Effect of Bile on the Action of Blood Against *Salmonella*

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ABSTRACT

KAYE, DONALD (New York Hospital-Cornell Medical Center, New York, N.Y.), MICHELLE PALMIERI, AND HEONIR ROCHA. Effect of bile on the action of blood against *Salmonella*. J. Bacteriol. 91:945-952. 1966.—Bile is superior to Trypticase Soy Broth (BBL) as a medium for isolation of *Salmonella* from blood. In addition to inhibitory activity against many of the common bacterial contaminants, bile has the additional advantages of a greater frequency of isolation of *Salmonella* and more rapid isolation. This study investigated the factors responsible for the superiority of bile. A 20% dilution of normal human blood in Trypticase Soy Broth was usually inhibitory or bactericidal against strains of *Salmonella*. Similarly, a 20% dilution in Trypticase Soy Broth of serum from patients with *Salmonella* bacteremia was frequently inhibitory or bactericidal for the salmonellae isolated from their blood. Bile inactivated the bactericidal activity of blood or serum by inactivating complement activity. Bile (13%) in Trypticase Soy Broth inactivated complement activity in 33% blood or serum. Although bile has anticoagulant and hemolytic effects, no evidence was found that these activities contribute to the superiority of bile over Trypticase Soy Broth as a culture medium for *Salmonella*. No evidence was found for a growth-promoting factor in bile for *Salmonella*. Bile was much more effective than 0.5 or 2.0% sodium taurocholate broth in inactivating the bactericidal activity of blood.

Bile has been recommended as a culture medium for isolation of *Salmonella* from blood (1, 6, 7, 8, 14). The reason usually stated for the superiority of bile is that it is inhibitory or lethal for many of the common bacterial contaminants (4, 6, 13).

Previous studies were undertaken to compare bile and Trypticase Soy Broth (BBL) as media for culture of blood from patients with *Salmonella* bacteremia. In these studies, there was a lower rate of contamination in bile. In addition, bile had the advantages of a greater frequency of isolation and more rapid isolation of *Salmonella*. The superiority of bile can be demonstrated by the fact that 88% of cultures were positive in bile, as compared with 68% in Trypticase Soy Broth. Furthermore, 78% of positive blood cultures in bile were positive after 24 hr of incubation, and all were positive after 72 hr, whereas none of the positive cultures in Trypticase Soy Broth was positive after 24 hr, and only 55% were positive after 72 hr.

Possible explanations for the more rapid and

higher frequency of isolation of *Salmonella* from blood cultures in bile are (i) destruction of inhibitory or bactericidal activity of blood, (ii) prevention of coagulation of blood, thereby preventing trapping of bacteria in clots or release of bactericidal factors by the process of coagulation, (iii) lysis of leukocytes containing *Salmonella*, and (iv) presence of a growth-promoting factor in bile. The present study was undertaken to investigate the factors responsible for the superiority of bile over Trypticase Soy Broth in the isolation of *Salmonella* from blood.

MATERIALS AND METHODS

Bacterial strains. All strains of *Salmonella* studied were isolated from blood of patients hospitalized at the Hospital Prof. Edgard Santos, Salvador, Bahia, Brazil, from January to May 1965. Each strain was classified serologically by J. W. Winter of the New York Salmonella Center at Beth Israel Hospital, New York. The inocula for all experiments were dilutions of 18-hr cultures in Trypticase Soy Broth.

Bacterial enumeration. The number of *Salmonella* per milliliter of medium was determined by preparing

serial 10-fold dilutions in Trypticase Soy Broth and plating 1-ml samples of each dilution in Trypticase Soy Agar pour plates. Colonies were counted after incubation of the plates for 24 hr at 37 C.

