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Effect of dilution on the growth of bacteria from blood cultures

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SYNOPSIS Artificial blood culture systems were set up to find the dilution with medium necessary to overcome the natural antibacterial effect of blood. The results indicate that blood should be diluted at least 1 in 50 in medium unless an additive such as Liquoid is used.

The inhibitory and lethal actions of blood on bacteria have been studied by many authors since Nuttall (1888) and have been reviewed recently by Austen and Cohn (1963). Clinical pathologists have long recognized the importance of overcoming the antibacterial action of blood when performing blood cultures, but of the several methods which have been advocated, none has been universally approved. Rosenow (1914) diluted the blood in sterile water and inoculated the centrifuged sediment into culture media, and sometimes succeeded in isolating pathogens when conventional methods failed. The antibacterial property of blood has been destroyed by the addition to the media of saponin (Elliott, 1938) and sodium polyanethol sulphonate (Liquoid) (Von Haebler and Miles, 1938).

Many pathologists still rely on simple dilution of the blood by broth to counteract its antibacterial property, but the required degree of dilution has not been established. The experiments described in this paper were performed in order to determine what the minimum safe dilution should be. Small inocula were used, since these would be more liable to inhibition by blood than larger inocula. In general, an inoculum of between 2 and 30 organisms was aimed at.

MATERIALS AND METHODS

The bacteria, except *Streptococcus viridans*, were grown overnight at 37°C in standard nutrient broth. Preliminary experiments established the viable counts that could be expected with each of the test organisms (Miles and Misra, 1938). For use, the broth culture was diluted in broth so as to give approximately the desired number of organisms, generally between 2 and 30, in a standard volume (0·02 ml) which was added to the broth without delay, using 50 dropper pipettes. With each experiment, the average size of the inoculum was determined by placing four drops of the broth dilution on each of four culture plates, which were incubated overnight.

Nutrient broth was dispensed and autoclaved in 60, 50, 40, 20, 10, 5, 2, and 1 ml volumes in screw-capped bottles. For volumes above 20 ml, bottles of 50 ml capacity were used. For 10 and 20 ml volumes, 30 ml universal containers were used, and for the others 6 ml bottles.

Blood was obtained from 19 healthy volunteers, some of whom were used several times. In each experiment (except those with Liquoid) four replicates of each volume of broth were used. Blood, 20 ml, was taken aseptically from the antecubital vein, and 1 ml quantities were added immediately to two of each of the broth bottles. (The other two bottles were used as controls.) All 36 bottles were then inoculated. In addition 1 volume of blood was incubated in 60 ml of uninoculated broth as a sterility check.

The bottles were incubated aerobically for five days at 37°C and then subcultured to blood agar (for staphylococci and streptococci) or MacConkey agar (for *Escherichia coli* and Proteus).

RESULTS

STAPHYLOCOCCUS ALBUS Nine experiments were performed with a strain which had been isolated from an acne pustule. The results are summarized in Table I.

ESCHERICHIA COLI The results of eight experiments using an organism from an infected urine are shown in Table II.

PROTEUS SPECIES Five experiments were performed with a strain from an infected urine (Table III).

STREPTOCOCCUS VIRIDANS Two different strains

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TABLE I

GROWTH FROM SMALL INOCULA OF STAPHYLOCOCCUS ALBUS IN BLOOD-BROTH MIXTURES[1]

Blood-broth Ratio	*Positive/Total Bottles Inoculated*
1/60	17/18
1/50	18/18
1/40	18/18
1/30	18/18
1/20	14/18
1/10	9/18
1/5	9/18
1/2	4/18
1/1	7/18

Average number of organisms inoculated per experiment varied from 4 to 24.
All control bottles (without blood) grew the organism in pure growth.

TABLE II

GROWTH FROM SMALL INOCULA OF ESCHERICHIA COLI IN BLOOD-BROTH MIXTURES

Blood-Broth Ratio	*Positive/Total Bottles Inoculated*
1/60	16/16
1/50	14/16
1/40	14/16
1/30	15/16
1/20	12/16
1/10	9/16
1/5	8/16
1/2	3/16
1/1	1/16

Average number of organisms inoculated per experiment varied from 2 to 5.

