tion. Subcortical white matter was one of the warmest sites in the brain and also had the greatest thermal inertia of all the intracranial structures in which temperature measurements were made. The genesis and functional significance of intracerebral temperature gradients are still unknown. In the past a major problem in evaluating these intracerebral gradients has been the continuous fluctuations of brain temperature. It would now appear that simultaneous measurements of arterial blood temperature and various brain temperatures will permit a more accurate assessment of those fluctuations in brain temperature due to altered neuronal or glial metabolism, *local* blood flow and *local* blood temperature in contrast to those changes that result simply from changes in the mean temperature of arterial blood coming to the brain.

Summary. Temperature measurements were made simultaneously in the arterial blood and various brain structures in 5 chronically prepared rhesus monkeys at rest in a lighted, sound-attenuated, thermoregulated chamber. The level of alertness of the animals was also noted by observing changes in overt behavior together with EEG and EMG recordings. Spontaneous and induced shifts in the level of arousal were followed in seconds by warming or cooling of the arterial blood and seconds to minutes later by parallel shifts in the temperature of the majority of the brain sites. Arterial blood temperature was thus found to yield a sensitive index of the behavioral state of the monkey in relation to sleep and arousal. Changes in the mean temperature of the arterial blood going to the brain appeared primarily responsible for temperature fluctuations in the brain. A number of steadily maintained but differing thermal gradients were found to exist between regional brain sites and the arterial blood.

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Enhanced Bacterial Virulence in Fluids Produced in Strangulation Intestinal Obstruction.* (30828)

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Fluids accumulating in the peritoneal cavity and in the small intestine during the irreversible course of strangulation intestinal obstruction are lethal when injected into healthy animals(1). These fluids are malodorous, red to reddish black in color, and laden with bacteria. Clostridia, coliforms, bacteroides and streptococci are respectively the most numerous flora(2). Supernates from centrifuged strangulation fluids often lose lethality when sterilized by filtration. In some cases sterile fluids retain their lethality owing to the presence of preformed clostridial exotoxins(3). Bacteria exert a role in the

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pathophysiology of human and experimental strangulation obstruction along with several interrelated determinants—site of the obstruction, length of the strangulated segment, fluid and electrolyte balance, blood loss, intraluminal pressure, and shock. Various authors have attributed the lethal bacterial factor to whole bacteria and bacterial endotoxins(4,5), clostridial exotoxins(3,6) and products of tissue breakdown(1). Current work suggests no one bacterial factor exists as the major lethal agent. Rather, under a variety of circumstances varying combinations of each bacterial factor probably contribute to the lethal outcome(3).

A specific synergism has been postulated to occur in mixtures of *Escherichia coli* and red blood cells, and to be necessary for strangulation fluid to be lethal(7). The primary toxic factor in strangulation fluid was reported to be achieved by adding a sufficient number of red blood cells to a broth culture of *E. coli*(5). This combination exerted the same physiologic effects on recipient animals as did strangulation fluids, and presented the clinical features of death following injection of Gram-negative bacterial endotoxin. This synergism has not been confirmed(3,8,9).

Amundsen and Midtvedt(8) observed that the number of *E. coli* found in strangulation fluids lethal for assay mice was far less than the number of *E. coli,* suspended in saline solution, needed to kill a mouse following intraperitoneal injection. Amundsen proposed that during strangulation obstruction disintegrating tissue releases an ultrafilterable factor that enhances the virulence of suspensions of *E. coli* injected intraperitoneally into healthy mice(10). Saltz, Haas, and Servadio(9) demonstrated that strangulation fluid served as an excellent culture medium for bacterial multiplication, and that clostridia in combination with sterile filtrate were the least invasive of the bacteria studied.

The present study examines the virulence-enhancing activity of sterile non-lethal ultrafiltrates of strangulation fluids from humans, dogs, and rats. Individual combinations of fluids with *E. coli, Clostridium welchii (Cl. perfringens),* and *Cl. sordelli (Cl. bifermentans)* were assayed. The likelihood that mucin and toxic substances such as bile, endotoxin, and exotoxin might contribute to the virulence-enhancing effect of strangulation fluids was investigated.

