Throughout these experiments, the animals appeared to be in excellent condition. They did not lose any weight, and showed *no* anorexia or lethargy. Body temperatures remained quite normal.

Conclusions. The oral administration of 2 mg. cobalt (as cobalt chloride) per kg. daily to dogs produces a significant increase (about 20%) in the erythrocyte number. No toxic symptoms were observed in dogs which were fed as much as 6 mg. cobalt per kg. daily for 3 weeks.

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Comparative Study of Fibrinolytic and Anticoagulating Properties of *Streptococcus hemolyticus* and *Streptococcus fecalis* (Enterococcus).

ERWIN NETER AND ERNEST WITEBSKY.*

From the Bacteriological Departments, Children's Hospital and Buffalo General Hospital, and the Department of Pathology and Bacteriology, University of Buffalo School of Medicine.

Tillett and Garner[1] made the interesting observation that hemolytic streptococci may produce a powerful fibrinolysin. Their findings were corroborated and extended by several authors, including Dart, Dennis and Berberian, Van Deventer, Hare and Colebrook, Hatfield, Magee and Perry, Lippard, Johnson and Wheeler, Madison, Meyers, Keefer and Holmes, Reich, Schmidt, Stuart-Harris, Tunnicliff and others. The fibrinolysin produced by hemolytic streptococci *in vitro* has the following main properties: It dissolves human but not animal plasma-clots, with the exception of *rhesus* plasma, as demonstrated by Van Deventer and Reich[2]; it is produced in infusion-broth in rather high concentration, and it may be neutralized by streptococcal antiserum as well as by serums of patients recovering from streptococcal infections. Besides the fibrinolysin, glucose-broth cultures of hemolytic streptococci may contain a second factor which inhibits coagulation of both human and animal plasma, as observed

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1 Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485; Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 239; Tillett, W. S., Edwards, L. B., and Garner, R. L., *J. Clin. Invest.*, 1934, **13**, 47; Tillett, W. S., *J. Clin. Invest.*, 1935, **14**, 276; Tillett, W. S., *J. Bact.*, 1935, **29**, 111.

2 Van Deventer, J. K., and Reich, T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 821.

by Dennis and Berberian,[3] Dart,[4] Witebsky and Neter.[5] Dennis and Berberian demonstrated such an anticoagulant also in a glucose-broth culture of one strain of *S. viridans*. These authors reported that type-specific (E1) antiserum neutralized the E1 anticoagulant when used in amount of 0.2 cc. or more. They concluded that there is evidence that fibrinolysin and anticoagulant are antigenic and exhibit some degree of type-specificity. The following facts, however, seem to cast doubt on the conception of the antigenic or even type-specific nature of bacterial anticoagulants: (1) Normal serum and heterologous antisera inhibit the streptococcal anticoagulant to about the same degree as does streptococcal antiserum. (2) Normal spinal fluid, known to be rather poor in normal antibody-functions, counteracts anticoagulant as well as normal serum. We, therefore, believe that this type of neutralization of the anticoagulant is due to non-specific factors operative in serum and spinal fluid respectively. The lack of antigenicity of the streptococcal anticoagulant would harmonize with the recent findings of Dennis and Adham,[6] according to which the effective factor of the anticoagulant is primarily lactic acid, perhaps with a minor admixture of other organic acids.

The production of anticoagulant is by no means limited to streptococci; a wide variety of bacteria, *e. g.*, staphylococcus,[7] enterococcus, pneumococcus, *B. coli, B. Friedländer, B. pyocyaneus* and others may produce anticoagulants in glucose-broth.[5] The following report is concerned with a comparative study on the fibrinolytic and anticoagulating properties of both *S. hemolyticus* and enterococcus *(S. fecalis)*. The microörganisms were obtained from human sources; the tests for the demonstration of fibrinolysin and anticoagulant were set up according to the original technic[1]: the supernatant fluid (in decreasing amounts, volume 0.5 cc.) of 18-hour plain infusion and 1% glucose-infusion-broth cultures respectively, were mixed with 1.0 cc. of 1:5 diluted, oxalated plasma. Then 0.25 cc. of 0.25% solution of calcium chloride in saline was added. The tubes were shaken thoroughly, incubated at 37°C., and read at various intervals: The fibrinolysin dissolves the plasma-clot; the anticoagulant prolongedly (24 hours) inhibits plasma-coagulation.

Sixty-seven strains of *S. hemolyticus* and 50 strains of enterococcus were examined. The results are summarized in Table I.

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[3] Dennis, E. W., and Berberian, D. A., *J. Exp. Med.*, 1934, **60**, 581.

[4] Dart, E. E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 285.

[5] Witebsky, E., and Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 858; Neter, E., and Witebsky, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 549.

[6] Dennis, E. W., and Adham, L. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 84.

[7] Neter, E., *J. Bact.*, 1937, **34**, 243.

Of 67 strains of *S. hemolyticus,* 61 (*ca.* 91%) produced fibrinolysin in plain infusion-broth, and 17 (*ca.* 25%) produced anticoagulant in 1% glucose-broth. In contrast, all 50 strains of enterococcus produced the anticoagulant in glucose-broth and failed to form the fibrinolysin. The high percentage of fibrinolytic strains of *S. hemolyticus* from human lesions is in accord with the findings of Tillett,[1] who reported that out of 157 strains, 98.1% were fibrinolytic. In a study of the relation of source to fibrinolytic activity of hemolytic streptococci, Madison[8] found 94% of strains isolated from internal lesions to be fibrinolytic, as contrasted with only 17% of strains isolated from superficial lesions. This latter figure was increased to 35% when the concentrative method was employed (Madison[9]).