Blood and serum. Blood was removed from patients with a sterile syringe and was immediately pipetted into tubes containing the different media. Blood from five healthy individuals (M.P., H.R., D.K., W.R., E.T.) was studied for inhibitory activity against six strains of *Salmonella*. M.P. and D.K. had received three injections of 0.5 ml of typhoid and paratyphoid vaccine (Wyeth Laboratories, Inc., Marietta, Pa.) 3 to 6 months prior to the experiments; E.T. had received one or more injections of a typhoid vaccine 11 years prior to the experiments; and H.R. and W.R. had never received immunization against *Salmonella*.

In addition, blood from six patients with *Salmonella* bacteremia (not receiving antibiotic therapy) was divided into samples for culture and samples for collection of serum. The sera were stored at -20 C, and each was later tested for inhibitory activity against the *Salmonella* strain simultaneously isolated from the same blood specimen. None of these patients had received previous immunization against *Salmonella*.

Inhibitory activity of blood or serum. The inhibitory activity of blood or serum diluted in Trypticase Soy Broth was tested by pipetting blood or serum into tubes containing Trypticase Soy Broth and then adding 0.1 ml of Trypticase Soy Broth containing a dilution of the test strain of *Salmonella*. The final concentrations of blood or serum were 1, 2, 5, 10, and 20% in a total volume of 5 ml for blood and 2 ml for serum. The inhibitory activity of blood or serum in bile was tested by pipetting blood or serum into tubes containing bile (10 g dissolved in 100 ml of sterile water, Difco) or dilutions of bile in Trypticase Soy Broth. After addition of *Salmonella* in 0.1 ml of Trypticase Soy Broth, the concentrations of blood or serum in bile were 20 or 33% in a total volume of 3 ml.

All tubes were subcultured after 1, 2, 7, and 14 days of incubation at 37 C (occasionally after 3, 4, and 5 days also). Subcultures were made by inoculating 0.1 ml from each tube into Trypticase Soy Agar pour plates and also by streaking with a platinum loop containing 0.001 ml on blood-agar and *Salmonella-Shigella* Agar (BBL) plates. The number of microorganisms per milliliter (from 10^1 to 10^8) was calculated from the plates after incubating at 37 C for 24 hr. When growth was obtained on subculture, the organisms were identified as *Salmonella* by appropriate differential media and *Salmonella* typing sera (*Salmonella* O Antisera, Difco).

If all subcultures from a tube were negative during the 14 days of incubation, 1 ml of bile was added to the contents of the tube and subculture was repeated after another 48 hr at 37 C.

Antibody and complement sources. Antibody and complement sources were prepared from fresh serum (D.K.) which was shown to have high bactericidal activity against *Salmonella typhosa* strain 1 (50% serum in Trypticase Soy Broth killed 3×10^8 per milliliter of *S. typhosa* strain 1). Antibody source was prepared by heating the serum for 30 min at 55 C to inactivate complement.

Complement source without antibody was prepared by absorption at 4 C with heat-killed *S. typhosa* strain 1 to remove antibody against this strain, but to leave complement. The *S. typhosa* was prepared by washing overnight cultures of *S. typhosa* strain 1 from agar slants with saline solution. A suspension of 1.6×10^{12} organisms in 16 ml of saline solution was heated at 60 C for 1 hr on each of 3 successive days. Subcultures of 0.1-ml samples in broth were sterile. The heat-killed *S. typhosa* were washed three times in large volumes of saline solution by centrifugation at $2,000 \times g$ for 30 min. The sedimented bacteria were suspended in 10 ml of serum (D.K.) which had been precooled to 4 C. After 1 hr at 4 C, the bacteria were sedimented by centrifugation at $2,000 \times g$ for 30 min at 4 C. The serum supernatant fluid was used as the source of complement. This complement source lacked bactericidal activity against *S. typhosa* strain 1 when inoculated with three microorganisms per milliliter. However, a 1:10 dilution had sufficient complement activity to hemolyze a 1% suspension of sensitized human erythrocytes.