Materials and methods. Specimens of either intestinal contents or peritoneal fluid, or both, were aspirated at operation from 4 patients with strangulation obstruction. Peritoneal fluids were collected at death from 3 dogs with experimentally produced strangulation obstruction. Peritoneal fluid collected at death from 10 Sprague-Dawley rats with experimental strangulation obstruction was pooled. The experimental surgical procedures have been described(1,2,9).

All raw strangulation fluids were lyophilized, resuspended to 6% with distilled water (w/v), and homogenized in a blendor(10). A portion of each was saved for assay of lethality; the remainder was centrifuged at a mean force of 80,000 $\times$ *g* for 60 minutes in a Spinco model L ultracentrifuge. The supernate was collected, a portion was withdrawn for assays, and the rest was sterilized by passage through Millipore filters. A sample of each ultrafiltrate was inoculated into a tube of thioglycollate broth. These cultures were sterile after five days' incubation, and confirmed the sterility of ultrafiltrates.

In addition, sterile ultrafiltrates of the following materials were prepared: canine normal small bowel contents, crystalline hemoglobin prepared from human blood(11), and canine peritoneal fluid aspirated when death occurred from bile peritonitis 6 hours after intraperitoneal injection of a 1:2 dilution of sterile cattle bile (30 ml/kg of body weight)(12). Female albino mice of the Rockland-All-Purpose strain (Rockland Farms, Gilbertsville, Pa.) were used throughout this study. All test substances were injected intraperitoneally at a dosage of 20 ml/kg of body weight, and mice were observed for 72 hours. The lethality of each strangulation fluid before and after ultracentrifugation and after sterilization was assayed in 2 mice. Groups of at least 6 mice were used to assay the ability of test substances to enhance the virulence of bacterial suspensions.

The following bacteria were studied: a

TABLE I. Enhanced Virulence of *E. coli* Suspended in Sterile, Non-Lethal Ultrafiltrates.

Specimen	No. of mice dead/No. of mice injected; scored at 24 hr
Human strangulation fluids:	
Loop fluid (Patient W.F.)	3/6
" " (Patient H.S.)	6/6
" " (Patient P.B.)	5/6
Peritoneal fluid (Patient P.B.)	6/6
" " (Patient H.B.)	3/6
Canine strangulation fluids:	
Peritoneal fluid (Dog #6516)	6/6
" " (Dog #3680)	5/6
" " (Dog #2678)	6/6
Rat strangulation fluid:	
Peritoneal fluid (pooled)	6/6
Canine bile peritonitis fluid (Dog #9399)	5/6
Canine normal small bowel contents	6/12
Human hemoglobin	0/6
Controls (not ultrafiltrates):	
Human serum (pooled)	0/6
Trypticase soy broth	0/6
Saline solution	0/16

strain of *E. coli* isolated from a hospital wound infection, a laboratory strain of *Cl. welchii* (*Cl. perfringens*), and *Cl sordelli* (*Cl. bifermentans*) A.T.C.C. strain #9715. Cells of *E. coli* were harvested by centrifugation from overnight broth cultures, washed twice with sterile saline solution and adjusted to read 56%T at 610 mμ in a Coleman Jr. spectrophotometer. Such suspensions contained 10^8 bacteria/ml, and were diluted 1:100 with either saline solution or test material for assays of virulence. Overnight cultures of the clostridial species in thioglycollate broth were diluted 1:10 for testing; these test suspensions contained approximately 10^7 bacteria/ml.

Gastric mucin and several toxic substances that might be involved in the virulence-enhancing effect of strangulation fluids were also studied. Sublethal dosages of the following preparations were used to suspend the test bacteria: sterile bile from yearling cattle, endotoxin prepared from acetone dried cells of *E. coli* by trichloroacetic acid extraction (13), lyophilized exotoxin prepared by ammonium sulfate fractionation of a clarified *Cl. welchii* broth(14), and gastric mucin (Granular Type 1701-W, Lot #70411, Wilson Laboratories, Chicago, Ill.) prepared as specified on the label.

