Joseph R. Melvin, Jr.,[1] *Ph.D.; Lois S. Cronholm,*[2] *Ph.D.; Laurence R. Simson,*[1] *Jr., M.D.; and Avrom M. Isaacs,*[3] *M.D.*

Bacterial Transmigration as an Indicator of Time of Death

This paper is dedicated to the memory of Dr. Avrom M. Isaacs.

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ABSTRACT: Time of death is difficult to evaluate in many forensic science situations. We have developed an animal model for assessing the time of death by evaluating the transmigration of normal microbiota through the wall of the small intestine. A segment of small intestine was removed from decapitated CF-1 mice (Carnsworth Farms) and suspended in vitro in a beaker containing sterile phosphate-buffered saline. Bacterial transmigration was evaluated in this model over a three-day period at select temperatures (4, 25, and 37°C) by microbiological cultures and scanning electron microscopy (SEM). Evidence of bacterial transmigration by SEM occurred within 2 to 3 h at 37°C, 5 to 6 h at 25°C, and 72 h at 4°C. Analysis of the microbiological data indicated a differential flux of select bacterial and mycotic organisms. Staphylococcal species were the first organisms to be cultured from the suspending saline. These organisms are known to elaborate powerful protease enzymes that may play an important role in the degeneration of gut tissues. Coliform-type organisms and *candida* species were found at later times after death. The last major groups of bacteria to be identified were a variety of anaerobic species. This model may be adaptable to certain situations in human forensic pathology.

KEYWORDS: pathology and biology, bacteria, death, bacterial transmigration, time of death, scanning electron microscopy, microbiological cultures

An accurate determination of time of death (TOD) is extremely useful in many medicolegal situations. Currently, there is no single method of making an exact determination of this time. Time of death has been traditionally estimated by select postmortem changes (for example rigor mortis or algor mortis) or physical-chemical observations. All of these approaches have intrinsic limitations and must be evaluated with care.

We present here an animal model using yet another approach to estimate time of death. This system is based on the postmortem transmigration of endogenous microbiota through the wall of the mouse small intestine. This approach may have future applications in human forensic pathology.

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[1]Forensic scientist and forensic pathologist, respectively, Department of Pathology, Edward W. Sparrow Hospital, Lansing, MI, and Great Lakes Institute of Forensic Science, Lansing, MI.

[2]Microbiologist, Department of Biology, University of Louisville, Louisville, KY.

[3]Deceased; formerly with Department of Pharmacology, University of Louisville School of Medicine, Louisville, KY.

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Materials and Methods

Gut Preparation

The small intestines of adult CF-1 mice were surgically resected after decapitation by strict aseptic technique. Three sections of the intestines were isolated with ligatures. One end, resected 10- to 15-mm from the duodenum, was placed immediately in 50 mL of cold 2.5% buffered glutaradehyde. This specimen served as the control to show the immediate postmortem status of the tissue. A 10- to 15-mm section from the opposite end of resected intestine was used for microbiological cultures of the luminal contents. The ends of the remaining intestine were cauterized with phenol and suspended in a sterile flask containing 700 mL of phosphate-buffered saline (Fig. 1) according to the method of Kellarman et al [*1*].

Scanning Electron Microscopy

Fixed tissues for scanning electron microscopy (SEM) were dehydrated in graded ethanol, treated with amyl acetate, and subjected to critical point drying in an Aminco Mark 40