Effect of bile on antibody and complement. The effect of bile on antibody and complement was studied by preparing six tubes containing 2 ml of 50% antibody source or complement source in bile or in Trypticase Soy Broth. Tubes 1, 3, and 5 contained 50% antibody source in Trypticase Soy Broth, tubes 4 and 6 contained 50% complement source in Trypticase Soy Broth, and tube 2 contained 50% antibody source in bile. An inoculum of 2×10^8 *S. typhosa* strain 1 in 0.1 ml of Trypticase Soy Broth was suspended in each tube, and the tubes were incubated for 1 hr at 37 C. Tubes 5 and 6 were refrigerated while the bacteria in tubes 1 to 4 were washed three times in large volumes of cold Trypticase Soy Broth by centrifugation at $2,000 \times g$ for 20 min. After removal of the supernatant fluid, 2 ml of 50% complement source in Trypticase Soy Broth was added to tubes 1 and 2, 50% complement source in bile was added to tube 3, and 50% antibody source in Trypticase Soy Broth was added to tube 4. The number of salmonella per milliliter was determined for all six tubes, and then the tubes were incubated for 2 hr at 37 C. The number of *Salmonella* was again determined. All dilutions of antibody or complement source were made immediately before addition to the tubes.

RESULTS

Inhibitory or bactericidal activity of blood or serum diluted in Trypticase Soy Broth. To compare the effect of Trypticase Soy Broth and bile on the inhibitory or bactericidal activity of blood, it was first necessary to determine the activity of varying concentrations of different blood specimens diluted in Trypticase Soy Broth against multiple strains of *Salmonella*.

The inhibitory activity of fresh blood from five healthy donors was tested against 3 to 8 viable units of each of six strains of *Salmonella* (i.e., two strains of *Salmonella typhosa*, two strains of

S. paratyphi A, and two strains of *S. choleraesuis*). Table 1 demonstrates the minimal concentrations of blood in Trypticase Soy Broth for which subcultures were sterile during a 14-day period of incubation. With only one exception, a 20% concentration of each blood specimen prevented growth of each of the six *Salmonella* strains. It is clear that the blood specimens varied in activity against the strains of *Salmonella*, and that the strains of *Salmonella* varied in susceptibility to the inhibitory activity of blood. For example, blood specimens M.P. and D.K. usually inhibited the *Salmonella* strains in lower concentrations than the other three bloods, and the two strains of *S. choleraesuis* were more susceptible to the inhibitory activity of all of the blood specimens than the other *Salmonella* strains. The greater activity of blood from M.P. and D.K. may have been related to recent immunization with typhoid and paratyphoid vaccine.

Serum from each of six patients with *Salmonella* bacteremia was tested for inhibitory activity against the *Salmonella* isolated from the bloodstream of the same patient. Each serum was diluted in Trypticase Soy Broth, and the dilutions were inoculated with 4 to 8 viable units of the appropriate *Salmonella* strain. Table 2 shows that in four of six patients, serum concentrations of 20% or less inhibited growth of the microorganism.

Studies were made of the effect of size of the bacterial inoculum on the inhibitory activity of blood. Table 3 demonstrates an experiment in which 3 to 8 or 30 to 80 viable units of each of six *Salmonella* strains was inoculated into tubes containing 20% dilutions of blood in Trypticase Soy Broth. A 0 indicates that no growth occurred during 14 days of incubation; a + indicates the growth of *Salmonella*. Growth of the smaller inoculum was inhibited in 29 of 30 tubes, but the

larger inoculum was inhibited in only 16 of 30 tubes.

In the tubes in Tables 1, 2, and 3 in which growth occurred, subculture with a 0.001-ml loop usually revealed confluent growth (i.e. $> 10^6$ per milliliter) after the first 24 hr of incubation. However, occasionally titers of this level were not reached for 2 to 5 days. Table 4 demonstrates two examples of delayed growth of *Salmonella* in 20% blood (M.P.) diluted in Trypticase Soy Broth.

