A GROWTH INHIBITORY SUBSTANCE FOR THE INFLUENZA GROUP OF ORGANISMS IN THE BLOOD OF VARIOUS ANIMAL SPECIES

The Use of the Blood of Various Animals as a Selective Medium for the Detection of Hemolytic Streptococci in Throat Cultures*

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The isolation of hemolytic streptococci from throat cultures is often rendered tedious and difficult by the presence of the so called bacillus X or *Hemophilus hemolyticus*, first described in 1919 by Pritchett and Stillman (1). Mueller and Whitman (2), in making routine throat cultures on horse blood agar plates, found it impossible to differentiate the colonies of these hemolytic bacilli on a crowded plate from those of beta hemolytic streptococci. These authors devised a special method for eliminating bacillus X by placing the swabs in alkalinized broth from 1 to 2 hours before plating.

The bacillus X described by Pritchett and Stillman (1) and further studied by Stillman and Bourn (3) is strictly hemophilic, requiring for growth both the heat-stable "X" factor associated with hematin and the vitamin-like "V" factor found in blood and plant cells. On blood agar this organism is readily differentiated from *Hemophilus influenzae* by its hemolytic property, but on oleate or chocolate agar the colonies are indistinguishable.

Further studies of these hemolytic bacilli resembling *H. influenzae* were made by Rivers and his coworkers (4–6) and Fildes (7). Rivers found that these organisms differ in their growth requirements, some requiring both X and V, some only V, and some only X. He suggested the name *Bacillus parainfluenzae* for those bacilli requiring only the addition of V to the medium. Rivers described these organisms as

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forming slightly opaque hemolytic colonies, often firm enough to be pushed about on the surface of the agar. He did not mention variations in the colony size. He stated that in fluid media flocculi similar to those seen in streptococcus cultures were usually formed. Upon microscopic examination the bacilli were very pleomorphic and tended to be larger than *H. influenzae*. Rivers noted that stock cultures of these organisms tended to die out very quickly.

EXPERIMENTAL

In making routine throat cultures for the isolation of hemolytic streptococci, it has been found that many children in this institution carry large numbers of hemolytic bacilli in their throats. These bacilli are of two varieties: one forming a moderately large colony about 1 to 2 mm. in diameter surrounded by a fairly wide zone of hemolysis, the other a much smaller colony very similar to that of *H. influenzae* except for a small zone of hemolysis. Microscopic examination of the bacilli obtained from these two types of colonies showed that they are Gram-negative, non-motile, pleomorphic bacilli. The organisms obtained from the larger colony tend to be somewhat longer than those from the smaller colony. Both varieties produce hemolysis when grown in rabbit blood broth. The larger bacilli form flocculi in fluid media, whereas the small bacilli usually grow diffusely. Both types stain irregularly. Stock cultures die out quickly and are best maintained by daily transplants.

It has been found that the bacilli forming the larger colony in most instances require only V as an accessory growth factor and correspond to Rivers' *B. parainfluenzae*. The majority of the strains forming the small type of colony require both X and V and correspond to bacillus X (*H. hemolyticus*) of Pritchett and Stillman.

The presence of these two types of hemolytic bacilli in the flora of the throats of most of our patients has made the examination of rabbit blood agar plates unsatisfactory for the isolation of beta hemolytic streptococci. At the time that we began this study we were unaware of the work of Mueller and Whitman. An effort was made to devise a selective medium which would eliminate the hemolytic bacilli and allow beta hemolytic streptococci to grow. The addition of potassium tellurite to rabbit blood agar plates was tried. It was found that potassium tellurite, even in high dilution, hemolyzes rabbit cells, and the medium is therefore useless for the detection of hemolytic streptococcus colonies. Since potassium tellurite does not hemolyze sheep cells, sheep blood was substituted for rabbit blood.

It was found that when sheep blood is used, even without the addition of potassium tellurite, both varieties of hemolytic bacilli either fail to grow or grow very poorly with little or no hemolysis. Hemolytic streptococci on the other hand grow well on sheep blood.

Brown (8) compared the appearance of beta hemolytic streptococci on human, rabbit and horse blood, and found no essential differences as far as true hemolytic streptococci were concerned. He did not include sheep blood in his studies.

