

Prevention of Blood Coagulation by Antibiotics *in vitro*. (20295)

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(Introduced by Reuben Kahn.)

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Antibiotics probably do not affect blood coagulation *in vivo* (1-4). The concentrations of antibiotics which can be attained in the blood of animals or man are limited. It is possible to demonstrate *in vitro*, however, that in suitable concentrations several antibiotics are anticoagulants.

Experimental. Streptomycin sulfate (Parke Davis), neomycin sulfate (Upjohn), oxytetracycline glycinate ("terramycin" Pfizer), Q-19 (Upjohn), potassium penicillin G (Pfizer), and sodium citrate were each dissolved in saline. Then 0.1 ml of each solution containing one of these agents was mixed with 1.0 ml fresh human blood to give final concentrations ranging from 0.5 to 50 mg per ml. Bloods containing antibiotics were tested for clotting time by the Lee-White method for one hour, refrigerated (4°C) for 23 hours, and examined again for clotting. The bloods containing antibiotics were examined in a Beckman pH meter. The pH of control bloods was adjusted to that of samples containing antibiotics. At the end of 24 hours, CaCl₂ was added to unclotted samples to give concentrations exceeding that of the antibiotic.

The results are summarized in Table I. They were reproduced with each antibiotic in many different samples of blood. As an anticoagulant, oxytetracycline was at least 8 times more effective than any of the other antibiotics. Glycinate was no anticoagulant and did not influence clotting when added to

the other antibiotics. Slight hemolysis was sometimes observed in unclotted blood 24 hours after adding oxytetracycline but not other antibiotics. Chloramphenicol did not dissolve in the desired concentrations in water. The anticoagulant action of streptomycin and penicillin was reversed by excess amounts of CaCl₂. It is suggested, therefore, that these agents prevented clotting by binding calcium. Calcium did not reverse the anticoagulant activity of neomycin or oxytetracycline. The prothrombin time (Quick's method) of blood containing 50 mg per ml of streptomycin was 56 seconds and of the same blood without streptomycin was 12.7 seconds; it was prolonged indefinitely by 100 mg per ml of neomycin. Overman and Wright(5) have also observed that streptomycin increases the prothrombin time and clotting time and suggested that streptomycin inhibited thromboplastin. The pH of bloods containing anticoagulant concentrations of antibiotics ranged from 6.9 (neomycin) to 8.8 (oxytetracycline). There was no delay in clotting of bloods with pH ranging from 6.9 to 8.8 in the absence of antibiotics.

Summary. When examined *in vitro*, streptomycin, penicillin, oxytetracycline ("terramycin") and neomycin were anticoagulants only when used in greater concentrations than can be attained *in vivo*.

TABLE I. Minimum Concentration of Antibiotics and Sodium Citrate (mg/ml of Blood) Which Prevented Clotting.

Sodium citrate	1
Oxytetracycline ("terramycin") glycinate	2.5
Neomycin sulfate	20
Streptomycin sulfate	25
Potassium penicillin G	30
Q-19*	Clotted with 50

* An antibiotic of the polymyxin type(6). Concentrations of 40 mg/ml delayed clotting for 1 hr.

1. Seegers, W. H., *Pharmacol. Rev.*, 1951, v3, 278.
2. Editorial, *New England J. Med.*, 1952, v246, 240.
3. Parker, J. W., and Wright, L. T., *Science*, 1952, v116, 282.
4. Triantaphyllopoulos, D. C., and Waisbren, B. A., *Arch. Int. Med.*, 1952, v90, 653.
5. Overman, R. S., and Wright, I. S., *J. Biol. Chem.*, 1948, v174, 759.
6. Murray, F. V., and Tetrault, P. S., *Proc. Soc. Am. Bact.*, 1948, v1, 20.

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