In those tubes in which no growth occurred during 14 days of incubation, the effect of the blood or serum was probably bactericidal rather than bacteriostatic. The evidence for this statement is that growth of *Salmonella* did not occur in these tubes even after addition of bile. Data presented below indicates that 13% bile will consistently destroy the inhibitory activity of 33% blood in Trypticase Soy Broth. At the end of 14 days, 1 ml of bile was added to the contents of all negative tubes containing blood diluted in Trypticase Soy Broth. This resulted in a bile concentration of more than 16%, and a concentration of blood or serum of less than 17%. After 48 hr at 37 C, the contents of these tubes were subcultured to blood-agar and *Salmonella-Shigella* Agar plates. *Salmonellae* were not recovered from any tube indicating probable death of the microorganisms.

Figure 1 shows a more direct demonstration of the bactericidal effect of blood and of the relationship to inoculum size. Different inocula of *S. typhosa* strain 1 were incubated at 37 C in a 33% suspension of blood (D.K.) in Trypticase Soy Broth. The number of bacteria per milliliter was determined immediately after adding the inocula and again after 6 and 24 hr of incubation. It is clear from Fig. 1 that this blood was bactericidal for inocula of 1,000 or fewer salmonellae

TABLE 1. Minimal inhibitory concentrations for *Salmonella* of blood in Trypticase Soy Broth*

Blood specimen	<i>S. typhosa</i>		<i>S. paratyphi</i> A		<i>S. choleraesuis</i>	
	Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 2
	%	%	%	%	%	%
M.P.†	5	5	20	10	2	2
H.R.	20	10	20	10	5	2
D.K.	10	20	20	20	2	1
W.R.	20	20	20	10	5	2
E.T.	20	20	>20	20	5	2

* Each tube contained 5 ml; inoculum was 3 to 8 viable units per tube.

† Initials of patient from whom blood was obtained.

TABLE 2. Minimal inhibitory concentration of serum from each of six different patients for the *Salmonella* isolated from the same patient*

<i>Salmonella</i> strain	Inoculum (viable units)	Minimal inhibitory concn
		%
<i>S. typhosa</i> strain 2.....	6	>20
<i>S. typhosa</i> strain 3.....	4	5
<i>S. typhosa</i> strain 4.....	5	>20
<i>S. paratyphi</i> A strain 1...	4	20
<i>S. paratyphi</i> A strain 2...	4	20
<i>S. choleraesuis</i> strain 2...	8	5

* Each tube contained 2 ml.

TABLE 3. Inhibitory effect of 20% blood in Trypticase Soy Broth on growth of *Salmonella**

Blood specimen	<i>S. typhosa</i> strain 1 (viable units)		<i>S. typhosa</i> strain 2 (viable units)		<i>S. paratyphi</i> A strain 1 (viable units)		<i>S. paratyphi</i> A strain 2 (viable units)		<i>S. choleraesuis</i> strain 1 (viable units)		<i>S. choleraesuis</i> strain 2 (viable units)	
	4 to 5	40 to 50	3 to 4	30 to 40	3 to 5	30 to 50	4 to 8	40 to 80	7 to 8	70 to 80	4 to 8	40 to 80
M.P.†	0	0	0	+	0	+	0	0	0	0	0	+
H.R.	0	0	0	+	0	+	0	+	0	0	0	0
D.K.	0	0	0	0	0	+	0	+	0	0	0	0
W.R.	0	0	0	0	0	+	0	0	0	+	0	+
E.T.	0	+	0	+	+	+	0	0	0	0	0	0

* Each tube contained 5 ml; 0 = negative subculture for 14 days; and + = growth of salmonella.

† Initials of patients from whom blood was taken.

TABLE 4. Delayed growth of *Salmonella*

Day of incubation	No. of bacteria per ml	
	<i>S. typhosa</i> strain 2*	<i>S. choleraesuis</i> strain 2†
1	2×10^3	2.4×10^3
2	$>10^5$	6×10^3
3		2×10^3
4		1.3×10^4
5		$>10^5$

* Inoculum was 33 viable units in 5 ml.