In order to be sure that typical hemolytic colonies are formed on sheep blood, the growth of several recently isolated strains of beta hemolytic streptococci has been compared on rabbit and sheep blood by surface streaking and pour plates. No difference in the number or size of the colonies has been observed, and the zones of hemolysis are comparable. It has been found that if the reaction of the agar base is slightly acid, pH 6.8, clearer zones of hemolysis are obtained than if agar with pH 7.4 is used.

Inhibitory Factor

The inhibition of the growth of the hemolytic bacilli on 5 per cent whole sheep blood plates has been so striking that it seemed of interest to try to analyze this phenomenon. The growth of two strains of the large hemolytic bacilli (*B. parainfluenzae*) and two strains of the small hemolytic bacilli (*H. hemolyticus*) have been compared with that of a stock culture of *H. influenzae*[1] throughout the following experiments. These five cultures constitute the test organisms referred to below.

Methods

Daily transplants of the test organisms were made on rabbit or guinea pig blood agar plates. Tubes of trypsin broth containing small quantities of rabbit blood were inoculated from the plate and grown overnight. Large amounts, 0.3 to 0.5 cc., were then inoculated from these tubes to plain trypsin broth the next morning. The experimental plates were streaked from the plain trypsin broth cultures which had grown from 4 to 6 hours, or until definite cloudiness could be observed. The growth of the test organisms on sheep and rabbit blood agar plates made with agar, pH 6.8 and 7.4, was compared. No difference in the growth was observed due to variations in pH. Therefore the same agar base, bacto Difco dehydrated blood agar base pH 6.8 (beef heart infusion agar) was used throughout. None of the test organisms grow on the plain agar base. The agar, tubed in 15 cc. amounts, was melted and cooled, and the substances to be tested were added before the plates were poured. When the quantity of blood used was less than 0.1 cc., a preliminary dilution of 1:10 was made. The results were recorded after 24 hours' incubation and again after 48 hours'. Defibrinated bloods were used in every instance.

1. Growth on the Blood of Various Species of Animals.—Rivers (4, 9) studied the growth of *H. influenzae* and closely allied hemolytic bacilli on various kinds of

[1] This culture was obtained from the Department of Bacteriology, New York Post-Graduate Hospital and Medical School.

blood. He found that these organisms grow well on rabbit, cat, dog, and pigeon blood, whereas on human and hen blood growth is unsatisfactory.

In view of the striking difference found by us between rabbit and sheep blood, it was thought of interest to compare the blood of animals closely related to the rabbit, such as the guinea pig and the rat, and that of animals closely related to the sheep, such as the goat and the cow. Horse and human blood were included for comparison. It was found that both varieties of hemolytic bacilli and *H. influenzae* grow well on various kinds of rodent blood. 5 per cent whole rabbit, guinea pig, or rat blood agar plates give equally good growth. When 5 per cent whole sheep, goat, or cow blood is used, the hemolytic bacilli and *H. influenzae*

TABLE I

Growth of Bacillus parainfluenzae hemolyticus, Hemophilus hemolyticus, and Hemophilus influenzae on Whole Unheated Blood of Different Animals

Medium: 5 per cent blood agar plates.

Organisms	Rodents			Artiodactylae			Perisso-dactyla	Pri-mate
	Rabbit	Guinea pig	Rat	Sheep	Goat	Cow	Horse	Man
B. parainfluenzae hemolyticus	+++*	+++	+++	±	±	±	++	±
H. hemolyticus (bacillus X)	+++	+++	+++	±	±	±	++	±
H. influenzae	+++	+++	+++	+	+	+	++	+

*++++ indicates excellent growth.
+++ " good "
++ " fair "
+ " poor "
± " doubtful "

These symbols have the same significance in all the tables.

fail to grow or grow very poorly. With 5 per cent whole horse blood the test organisms grow fairly well, but the colonies are smaller than on rodent blood. It had been noted by Rivers (9) that human blood is often inhibitory for *H. influenzae*. With 5 per cent whole human blood somewhat variable results are obtained, but the growth in the majority of instances is poor and is inferior to that obtained on rodent blood.

These results are summarized in Table I. It is of interest that animals of closely related species such as the rodents behave alike in allowing good growth, whereas the three artiodactylae, sheep, goat, and cow, all show marked inhibition.