Results. None of the strangulation fluids was lethal for mice after ultracentrifugation or after sterilization, even though several were lethal before ultracentrifugation. Sterile ultrafiltrates of strangulation fluids enhanced the virulence of *E. coli* for mice (Table I). Amundsen's(10) finding with peritoneal fluid from rats was confirmed and extended to include fluids from humans and dogs. Virulence was also enhanced by sterile ultrafiltrates of normal small bowel contents and bilious peritoneal fluid, but not by hemoglobin. *E. coli* was not virulent when suspended in serum, Trypticase Soy Broth, or saline solution.

Individual ultrafiltrates of the strangulation fluids were pooled for subsequent studies with *E. coli* and the 2 clostridial species (Table II). The virulence-enhancing effect on *E. coli* was demonstrated again. However, the clostridial species killed only when suspended in the strangulation fluids, and then only a few mice. Serial dilutions of the pooled ultrafiltrates of human and canine peritoneal

TABLE II. Enhanced Virulence of Test Bacteria Suspended in Sterile, Non-Lethal Ultrafiltrates.

Specimen	No. of mice dead/No. of mice injected; scored at 24 hr		
	E. coli	*Cl. welchii*	*Cl. sordelli*
Sterile ultrafiltrate:			
Human loop fluid (pooled)	6/6	0/6 (2/6)*	0/6
" peritoneal fluid (pooled)	4/6	1/6	3/6
Canine peritoneal fluid (pooled)	5/6	0/6 (1/6)	0/6
" bile peritonitis fluid	5/6	0/6	0/6
" normal small bowel contents	5/6	0/6	0/6
Controls:			
Human serum (pooled)	0/6	0/6	0/6
Saline solution	0/6	0/6	0/6

* Figures in parentheses refer to 48 hr observations when different.

TABLE III. Enhanced Virulence of Test Bacteria Suspended in Sublethal Doses of Toxic Substances Possibly Present in Strangulation Fluids.

Specimen	No. of mice dead/No. of mice injected; scored at 24 hr		
	E. coli	*Cl. welchii*	*Cl. sordelli*
E. coli endotoxin	4/6	0/6	2/6 (3/6)*
Cl. welchii exotoxin	3/6	0/6	0/6
Yearling cattle bile	5/6	5/6	2/6 (5/6)
Gastric mucin	6/6	5/6	6/6
Saline solution	0/6	0/6	0/6

* Figures in parentheses refer to 48 hr observations when different.

fluids were used to titrate the virulence-enhancing effect. Virulence-enhancement was lost when fluids were diluted 1:2.

In an attempt to identify the factor in strangulation fluids, mucin and several toxic substances which might possibly contribute to the virulence-enhancing effect were studied (Table III). The 3 bacterial species were lethal when suspended in sublethal dosages of mucin or bile. *E. coli* and *Cl. sordelli* killed when suspended in a sublethal dosage of coli endotoxin, and only *E. coli* killed when suspended in a sublethal dosage of *Cl. welchii* exotoxin.

Discussion. These data indicate that strangulation fluids from humans, dogs, and rats promote a rapid, lethal, bacterial infection in healthy mice by fewer bacteria than is possible without the fluids. The virulence-enhancing factor enhances lethality of *E. coli* and has a limited effect on the virulence of *Cl. welchii* and *Cl. sordelli*. Opportunists such as *E. coli* produce disease when the resistance of the host is lowered sufficiently to allow invasion of tissue. Alternatively, the pathogenic clostridia are not particularly invasive, and cause an initial local infection only when introduced into traumatized tissue. Their pathogenicity depends upon production of powerful exotoxins. Accordingly, the intraperitoneal injection of clostridia, suspended in non-lethal strangulation fluid, would not be expected to be virulent. This does not, however, exclude a lethal role for clostridial exotoxins produced during strangulation intestinal obstruction(3,6).