† Inoculum was 61 viable units in 5 ml.

FIG. 1. Number of *Salmonella* per milliliter after inoculation of different numbers of *Salmonella typhosa* strain 1 into 33% blood in Trypticase Soy Broth.

per milliliter. Although 90% or more of the bacteria in the two higher inocula had been killed by 6 hr, maximal growth occurred by 24 hr.

Effect of bile on the inhibitory or bactericidal activity of blood or serum. Addition of bile inactivated the inhibitory activity of blood against *Salmonella*. Three to eight viable units of *Salmonella* were inoculated in tubes containing 20 or 33% blood in bile or 20 or 33% blood in various dilutions of bile in Trypticase Soy Broth. The

cultures were incubated at 37 C for 24 hr and were then subcultured to the surface of blood agar and *Salmonella-Shigella* Agar plates with a .001-ml platinum loop. Each of the six *Salmonella* strains in Table 1 was tested against each of the five blood specimens listed in Table 1. In each instance, subculture after 24 hr revealed confluent growth of *Salmonella* from tubes containing 13% or more bile.

Similarly, each of the six *Salmonella* strains in Table 2 was tested against serum of the patient from whom the *Salmonella* had been isolated. Four to eight viable units of *Salmonella* were incubated in tubes containing 20 or 33% serum in bile or in 20 or 33% serum in various dilutions of bile in Trypticase Soy Broth. Subculture to agar plates after 24 hr revealed confluent growth from each tube containing 13% or more bile.

Mechanism of inactivation of bactericidal activity by bile. The fate of *S. typhosa* strain 1 was studied after exposure to antibody and complement sources diluted in Trypticase Soy Broth or bile as detailed in Materials and Methods and in Table 5. As shown in Table 5, exposure to antibody in Trypticase Soy Broth or bile, followed by washing and then exposure to complement in Trypticase Soy Broth (tubes 1 and 2), resulted in killing of more than 99% of the inoculum. Therefore, bile did not destroy the antibody activity. However, exposure to antibody in Trypticase Soy Broth, followed by complement in bile (tube 3), resulted in no killing. Therefore, bile did inactivate complement. Tubes 4 to 6 were controls in which *Salmonella* were exposed to complement in Trypticase Soy Broth, followed by antibody in Trypticase Soy Broth (tube 4) or to antibody alone (tube 5) or complement alone (tube 6). There was no killing of bacteria in any of these tubes. It is of interest that at the end of the experiment there was almost 10-fold more growth in tubes 4 to 6 as compared with tube 3, indicating perhaps that the complement in tube 3 was

TABLE 5. *Effect of bile on antibody and complement**

Tube no.	Tube contents before treatment	Treatment of tube contents	No. of bacteria/ml	
			After indicated treatment	After 2 hr of additional incubation at 37 C
	<i>Salmonella typhosa</i> (10 ⁵ /ml) in	Incubated 1 hr at 37 C; bacteria washed in cold TSB; bacteria resuspended in		
1	50% A in TSB	50% C in TSB	10 ⁵	6 × 10 ²
2	50% A in bile	50% C in TSB	10 ⁵	5 × 10 ¹
3	50% A in TSB	50% C in bile	10 ⁵	1 × 10 ⁵
4	50% C in TSB	50% A in TSB	10 ⁵	9 × 10 ⁵
	<i>S. typhosa</i> (10 ⁵ /ml) in			
5	50% A in TSB	Incubated 1 hr at 37 C; refrigerated while bacteria in tubes 1-4 were washed	10 ⁵	9 × 10 ⁵
6	50% C in TSB		8 × 10 ⁴	1 × 10 ⁶

* A = antibody source; C = complement source; TSB = Trypticase Soy Broth.

effective in lysing some bacteria before inactivation by bile occurred.