TABLE III

GROWTH FROM SMALL INOCULA OF PROTEUS SPECIES IN BLOOD-BROTH MIXTURES

Blood-Broth Ratio	*Positive/Total Bottles Inoculated*
1/60	12/12
1/50	12/12
1/40	12/12
1/30	12/12
1/20	12/12
1/10	11/12
1/5	10/12
1/2	0/12
1/1	0/12

Average number of organisms inoculated per experiment varied from 6 to 7.

were used. One was isolated from saliva and the other was grown from the blood of a patient with subacute bacterial endocarditis. They were grown overnight in nutrient broth containing 0·1% glucose and 10% sterile horse serum. Three experiments were done with the first strain and four with the second.

EXPERIMENTS WITH LIQUOID In order to compare the anti-inhibitory effect of Liquoid with that of simple dilution, experiments with a strain of *Escherichia coli* recently isolated from a blood culture were performed as previously described, except that half

TABLE IV

GROWTH FROM SMALL INOCULA OF STREPTOCOCCUS VIRIDANS IN BLOOD-BROTH MIXTURES[1]

Blood-Broth Ratio	*Positive/Total Bottles Inoculated*
1/60	17/18
1/50	18/18
1/40	18/18
1/30	18/18
1/20	14/18
1/10	9/18
1/5	9/18
1/2	4/18
1/1	7/18

Average number of organisms inoculated per experiment varied from 2 to 14.

TABLE V

GROWTH FROM SMALL INOCULA OF ESCHERICHIA COLI WITH AND WITHOUT 0·05% LIQUOID[1]

Blood-Broth Ratio	*Positive/Total Bottles Inoculated*	
	With Liquoid	*Without Liquoid*
1/60	3/3	3/3
1/50	3/3	3/3
1/40	3/3	3/3
1/30	3/3	3/3
1/20	3/3	3/3
1/10	3/3	3/3
1/5	3/3	0/3
1/2	3/3	0/3
1/1	3/3	0/3

[1]Average number of organisms inoculated per experiment varied from 4 to 25.
All control bottles (both with and without Liquoid, but with no blood added) yielded growth of the organisms.

of the broth bottles contained 0·05% Liquoid, before the addition of blood. The results obtained are shown in Table V.

DISCUSSION

In these experiments, the antibacterial action of normal blood against small inocula of bacteria was nearly always overcome by a dilution of 1/30. This must be regarded as a minimum value, since other organisms might be more sensitive than those tested, and also because blood from infected patients might be more actively bactericidal than normal blood. If 5 ml blood is taken into a blood culture bottle without Liquoid, therefore, the minimum volume of broth should be 150 ml. This is more than is used in many hospital laboratories, where volumes of 100 or even 50 ml are sometimes recommended (Cruickshank, 1965). In this laboratory when Liquoid is not used standard blood culture bottles contain 300 ml nutrient broth, giving a dilution of 1/60 when 5 ml of blood is taken.

The number of bacteria in the blood of a patient with bacteraemia is often greater than was used in these experiments, and the antibacterial action of blood may not be so pronounced. It would be

difficult, therefore, to establish what is the optimum volume of broth for blood culture purposes, using clinical observation.

Liquoid broth certainly has much to recommend it. Our experiments confirm that it allows small inocula of bacteria to grow from blood in small volumes of broth. Garrod (1966) found it satisfactory in overcoming the bactericidal effect of blood on small inocula of *Streptococcus viridans*, but Penfold, Goldman, and Fairbrother (1940) isolated *Streptococcus viridans* in only six out of 10 blood cultures in Liquoid-containing medium in a series in which 11/11 cultures using their best medium not containing Liquoid were positive. Furthermore, von Haebler and Miles (1938) found that if the concentration of Liquoid in the medium were increased, *Neisseria meningitidis* would not grow, and Hoare (1939) found that Liquoid was unfavourable to the growth of anaerobic streptococci.

Perhaps the most suitable compromise solution to the problem would be to use large volumes of broth or Liquoid broth for most blood culture work while employing both methods in cases where blood cultures remained persistently negative despite clinical evidence of bacteraemia.

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