2. Is the Inhibitory Factor in the Cells or in the Serum?—Red blood cells of the rabbit, guinea pig, sheep, goat, cow, and man were washed three times and made up to volume with physiological salt solution. Agar plates containing 5 per cent washed cells of the various kinds of blood give the same results in every instance

TABLE II

Comparison of Whole Blood and Washed Erythrocytes of Various Animals

Media: 5 per cent whole blood agar plates.
5 per cent washed cell agar plates.

Organisms	Whole rabbit blood	Washed rabbit cells	Whole guinea pig blood	Washed guinea pig cells	Whole sheep blood	Washed sheep cells	Whole goat blood	Washed goat cells	Whole cow blood	Washed cow cells	Whole human blood	Washed human cells
B. parainfluenzae hemolyticus	+++	+++	+++	+++	±	±	±	±	±	±	±	±
H. hemolyticus (bacillus X)	+++	+++	+++	+++	±	±	±	±	±	±	±	±
H. influenzae	+++	+++	+++	+++	+	+	+	+	+	+	+	+

TABLE III

Comparison of Washed Intact Erythrocytes and Laked Erythrocytes of Different Animals

Medium: 5 per cent cell suspension or the equivalent amount of laked cells in agar.

Organisms	Intact rabbit cells	Laked rabbit cells	Intact guinea pig cells	Laked guinea pig cells	Intact sheep cells	Laked sheep cells	Intact goat cells	Laked goat cells	Intact cow cells	Laked cow cells	Intact human cells	Laked human cells
B. parainfluenzae hemolyticus	+++	+++	+++	+++	±	±	±	±	±	±	±	±
H. hemolyticus (bacillus X)	+++	+++	+++	+++	±	±	±	±	±	±	±	±
H. influenzae	+++	+++	+++	+++	+	+	+	+	+	+	+	+

as plates containing whole blood, indicating that the inhibitory factor resides in the erythrocytes of these animals.

3. Is the Inhibitory Factor Present in Laked Cells?—Red blood cells of the rabbit, guinea pig, sheep, goat, cow, and man were laked by replacing the serum with distilled water. Agar plates containing 5 per cent of the solution of laked cells give the same results as plates containing 5 per cent washed intact cells of these ani-

mals, indicating that the inhibitory factor is not destroyed by the disruption of the erythrocyte.

4. Can the Inhibitory Factor in Sheep Blood Be Overcome by the Addition of Excess V?—V was prepared from fresh yeast according to the directions of Thjötta and Avery (11). 100 gm. of fresh yeast were emulsified in 400 cc. distilled water and adjusted to pH 4.6. The suspension was boiled for 10 minutes and allowed to settle. The clear supernatant fluid was tested for sterility, decanted, and stored in the ice box. In some experiments the unneutralized supernatant fluid was added to the medium, in others the yeast extract was neutralized just before use. The same results have been obtained with the unneutralized yeast extract as with the neutralized. It has been found that 0.5 cc. of the yeast extract added to 15 cc. of agar containing a minimal quantity of X is more than sufficient to assure good growth of *H. influenzae*. The addition of 3 cc., or six times the required amount of the neutralized yeast extract, to 15 cc. of agar containing 5 per cent sheep blood fails to overcome the inhibitory action of the sheep blood. In subsequent experiments, in which minimal amounts of the sheep inhibitor were used, a large excess of V also failed to give growth of the test organisms.

5. What is the Minimal Quantity of Sheep, Goat, Cow, and Human Blood Which Will Inhibit?—Decreasing amounts of sheep, goat, and cow blood were titrated in 15 cc. amounts of agar. Since with the diminishing quantities of blood, the amount of V supplied might be inadequate, a constant amount of V in the form of yeast extract (0.5 to 15 cc. of agar) was added. At first minimal quantities of X were also added but it was found that even the smallest quantities of blood used always contain sufficient X.

Table IV shows the inhibitory action of sheep blood. It will be seen that a concentration of 0.3 per cent sheep blood (0.05 cc. added to 15 cc. agar) is usually sufficient to inhibit growth in the presence of the standard amount of V. If the concentration of sheep blood is reduced further to 0.15 per cent (0.025 cc. added to 15 cc. of agar) growth is obtained indicating that this amount of sheep blood does not contain enough of the inhibitory factor to prevent growth, but that a sufficient quantity of X is still present. Inhibition of growth of the test organisms has been obtained with the same quantities of cow and goat blood (0.05 cc. added to 15 cc. of agar).