The inactivation of complement by bile could not be attributed to an effect of pH or osmolality. The pH of bile (a 10% solution of Difco Oxgall) is 7.3, which is the same as for Trypticase Soy Broth. Bile is hyperosmolar, as compared with serum or Trypticase Soy Broth. Bile has an osmolality of 540 milliosmoles per liter, as compared with 280 for Trypticase Soy Broth. However, 50% bile in water (270 milliosmoles per liter) was effective in eliminating the bactericidal effect of blood.

Effect of bile on coagulation of blood. A 5-ml amount of blood from three different individuals (M.P., H.R., D.K.) was placed in tubes containing 15 ml of bile. After 30 min at room temperature, the blood was completely hemolyzed, and only friable sediment remained at the bottom of each tube. Coagulation did not occur during 14 days of observation. In contrast, blood coagulated within 1 to 2 hr after addition to Trypticase Soy Broth.

The inhibitory effect of serum or heparinized blood (10 USP units of heparin sodium per ml of blood) against *Salmonella* was compared with the activity of clotted blood. In repeated experiments, 3 to 8 viable units of *S. typhosa* strain 1 was added to tubes containing blood or serum (D.K.) diluted in Trypticase Soy Broth. Each tube contained a final total volume of 5 ml. Clotted or heparinized blood prevented growth at a concentration of 10% and allowed growth at 5%, whereas serum prevented growth at 5% and allowed growth at 2.5%. Therefore, heparinized blood, clotted blood, and serum (from the same volume of blood) were equally active against the test strain of *Salmonella* when the bacteria were

added to the blood or serum. Similarly, when blood was added to tubes already containing *Salmonella*, the inhibitory activity of heparinized blood was equivalent to that of blood without an anticoagulant.

Effect of bile on leukocytes. Bile rapidly and completely hemolyzes blood. Bile also has a powerful lytic action on leukocytes. Blood specimens from three normal individuals with leukocyte counts of 5,000 to 9,000/mm³ were diluted in bile or Trypticase Soy Broth. After 30 and 60 min at room temperature, the red and white blood cells were counted in a bright-line hemocytometer. There was no lysis of erythrocytes or leukocytes in Trypticase Soy Broth containing 5%, 25%, or 50% blood. However, within 30 min there was complete lysis of erythrocytes and leukocytes in bile containing 5%, 25%, or 50% blood.

To study the effect of lysis of leukocytes on the results of blood cultures, blood (D.K.) was diluted in Trypticase Soy Broth and then frozen and thawed six times, resulting in complete lysis of leukocytes. These dilutions and dilutions of freshly drawn blood (D.K.) in Trypticase Soy Broth were tested for inhibitory activity against *S. typhosa* strain 1. Freezing and thawing the blood did not reduce inhibitory capacity.

Lack of a growth-promoting factor in bile. Inocula of *S. typhosa* strain 1 were incubated in Trypticase Soy Broth, bile, 33% blood (D.K.) in Trypticase Soy Broth, or 33% blood (D.K.) in bile. The number of salmonellae per milliliter of culture medium was determined periodically by serial dilution and pour plates.

Figure 2 demonstrates an experiment in which the inoculum was 20 *S. typhosa* per milliliter of medium. The salmonellae grew rapidly in Trypti-

FIG. 2. Number of *Salmonella* per milliliter after inoculation of 20 *Salmonella typhosa* strain 1 per milliliter of Trypticase Soy Broth, bile, 33% blood in Trypticase Soy Broth, or 33% blood in bile.

case Soy Broth, but were killed by 33% blood in Trypticase Soy Broth. Growth was equivalent in bile with or without blood. However, the peak growth in the bile media was less than 10% of the peak growth in Trypticase Soy Broth without blood. In similar experiments with different size inocula of *S. typhosa* strain 1, growth was always equivalent in bile and in 33% blood, serum, or heat-inactivated serum in bile; furthermore, the salmonellae consistently reached titers at least 10-fold higher in Trypticase Soy Broth without blood than in the bile media.