In view of the different fibrinolytic and anticoagulating properties of *S. hemolyticus* and enterococcus, the question arises as to the respective properties of hemolytic enterococci. The lack of fibrinolytic activity of Lancefield's group D—representing hemolytic strains of the *S. fecalis* group—was reported by Lancefield and Hare,[10] Hare and Maxted.[11] The characteristics of different members of the *S. fecalis* group were extensively studied by Sherman and his coworkers.[12, 13, 14] Sherman, Stark and Mauer[13] also established the identity of Lancefield's group D with *S. zymogenes.*

Five strains of hemolytic enterococci were isolated in this laboratory from human sources and examined for fibrinolytic and anticoagulating properties. All failed to show fibrinolytic activity when tested according to the original technic, but produced anticoagulant in glucose-broth. Through the courtesy of Dr. Sherman, 4 more strains of hemolytic enterococci from human sources were obtained, 2 of them identified as *S. zymogenes,* the other 2 as *S. durans.* The lack of fibrinolytic activity was observed by Dr. Sherman (personal communication) when secondary growth was inhibited by incubating at 53°C. after coagulation had occurred. When tested in this laboratory, they were found to produce anticoagulant in glucose-broth, but failed to exhibit fibrinolytic activity. Thus, 9 strains of hemolytic enterococci produced the anticoagulant and failed to show fibrinolytic activity when tested with the original Tillett-Garner technic. Further experiments will deter-

8 Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1018.

9 Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 445.

10 Lancefield, R. C., and Hare, R., *J. Exp. Med.*, 1935, **61**, 335.

11 Hare, R., and Maxted, W. R., *J. Path. Bact.*, 1935, **41**, 513.

12 Sherman, J. M., Mauer, J. C., and Stark, P., *J. Bact.*, 1937, **33**, 275.

13 Sherman, J. M., Stark, P., and Mauer, J. C., *J. Bact.*, 1937, **33**, 483.

14 Sherman, J. M., and Wing, H. N., *J. Dairy Science,* 1937, **20**, 165.

TABLE I.

Fibrinolytic and Anticoagulating Properties of *Streptococcus hemolyticus* and *Streptococcus fecalis* from Human Sources.

No. of Strains		Number of Strains Producing: Fibrinolysin* and Anticoagulant†	Fibrinolysin Only	Anticoagulant Only	Neither Fibrinolysin nor Anticoagulant	Total: Fibrinolytic	Total: Anticoagulant Strains
67	*Streptococcus hemolyticus*	13	48	4	2	61 (Ca 91%)	17 (Ca 25%)
50	*Enterococcus (Streptococcus fecalis)*	0	0	50	0	0	50 (100%)
9	*Enterococcus hemolyticus*	0	0	9	0	0	9

*Dissolution of plasma clot within 24 hr.

†Constant (24 hr.) inhibition of plasma coagulation.

mine whether the difference in fibrinolytic activity is qualitative or quantitative and whether a relation exists between the apparently constant production of anticoagulant in glucose-broth by hemolytic enterococci and the final low pH of the broth, characteristic of the *S. fecalis* group.

The anticoagulant of enterococci is not necessarily an artificial product occurring *in vitro* only. Recently it was shown[15] that the *Enterococcus hemolyticus* anticoagulant may occur *in vivo:* the fluid obtained from the lesion in a case of Ludwig's angina, caused by hemolytic enterococci showed anticoagulating properties as did the isolated strain when cultured in glucose-broth. Meanwhile, we found bacterial anticoagulants in 17 other exudates of various origin such as abscesses, empyemic fluid, etc. In 5 of them the culture revealed enterococci, which were found in pure culture in one case and in the remaining 4 cases together with *B. coli, B. welchii* and staphylococcus respectively. It cannot yet be decided whether the anticoagulating properties of such exudates are due to one factor or to different factors and whether the anticoagulant produced *in vivo* is identical with that *in vitro*. The demonstration of bacterial anticoagulants in natural infections supplements the recent finding of *S. hemolyticus* fibrinolysin in human lesions.[16]

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Chick Embryo Broth and Chick Embryos, Held in Cold Storage, as Sources of Growth Stimulants for Tissue Cultures.

MARY E. SHIPP. (Introduced by F. H. Swett.)

From the Department of Anatomy, Duke University School of Medicine.

It is frequently difficult during the winter months in certain localities to obtain the adequate and inexpensive supply of fertile eggs so essential where constant supplies of embryo juice are required for procedures in tissue culture. Two methods were devised for utilizing chick embryos from which growth-promoting extracts might be made while a plentiful supply of embryos was available.

Embryo Broth. Five hundred grams of finely chopped embryos

[15] Neter, E., and Young, G. S., *Am. J. Dis. Child.*, 1937, **53**, 1531; Neter, E., *Arch. Path.*, 1937, **23**, 295.

[16] Neter, E., and Witebsky, E., *J. Bact.*, 1936, **31**, 77; Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 735.