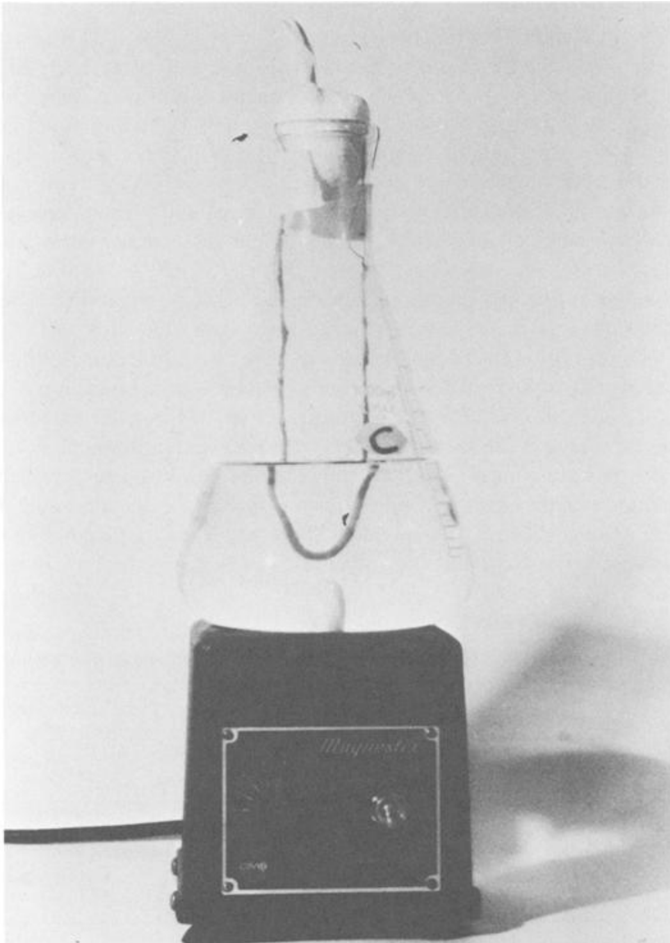

FIG. 1—*Mouse intestine preparation.*

(American Instrument Co.). The dried specimens were mounted on stubs, coated with 20 nm of gold, and viewed in a Cambridge Stereoscan (Mark 2-A) electron microscope operated at 20 kV.

Experimental Model

A series of these gut preparations were incubated at 4, 25, and 37°C to generate a model for postmortem bacterial transmigration. In one set of experiments, the surface topography of the degenerating gut was examined after incubation at these temperatures. These examinations were conducted at select intervals over a 72-h period by using gut tissue above the saline level of the preparation (Fig. 1). The second set of experiments evaluated the transmigration of normal microbiota into the physiologic saline of the gut preparatation. Aerobic, mycotic, and anaerobic cultures were performed by standard methods of the same test period.

Results and Discussion

The postmortem transmigration of microorganisms to the outer surface of the gut preparation in our model was found to be time- and temperature-dependent. Microbes first began to appear on the gut surface within 2 to 3 h after death for samples at 37°C, 5 to 6 h at 25°C, and 72 h at 4°C. A detailed breakdown by species is given in Table 1. By SEM, bacterial transmigration appeared to be associated with a concatenation of surface changes. These surface changes were consistent for the various test temperatures and could be categorized into the following stages: (1) initial degeneration and loss of the outer serosal covering, (2) focal exposure of underlying connective and muscular tissues, (3) formation of surface furrows with a concommitant penetration of microorganisms, and (4) the presence of a variety of bacterial morphologies on the surface with marked disorganization of the subsurface structures (Figs. 2 to 4).

The microbiological analysis closely paralleled the SEM observations. The presence of microbes on the surface of the gut by SEM was always associated with positve bacteriologic cultures. Bacteria and fungi found on the surface were also cultures from the luminal contents. There appeared to be a differential flux of bacteria and fungi into the physiologic saline (Table 1). Regardless of the test temperature, coagulase-positive bacteria of the genus *Staphylococcus* were always the first organisms to be detected in the sequential culturs. Organisms of this genus are known to elaborate a variety of powerful protease and collagenase enzymes [*2*]. These enzymes may play an important role in the degeneration of the devitalized intestine. As the gut further degenerated, there was a gradual change to cultures with coliform organisms as the dominant flora. At these times, it was also possible to detect my-

TABLE 1—*Microbiological analysis: appearance of specific groups of microbes.*[a]

	Time of Appearance After Death of Different Organisms		
Temperature, °C	*Staphylococcus* Species	Coliforms and Fungi	Coliforms and Anaerobes
4	66–68 h	few species at 68–72 h	rare at 72 h
25	5–6 h	8–10 h	12–16 h
37	2–3 h	4–5 h	6–8 h

[a]Data are for cultures taken from the saline of the gut preparation. The saline and the surface of the resected gut were both found to be sterile before the experiment. The major groups of bacteria found on the surface of the gut after transmigration were also found in the cultures of the luminal contents.