Comparison of bile and sodium taurocholate solution. Studies were undertaken to investigate the relative effectiveness of bile and sodium taurocholate solution in inactivating the bactericidal activity of blood. Blood (D.K.) was suspended in tubes containing bile, Trypticase Soy Broth, 0.5% sodium taurocholate in Trypticase Soy Broth, or 2% sodium taurocholate in Trypticase Soy Broth to make 20%, 40%, 60%, 80%, and 90% concentrations of blood in each medium. A 0.1-ml amount of Trypticase Soy Broth containing 6 viable units of *S. typhosa* strain 1 was inoculated into each tube. The final volume in each tube was 2.5 ml. The contents of each tube were subcultured to blood-agar plates and *Salmonella-Shigella* Agar plates after 1, 2, 7, and 14 days of incubation at 37 C.

As shown in Table 6, growth of *Salmonella* occurred in all concentrations of bile (even with 90% blood and 10% bile). In contrast, inhibition of growth was noted with 40% or more blood in 0.5% sodium taurocholate and with 80% or more blood in 2.0% sodium taurocholate. These same experiments were repeated with 0.5% and 2% sodium taurocholate dissolved in distilled water, with identical results.

DISCUSSION

The results of the present investigation and those of other studies (3, 9, 10, 12) clearly demon-

TABLE 6. Inhibitory concentrations of blood in different media*

Medium	Concn of blood†				
	20%	40%	60%	80%	90%
Trypticase Soy Broth.....	0	0	0	0	0
Bile.....	+	+	+	+	+
Sodium taurocholate, 0.5%.....	+	0	0	0	0
Sodium taurocholate, 2.0%.....	+	+	+	0	0

* Inoculum was 6 viable units of *Salmonella typhosa* strain 1 in each tube containing 2.5 ml.

† Symbols: 0 = negative subculture for 14 days; + = growth on subculture after 24 hr of incubation.

strated that human blood or serum has bactericidal activity against strains of *Salmonella*. Furthermore, the blood or serum of patients with *Salmonella* bacteremia is frequently bactericidal against the *Salmonella* isolated from their blood. Khairat (9) tested the bactericidal activity of defibrinated whole blood from patients with *Salmonella* bacteremia against the *Salmonella* isolated from the patient's blood. He found that 80% blood in broth was bactericidal for small inocula of three of five strains tested. Similar results were found in the present study.

The number of *Salmonella* circulating in the blood of patients with *Salmonella* bacteremia is small. Watson (12) found 0.5 to 22 bacteria per milliliter of blood in 15 patients with typhoid fever. Furthermore, blood from 11 of the 15 contained fewer than 10 microorganisms per milliliter. In other studies (Kaye, et al., submitted for publication), 90% of 80 positive blood cultures from patients with *Salmonella* bacteremia contained less than two salmonellae per milliliter of blood. Therefore, when evaluating the effect of presence of blood or serum on results of blood cultures in *Salmonella* bacteremia, it seems reasonable to use inocula of less than 10 bacteria per milliliter of blood. Furthermore, it is important to use small inocula of *Salmonella* in the test system, as inocula only 10-fold larger can overcome the inhibitory or bactericidal effect and therefore result in a falsely low appraisal of the antibacterial activity of a blood specimen.

It has usually been assumed that the activity of serum or blood against *Salmonella* is bactericidal and not inhibitory. In studies by others (3, 9, 10, 12) on the effect of serum or blood on *Salmonella*, the last subculture was usually performed after 24 hr of incubation. Although bactericidal activity would be apparent before 24 hr, inhibition of growth over a period of days could not be evaluated unless daily subcultures were contin-

ued. In the present study, where subcultures were continued over a 14-day period, growth of *Salmonella* was occasionally delayed for days.