A wide variety of substances is known to potentiate the intraperitoneal invasiveness of bacteria. These include polysaccharides, proteins, gastric mucin, whole bacterial cells, bacterial cell wall preparations, cellular constituents, and endotoxins(15,16,17). The ultrafiltrates were prepared from aqueous extracts of lyophilized mixtures of bacteria, serum and tissue proteins, small intestinal contents, and blood. Their ability to promote a lethal infection was not surprising. The factor in strangulation fluids was without effect once the ultrafiltrate was diluted in half, and initially was either present in low concentration or not very active. Each test microorganism killed when suspended in mucin presumably owing to the ability of mucin to lower host resistance(15). Accordingly, the factor in strangulation fluid may not function in the same manner as does mucin. The identity of the factor(s) in strangulation fluid is unknown although it has been suggested that it is a protein released from disintegrating tissue(10).

The demonstration that sub-lethal dosages of *Cl. welchii* exotoxin enhanced the virulence of *E. coli* for mice points to the possible occurrence of a secondary role for clostridial toxins that are produced in strangulation obstruction, but are not actively toxic. Similarly, the enhanced virulence of *Cl. sordelli*, suspended in a sublethal dosage of *E. coli* endotoxin, suggests a secondary infective role for *Cl. sordelli* in strangulation obstruction in addition to toxigenesis.

A sublethal dosage of bile enhanced the virulence of all the test bacteria. Previous study of experimental bile peritonitis has shown that an overwhelming bacterial peritonitis is favored during a slow continual spillage of bile into the peritoneal cavity from an internal bile fistula(18). Alternatively, bile peritonitis due to injecting bile intraperitoneally produces an acute "chemical burn" causing the peritoneal surface to exude large quantities of plasma-like fluid. Neither injury nor exudate follow injection of diluted bile, and animals can tolerate dosages of diluted bile far in excess of the undiluted lethal dosage(12). Accordingly, an innocuous accumulation of bile in a strangulated and obstructed segment of intestine may potentiate polymicrobic infections.

Our present evidence is not adequate to

establish a role for a bacterial virulence-enhancing factor in the pathophysiology of strangulation obstruction, but suggests that synergism between bacteria of one species and products of another can occur in this polymicrobic surgical disease. Further work is needed to determine the nature of the virulence-enhancing factor in strangulation fluids, and whether the factor(s) acts in strangulation obstruction by directly enhancing the virulence of endogenous intestinal bacteria, or by depressing host resistance to infection.

Summary. Sterile, non-lethal ultrafiltrates of strangulation fluids from humans, dogs, and rats promoted a rapid, lethal *E. coli* infection in healthy mice by fewer bacteria than was possible with saline suspensions of the same organisms. *Cl. welchii* and *Cl. sordelli,* similarly suspended, killed only a small proportion of test mice. Sublethal dosages of several substances that might contribute to the virulence-enhancing activity of strangulation fluids were also studied: *E. coli* endotoxin enhanced the virulence of *Cl. sordelli* and *E. coli; Cl. welchii* exotoxin enhanced only *E. coli;* bile and mucin enhanced all 3 test microorganisms. Synergism between bacteria of one species and products of another may contribute to the severity of mixed bacterial infections. The identity of the factor in strangulation fluids is unknown. There is as yet no evidence of a role for a bacterial virulence-enhancing factor in the pathophysiology of strangulation intestinal obstruction.

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Thyro-Adrenal Relationships in Maternal Fetal and Neonatal Guinea Pigs: Effects of Goitrogens* (30829)

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Knowledge regarding placental permeability to maternal hormones and the maturation and functional interrelationships of the mammalian fetal endocrine systems is incomplete. Jost(1) and Mitskevich(2) have summarized pertinent experimental data on the pituitary-thyroid and adrenal systems in the rodent. Critical analysis indicates that there are mutual compensations in hormone function of mother and fetus and that these reflect differences in placental transmission for different hormones, as well as for type of placenta and stage of pregnancy. Information on the thyro-adrenocortical relationship in maternal

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