the superficial lymphatic vessels of the intestine are readily permeable to particles of bacterial size (1), and that pathogenic bacteria placed in the intestine can rapidly pass to other body tissues by this route (3, 5, 16). It has therefore been generally assumed that, unless the viscera of slaughtered pigs, cattle, and sheep are removed soon after death, the meat will become contaminated by bacteria from the intestinal flora (9). However, it is clear that if aseptic precautions are observed sterile tissue can be readily obtained from normal healthy animals (6). Also, it has been shown that muscle from game birds and poultry stored for several days with the viscera in place remained sterile (4, 11). The present study was undertaken to resolve the apparent discrepancy between these observations.

MATERIALS AND METHODS

Cultures. Escherichia coli strain B, NCIB 9484, Salmonella typhimurium, a gift from Mannings Vaccine Laboratory, Hamilton, N. Z., and Bacillus cereus NCTC 8035 were maintained on nutrient agar slopes and grown in shake culture on Oxoid nutrient broth. A strain of Clostridium perfringens isolated from sheep was maintained on Difco cooked meat medium and grown on Oxoid reinforced clostridial medium.

Preparation of live and 14C-labeled bacterial and spore suspensions. All suspensions were prepared in 0.9% saline. Live bacterial suspensions were prepared from log-phase cells washed three times with saline. The final suspensions contained approximately 109 cells/ml. 14C-labeled bacteria were prepared by growing E. coli to the stationary phase in medium containing 10 µCi of [U-14C]glucose per ml. The cells were washed twice and then shaken with 2% formalin solution for 20 min. The fixed cells were washed twice and stored at -5 C. Immediately before use the cells were washed three times with saline.

14C-labeled spores were obtained by addition of [14C]lysine (20 µg/2 µCi per ml of broth) to a culture of B. cereus in which sporulation had been initiated. The spores were harvested and freed from cell debris by the method of Grecz et al. (7).

Live cell numbers were determined by plate counts, and fixed cell and spore numbers were determined by counting cells in a hemocytometer.

The 14C contents of the suspensions were determined by liquid scintillation counting of 1 ml of a suitable dilution of a suspension in 10 ml of Bray's scintillation fluid.

Inoculation of guinea pig intestine. Gut sacs were prepared from 5-cm lengths of small intestine washed with 0.9% saline. The sacs containing 1 ml of bacterial or spore suspension were suspended in 20 ml of saline with the cut ends above the surface of the bathing fluid. Samples of bathing fluid (1 ml) were removed as required, and 14C was determined by scintillation counting, using Bray's scintillation fluid.

For in situ experiments, guinea pigs were killed with chloroform vapor, the abdomen was opened, and 1 ml of the bacterial suspension was injected slowly into the small intestine or colon. The intestine was ligatured just below the point of entry of the needle.

Immunization and determination of antibody ti-
ters. Guinea pigs were immunized against S. typhi-
murium by two subcutaneous injections of 0.5 ml of
Mannings bivalent Salmonella vaccine, with a 10-
day interval between injections.

Serum antibody titers were determined by using
cell suspensions standardized against Brown's opac-
ity tube no. 1.

Sampling and enumeration of bacteria. Samples
of muscle (20 g) from the leg and loin and the in-
guinal lymph nodes were removed from lamb car-
casses using the aseptic method of Gardner and
Carson (6). Samples (2 g) from guinea pigs were
removed using sterile instruments but without prior
searing of the flesh.

Tissue samples for viable counts were homogen-
ized with peptone water (10 ml/g, wet weight) in a
Waring blender. Suitably diluted samples (0.1 ml)
were spread in triplicate on nutrient agar (Difco)
plates, which were incubated at 37 C for both aero-
bic and anaerobic counts. Test organisms were iden-
tified by growth on selective agar plates: E. coli
were identified on MacConkey's agar and C. perfrin-
gens on Shahidi and Ferguson perfringens agar (13).
Triple sugar iron agar and lysine broth were used
for the preliminary screening of S. typhimurium,
and the identification was confirmed by agglutina-
tion of cells with specific antisera.

For the estimation of 14C in tissues, samples (200
mg) were oxidized by the method of Van Slyke and
Folch (15). Carbon dioxide was trapped on fiber
glass paper wetted with hyamine hydroxide solution
(1 M in methanol), and 14CO2 was determined by
liquid scintillation counting, using a solution of 2:5
diphenylloxazole in toluene (4 g/liter) as the scintil-
lation fluid.

RESULTS

Passage of bacteria across the intestinal wall.
Guinea pigs were examined for the presence of antib-
odies against the test organisms. Titers against E. coli
varied from 60 to 460 antibody units/ml of serum, but no
titers were obtained against C. perfringens.

To determine whether immunization against
individual bacteria is necessary for the mainte-
nance of tissue sterility after death, live S.

The sera of the guinea pigs were examined
(Table 1). Similar results were obtained with two other
carcasses.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>14CO2 (dpm/g, wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2,600</td>
</tr>
<tr>
<td>10</td>
<td>3,200</td>
</tr>
<tr>
<td>15</td>
<td>6,200</td>
</tr>
<tr>
<td>30</td>
<td>5,500</td>
</tr>
<tr>
<td>60</td>
<td>5,800</td>
</tr>
</tbody>
</table>

* Similar results were obtained with two other
carcasses.