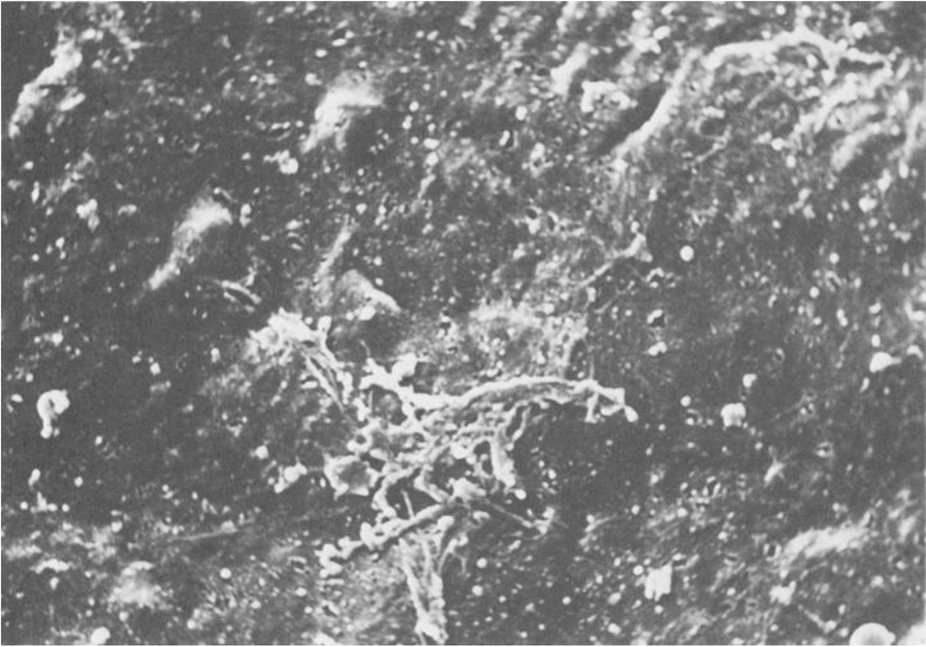

FIG. 2— SEM, ×6000. *High-magnification image of the surface of the control section of intestine. The serosal surface is intact and there is no evidence of underlying fibrocollagen structures.*

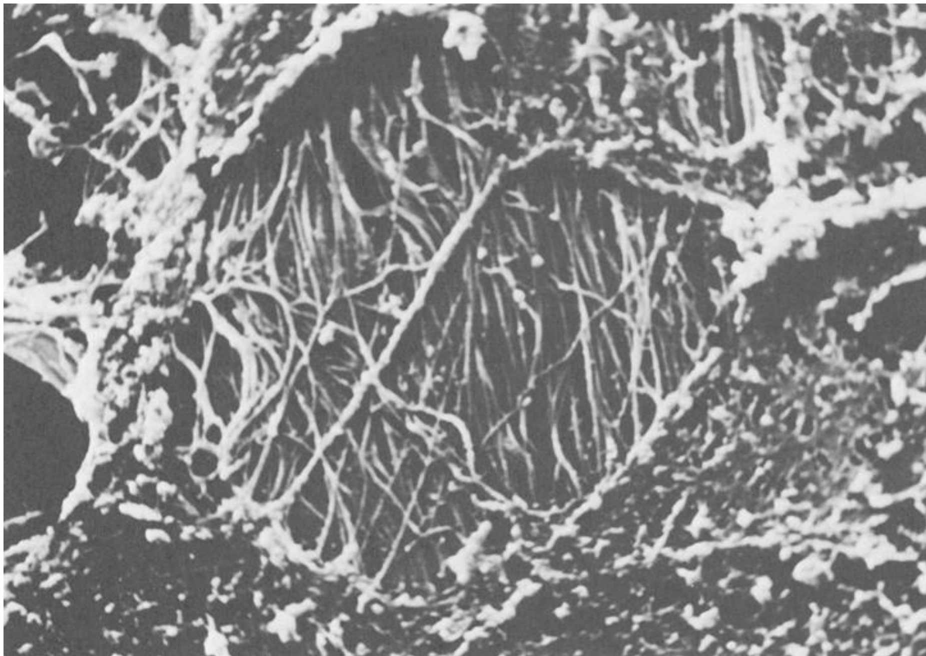

FIG. 3—SEM, ×6000. *High magnification of the surface of the gut 4 h after death at 25°C. There is loss of the serosal covering and exposure of fibrocollagen tissue.*