Human Blood.—Several specimens of human blood obtained from children with rheumatic heart disease, both in the active and in the quiescent stage, were compared with blood obtained from normal adults for the presence of the inhibitory factor. No differences have been noted between these various kinds of human blood. Human blood contains less inhibitor than sheep blood. It requires 3.3 per cent or ten times as much human blood (0.5 cc. added to 15 cc. of agar) as sheep blood to inhibit growth.

6. Is the Inhibitory Factor Thermolabile?—Fleming (10) first pointed out that the growth of *H. influenzae* is enhanced when blood agar is heated so as to produce a chocolate color. Chocolate agar is usually made by heating blood agar to a temperature of 90°C. for a few minutes. The improved growth obtained on

chocolate agar has generally been attributed to the fact that the red cells are broken up and their modified contents distributed throughout the medium. Rivers (9) noted that, whereas *H. influenzae* and allied organisms grow poorly on unheated human blood, excellent growth is obtained on heated human blood.

The effect of heating rodent blood and the bloods of other animals was compared. Although good growth is obtained on unheated rodent blood agar, the growth is enhanced when this medium is made chocolate. When sheep and goat blood are used, the difference between heated and unheated blood is striking. In contrast to the marked inhibitory effect of the unheated bloods, excellent growth is obtained on 5 per cent sheep or goat blood agar which has been heated to 90°C. for a few minutes. With cow and human blood a similar result is obtained, but care must be taken to heat the blood agar mixture at 90°C. for 15 minutes.

7. At What Temperature Is the Inhibitory Factor in Sheep and Cow Blood Destroyed?—As stated above it was found that if sheep or goat blood is heated to 90°C. for a few moments to make it chocolate, good growth is obtained. Using

TABLE IV

Effect of Decreasing Amounts of Sheep Blood in Agar in the Presence of a Constant Amount of V

Organisms	5 per cent sheep blood	1 per cent sheep blood	0.5 per cent sheep blood	0.3 per cent sheep blood	0.15 per cent sheep blood	5 per cent rabbit blood
B. parainfluenzae hemolyticus	±	±	±	±	++	+++
H. hemolyticus (bacillus X)	±	±	±	±	++	+++
H. influenzae	+	+	+	+	++	+++

minimal quantities (0.05 to 15 cc. of agar) of sheep or cow blood, it was found that 68°C. for 30 minutes is sufficient to destroy the inhibitory factors. Exposure to 56°C. for 1 hour fails to reduce the inhibitory action with these two types of blood. Temperatures between 56°C. and 68°C. were not tried. The inhibitory action of human blood is also destroyed at 68°C. for 30 minutes (Table VI).

8. Is It Possible to Destroy the Inhibitory Factor by Other Means: Acidification and Alkalinization?—Acidification.—Fleming (10) found that if blood is digested with normal sulfuric acid and neutralized and a small amount of the fluid thus procured is added to the medium, good growth of *H. influenzae* is obtained. This observer did not state what kind of blood he used.

In these experiments sheep blood was diluted 1:10 with salt solution. N/1 HCl was then added until the solution turned brown and no pinkish tinge remained (about 0.1 cc. of acid per 1 cc. of diluted blood). The mixture was shaken and allowed to stand for 30 minutes at room temperature. It was then neutralized with N/1 NaOH. 0.5 cc. of the acidified and neutralized blood was added to 15 cc. of agar containing the standard amount of yeast extract and a plate poured.

TABLE V

Comparison of Unheated and Heated Blood of Various Animals

Media: 5 per cent blood agar plates.
5 per cent chocolate agar plates heated at 90°C. for 15 minutes.

Organisms	Rabbit		Guinea pig		Rat		Sheep		Goat		Cow		Horse		Man	
	Unheated	Chocolate	Unheated	Chocolate	Unheated	Chocolate	Unheated	Chocolate	Unheated	Chocolate	Unheated	Chocolate	Unheated	Chocolate	Unheated	Chocolate
B. parainfluenzae hemolyticus	+++	++++	+++	++++	+++	++++	±	++++	±	++++	±	++++	++	++++	±	++++
H. hemolyticus (bacillus X)	+++	++++	+++	++++	+++	++++	±	++++	±	++++	±	++++	++	++++	±	++++
H. influenzae	+++	++++	+++	++++	+++	++++	+	++++	+	++++	+	++++	++	++++	+	++++

All the test strains grow well on this medium. The same result has been obtained when twice the quantity of the acidified and neutralized sheep blood is added to 15 cc. of agar.