Table 2. Distribution of 14C in guinea pig carcass
tissues 15 min after injection of a suspension of 14C-
abeled bacteria (10^5 cells/ml; 1.1 x 10^6 dpm/ml) or
spores (3.9 x 10^5 spores/ml; 1.3 x 10^6 dpm/ml)*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>14CO2 (dpm/g, wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart blood</td>
<td>1,300</td>
</tr>
<tr>
<td>Lung</td>
<td>2,000</td>
</tr>
<tr>
<td>Liver</td>
<td>2,700</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>600</td>
</tr>
<tr>
<td>Spleen</td>
<td>1,100</td>
</tr>
<tr>
<td>Muscle</td>
<td>1,100</td>
</tr>
</tbody>
</table>

* Similar results were obtained with nine other
carcasses inoculated with bacterial suspensions and
two inoculated with spore suspensions.
typhimurium suspensions were injected into the intestines of carcasses from seven guinea pigs immunized against the organism and seven nonimmunized animals. Samples were removed from the carcass 30 min after injection of the suspensions.

A measurable antibody titer against S. typhimurium was not obtained with serum from any of the animals. However, S. typhimurium could not be isolated from any tissue of the immunized animals, whereas the bacteria were present in numbers in excess of 10^4/ml in the spleen and lymph nodes of three of the control animals. The tissues of the other four control animals were free of bacteria.

Microbial contamination of uneviscerated lamb carcasses. The above observations suggest that delay before evisceration should not result in any microbial contamination of carcasses by invading bacteria from the normal intestinal flora. To test this hypothesis, six skinned lamb carcasses with the viscerum in place were obtained from a local abattoir and hung at room temperature (20 C) for 24 h before samples of muscle and lymph nodes were removed aseptically. No bacterial growth occurred on any of the nutrient agar plates spread with undiluted tissue homogenates. The homogenates were incubated overnight at 37 C, but plates prepared from these incubated homogenates were again free of bacteria.

DISCUSSION

Examination of the effect of delayed evisceration on the microbial quality of sheep carcasses has confirmed the observations made by Barnes and Shrimpton (4) and Mead et al. (11) with birds, i.e., that leaving the viscerum in carcasses for 24 h does not result in the invasion of muscle or lymph nodes by viable organisms from the intestine.

There would appear to be two possible hypotheses to explain why, in spite of the ease with which bacteria can enter the lymphatic system from the intestine, no invasion of other tissues by bacteria of the normal intestinal flora was observed in uneviscerated carcasses. It could be that the spread of bacteria from the intestine is so slow that the duration of the experiments was too short for the bacteria to reach other tissues or that the death of the animal does not impair all immunity mechanisms, which continue to rapidly kill any bacteria reaching the lymphatics.

The observations reported here favor the latter hypothesis. Radioactively labeled bacteria and spores appeared to spread rapidly to all tissues after their injection into the intestines of carcasses. No explanation can be offered for the mechanisms involved in this rapid spread of bacteria when there is no blood circulation. Live bacterial suspensions were also injected into the intestines of carcasses, and tissue samples were removed 30 min later. In cases where the animal had been immunized against the test organism, or when the test organism was of a type normally present in the intestine, no viable bacteria could be isolated from any tissue examined. However, in three out of seven cases where carcasses from nonimmunized animals were inoculated with the pathogen S. typhimurium, large numbers of viable organisms were isolated from the lymph nodes and spleen. This seems to confirm that bacteria can spread to carcass tissues from the intestine at the rate indicated by the experiments with radioactively labeled particles. It also implies that both specific and nonspecific immunity mechanisms, which have been shown to operate in resistance to Salmonella infection (8), are involved in the destruction of invading bacteria, since not all carcasses from nonimmunized animals became infected. Although a high titer of specific circulating antibody is not necessary for the prevention of bacterial invasion, in the absence of acquired immunity to specific bacteria successful invasion can occur. Since bacteria can enter the lymphatics of the intestine during life, animals must be immune to the organisms of their normal intestinal flora and therefore carcasses would not be successfully invaded by these organisms until the immune defense mechanisms had ceased to function.

A possible objection to the hypothesis is the observation that small numbers of nonpathogenic bacteria can be isolated from the lymph nodes of about 30% of animals after slaughter, although no evidence has been presented to show that these organisms are of intestinal origin and some have been identified as belonging to genera that cannot grow in the intestine (10, 12).

Salmonella is also found in the lymph nodes of pigs, sheep, and cattle, but pathogens are by definition not part of the normal flora. It has been shown that the ingestion of Salmonella by susceptible animals can result in a rapid spread of this organism throughout the body, but relatively high doses are generally required (5, 14).

This seems to indicate that nonspecific immunity mechanisms prevent successful invasion by small numbers of Salmonella from the intestine. Small doses of Salmonella may stimulate the defense mechanisms to protect the animal against later, larger doses of infection, and the tissues of these immune animals re-
main free of the pathogen (5). Animals often become infected after entering the abattoir, that is, within a day or so of slaughter (2), and infection of lymph nodes, spleen, and other organs occurs after invasion through the upper respiratory tract (14). Presumably the nonpathogenic bacteria found in lymph nodes could have entered the body in a similar manner.

The processes involved in the initial stages of bacterial invasion are little understood (17), and the persistence of immunity mechanisms after death does not appear to have been the subject of extensive study (18), so the explanation of our observations can only be advanced as a basis for further research. However, the assumption that delay in evisceration of carcasses can lead to contamination of the meat by bacteria of the normal intestinal flora appears to be incorrect, and therefore it is not a valid reason for the present practice of condemning such carcasses.

ACKNOWLEDGEMENT

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LITERATURE CITED