Conradi (1) first used bile for culture of blood in patients with *Salmonella* bacteremia. He attributed the effectiveness of bile as a culture medium at least in part to its effect in preventing coagulation of blood. The present study does not provide evidence to suggest that the anticoagulant effect of bile is an important factor in the superiority of bile over Trypticase Soy Broth as a blood culture medium for *Salmonella*.

Eppenstein and Korte (5) and Conradi (2) demonstrated that bile decreases the bactericidal activity of serum. The present study has confirmed and expanded this observation. The bactericidal activity of human serum against *Salmonella* and other *Enterobacteriaceae* is due to the presence of antibody and complement (10). The results of the present study indicate that bile destroys the bactericidal activity of serum by destruction or inactivation of complement activity.

In the present study, destruction of leukocytes did not decrease the inhibitory or bactericidal activity of blood. However, it must be noted that under the conditions of this study, phagocytosis of *Salmonella* by leukocytes would not occur, and therefore no decrease in the antibacterial activity of blood should be expected from destruction of leukocytes. In patients with *Salmonella* bacteremia where *Salmonella* may be present in leukocytes, rapid release from leukocytes by bile might be a very important factor contributing to the advantage of bile over Trypticase Soy Broth as a culture medium.

It has been asserted (13) that bile-containing media have a growth-promoting effect on *S. typhosa*. The present study failed to provide evidence for a growth-stimulating factor in bile and in fact demonstrated that bile, in the absence of blood, is inferior to Trypticase Soy Broth as a culture medium for *S. typhosa*.

Sodium taurocholate in a 0.5% solution in water or broth ("bile salt broth") has generally been considered equivalent to bile as a medium for blood culture (13). However, investigators who have studied sodium taurocholate in vitro have reached opposite conclusions concerning the activity of a 0.5% solution in inactivating the bactericidal effect of blood. Cummins (3) demonstrated that blood diluted 1:8 in 0.5% sodium taurocholate solution lost its bactericidal activity against one strain of *S. typhosa*; in contrast, Watson (11) found that a 2:7 dilution of blood in 0.5% sodium taurocholate broth did not inactivate the bactericidal effect of blood against five of seven strains of *S. typhosa*.

It is clear from the present studies that bile is

much more active than 0.5% or 2.0% sodium taurocholate solution in eliminating the inhibitory effect of blood. Watson (12) demonstrated an advantage of clot culture in 0.5% sodium taurocholate-streptokinase broth over culture of whole blood in 0.5% sodium taurocholate broth. It would be of interest to repeat these studies with use of bile instead of sodium taurocholate broth. It is possible that there would be no advantage of clot culture over culture of whole blood in bile. It would have been difficult to improve the yield of cultures in bile in the in vitro system presented in the present paper. All cultures in bile were consistently positive within 24 hr, even with the largest concentration of blood (33%) and the smallest inoculum of bacteria (three cells per milliliter of blood).

There were some major differences between the results of the present study and those of a former study (Kaye et al., *submitted for publication*) in which blood from patients with *Salmonella* bacteremia was simultaneously cultured in bile and in Trypticase Soy Broth. In the present study, subcultures from all of the bile cultures and from most of the positive cultures in Trypticase Soy Broth were positive after 24 hr of incubation. In contrast, in the former study, only 78% of the blood cultures in bile were positive after 24 hr (the other 22% took 2 to 3 days to turn positive). Furthermore, none of the cultures in Trypticase Soy Broth were positive after 24 hr, and, in fact, required 2 to 11 days of incubation to become positive. The longer period of incubation required for *Salmonella* to grow from blood cultures may be related to alterations in the bacteria. *Salmonella* isolated from the bloodstream of patients have already been exposed to undiluted blood and may grow slower than the same bacteria after passage in laboratory media (as in the current study). Another factor that may delay growth on initial isolation in Trypticase Soy Broth could be intracellular residence of *Salmonella*. If viable salmonellae are inside leukocytes, it may require time for the bacteria to "grow out" in Trypticase Soy Broth.

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