Alkalinization.—In this experiment the process was reversed. Diluted sheep blood was alkalinized with N/1 NaOH and then neutralized with N/1 HCl. 0.5 cc. of this mixture was added to 15 cc. of agar and a plate poured. No growth or very poor growth was obtained when the test organisms were streaked on this medium (Table VII).

TABLE VI

Thermolability of the Inhibitory Factor in Sheep and Cow Blood

Media: 0.3 per cent sheep blood agar with a constant amount of V.
0.3 per cent cow " " " " " " " "

Organisms	Sheep		Cow	
	56°C. for 1 hr.	68°C. for 30 min.	56°C. for 1 hr.	68°C. for 30 min.
B. parainfluenzae hemolyticus	±	++++	±	++++
H. hemolyticus (bacillus X)	±	++++	±	++++
H. influenzae	+	++++	+	++++

TABLE VII

Comparison of Growth on Sheep Blood Treated with Acid and Treated with Alkali

Media: 0.3 per cent acidified and neutralized sheep blood plate with a constant amount of V.
0.3 per cent alkalinized and neutralized sheep blood plate with a constant amount of V.

Organisms	Acidified and neutralized sheep blood	Alkalinized and neutralized sheep blood
B. parainfluenzae hemolyticus	+++	±
H. hemolyticus (bacillus X)	+++	±
H. influenzae	+++	+

These experiments seem to indicate that the inhibitory factor can be destroyed by acidification but not by alkalinization. It is of interest that Fildes (12) found trypsin digestion of sheep or ox blood unsatisfactory as a means of improving the growth of *H. influenzae.* This author obtained good results by using peptic digestion of sheep or ox blood. In the case of peptic digestion the amount of acid (0.12 cc. pure HCl to 1 cc. of sheep blood) added by Fildes should be in itself sufficient to destroy the inhibitory action of sheep blood, whereas with trypsin digestion an alkaline reaction is used.

9. Is the Inhibitory Factor Active in Fluid as Well as in Solid Media?—It seemed probable that if the failure of the influenza group of organisms to grow on sheep blood is due to an inhibitory factor, it should be possible to demonstrate the inhibitory action in fluid as well as in solid media. The basic fluid medium consisted of Difco dehydrated beef heart infusion broth (pH 7.5) to which a sufficient amount of yeast extract was added.

It has been found that the addition of washed laked sheep cells to broth inhibits the growth of the test organisms markedly, both aerobically and anaerobically. The reduction in growth has been confirmed by pour plates. No inhibitory action has been noted with intact washed sheep cells which tend to settle at the bottom of the tube. Large quantities of sheep serum added to broth have no inhibitory effect.

10. Nature of the Inhibitory Factor.—Adsorption with Charcoal.—2 gm. of bone charcoal were added to 5 cc. of laked sheep cells diluted 1:10 with distilled water and the mixture was incubated overnight at 37°C. Although the charcoal removes all the pigment from the diluted blood, the inhibitory action of the colorless fluid thus obtained is still marked.

Filtration through a Berkefeld Filter.—Sheep cells were laked by diluting 1:10 with distilled water. This solution was then filtered through a Berkefeld filter (N). Filtration failed to remove the pigment. The first and last portions filtered were tested separately. No difference has been noted between the two lots. The inhibitory factor is removed from both.

Symbiosis.—It was noted by Rivers (4) that symbiosis improved the growth of *H. influenzae* on human blood. On rabbit and cat blood on the other hand, on which *H. influenzae* grows well in pure culture, the size of the colony is not increased by the presence of other organisms. We have observed the same phenomenon with sheep blood: the activity of the inhibitory factor is reduced in the vicinity of accidental contaminants such as the staphylococcus.

11. Mechanism of the Inhibitory Action of Sheep Blood.—It was thought possible that the inhibitory action of sheep blood might be due to a destruction of the V factor by enzymes present in the erythrocytes. However, no definite experimental evidence has been obtained for this hypothesis.