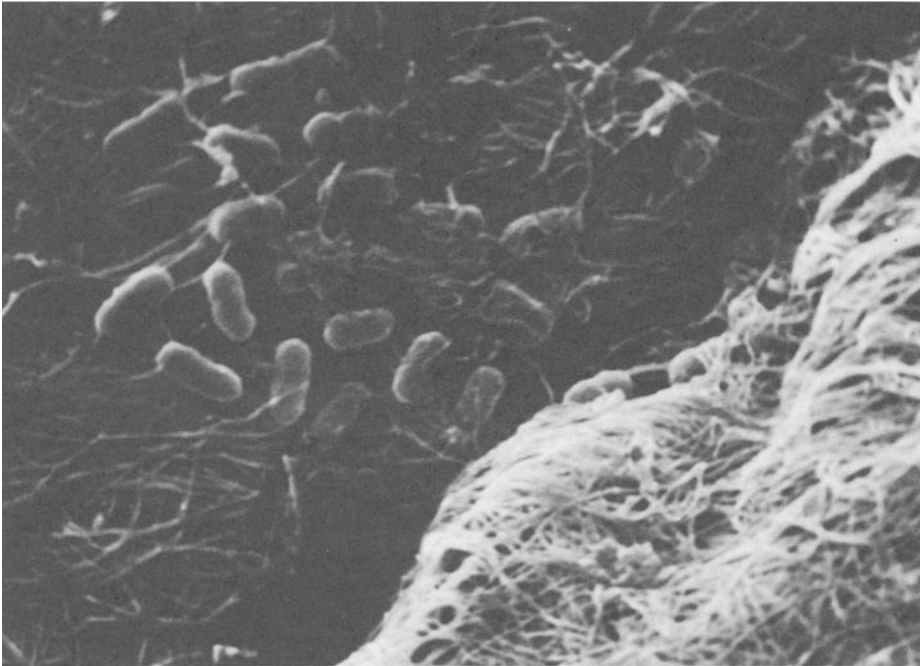

FIG. 4—SEM, ×6000. *High-magnification profile of the surface of the gut 20 h after death at 25°C. Image shows a group of morphologically similar bacterial forms associated with a furrow. The fibrocollagen matrix is very dispersed and disorganized in reference to earlier degenerative changes.*

cotic organisms, principally *candida* species. Lastly, there was an admixture of coliforms and organisms regarded as faculative anaerobes.

The development of a reliable method that can accurately assess the time of death in many settings has been an elusive goal for forensic pathology. Indeed, an exact determination of this time may be beyond our current scientific capabilities. This is due largely to the numerous parameters that must be analyzed and controlled in such determinations. Temperature, humidity, rate of body cooling, pre-existing disease, antemortem immune status, and countless other factors undoubtedly affect any such assessments.

Our model attempted to control some of these variables by exactly regulating the time of death, temperature of the gut preparation, and the antemortem status of the test animal. It is based on changing ecological relationships between living, endogenous microbiota and a dead host. This model demonstrated that there was a sequence of predictable postmortem events resulting int he transmigration of certain classes of microorganisms through the gut wall. In our laboratory, an analysis of the surface topography and bacteriologic status of the gut could be used to estimate the time of death in a retrospective fashion.

How this model could be applied to human forensic pathology is dependent on subsequent research using human cadavers. Obviously, the surface topography and microbial ecology of the human small intestine are different than those of the mouse. Work by Kellerman et al [*1*], however, suggests that there is a similar mode of postmortem bacterial transmigration in the human gut. Postmortem culturing of the surface of the human gut may therefore be predictive in an analogous fashion to our model. This system may also generate useful information in correlating antemortem-postmortem bacteriology, evaluating the transplantation of organs from cadavers, and studying the pathology-clinical medicine of certain intestinal obstructions (for example, intussusception or volvulus) resulting in tissue devitalization.

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Address requests for reprints or additional information to
Joseph R. Melvin, Jr., Ph.D.
Department of Pathology
Edward W. Sparrows Hospital
1215 E. Michigan Ave.
Lansing, MI 48909