DISCUSSION

Many types of selective media have been used to facilitate the isolation of various kinds of bacteria: aniline dyes for members of the typhoid-paratyphoid group, potassium tellurite for *Corynebacterium diphtheriae*, and sodium oleate for *H. influenzae*. The appearance of beta hemolytic streptococci on blood agar plates is usually so characteristic that no special selective medium has been considered necessary. When, however, the bacillus X (Pritchett and Stillman) or *B. parainfluenzae hemolyticus* (Rivers) are present in the throat

flora, colonies of beta hemolytic streptococci cannot be recognized with certainty.

Attempts were therefore made to find a selective medium which would eliminate *H. hemolyticus* and closely allied organisms. It has been found that if sheep blood is used instead of rabbit blood, streptococci produce characteristic hemolytic colonies while the hemolytic bacilli fail to grow. 5 per cent sheep blood agar thus proves to be a selective medium which facilitates the isolation of *Streptococcus hemolyticus* when large numbers of hemolytic bacilli are present in the throat flora.

It has been found that although unheated sheep blood has a marked inhibitory action on the growth of *H. hemolyticus*, *B. parainfluenzae hemolyticus*, and true influenza bacilli, heated (chocolate) sheep blood gives excellent growth. Even unheated rabbit blood which gives good growth of these organisms is improved by heating.

The most commonly accepted explanation of the improved growth on heated blood is that the red cells are broken down, and that their modified contents are distributed throughout the medium. If the disruption of the red cells alone were responsible for the improved growth, then a similar result might be expected from laking the cells. No improvement, however, results from laking alone. The enhanced growth seems to be related to heating the blood, suggesting the presence of a thermolabile inhibitory substance.

Rabbit blood appears to have a very slight inhibitory action since somewhat better growth is obtained with heated rabbit blood than unheated. Sheep blood contains a much larger amount of inhibitory substance, since a relatively small quantity of unheated sheep blood is sufficient to inhibit growth.

It has been shown that the inhibitory factor in sheep blood resides in the erythrocytes and is thermolabile. Its presence can be demonstrated with laked and intact cells in solid media, and with laked cells in fluid media. A concentration of 0.3 per cent sheep blood is sufficient to inhibit growth. The addition of excess V does not overcome the inhibitory factor. It is inactivated by acid, but not by alkali. Although adsorption with charcoal removes all the pigment from a solution of laked sheep cells, the inhibitory factor is present in the colorless fluid thus obtained. Filtration of a solution of laked sheep

cells through a Berkefeld filter removes the inhibitory factor without removing the pigment. These experiments seem to indicate that the inhibitory factor is not bound to the pigment of the erythrocyte.

It was thought of interest to examine the bloods of other animals to see how they compare with the rabbit and sheep. Blood was obtained from goats and cows, animals closely related to sheep, and from guinea pigs and rats for comparison with the rabbit. It has been found that goat and cow bloods inhibit the growth of the hemolytic bacilli and *H. influenzae* in the same way as sheep blood. No essential differences have been noted between the properties of the inhibitory factors in cow and goat blood and those described above for the inhibitor in sheep blood.

Unheated guinea pig and rat bloods are like rabbit blood in that they give good growth of *B. parainfluenzae*, *H. hemolyticus*, and *H. influenzae*. These organisms also grow moderately well on unheated horse blood. The growth of these bacilli on media containing 5 per cent human blood is poor and irregular. The inhibitor present in human red cells is, however, less powerful than that in sheep blood; it requires approximately ten times as much human blood to inhibit as it does sheep blood. The inhibitor in human blood, like that in sheep blood, is destroyed by heating at 68°C. for 30 minutes.

CONCLUSIONS

1. 5 per cent sheep blood agar is a selective medium for beta hemolytic streptococci in throat cultures since sheep blood inhibits the growth of bacillus X (*H. hemolyticus*) and *B. parainfluenzae hemolyticus*. The growth of *H. influenzae* is also inhibited by sheep blood.

2. The inhibitory action of sheep blood resides in the erythrocytes and is thermolabile. Disruption of the cell by laking has no effect upon the inhibitor.

3. The bloods of animals closely related to the sheep, such as the goat and the cow, have a similar inhibitory action on the growth of hemolytic and non-hemolytic members of the influenza group, while human blood contains a similar but less powerful inhibitor for these organisms.

4. Members of the influenza group grow well on unheated rodent blood: rabbit, guinea pig, and rat.

5. These organisms also grow fairly well on unheated horse blood.

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