Technical Papers

Some Physical and Biologic Properties of Subtilin and Other Antibiotics[1]

HAMILTON H. ANDERSON, GILBERTO G. VILLELA, EDER LINDSAY HANSEN, and RACHEAL K. REED

Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco

Subtilin, an antibiotic obtained from *Bacillus subtilis* by Jansen and Hirschmann in 1944 (*4*), was found to be active *in vitro* against *Staphylococcus aureus, Lactobacillus casei, Micrococcus conglomeratus,* and *Streptococcus viridans.* Salle and Jann (*10*) have indicated that it is also active *in vitro* against gram-positive bacteria including *Mycobacterium tuberculosis.* It was reported by them to have a cytotoxic index of about 20. Because of its favorable antibiotic activity we desired to investigate possible modes of action against a variety of organisms. Effects on *Trypanosoma equiperdum, Leishmania donovani, Endamoeba histolytica, Lactobacillus plantarum,* and *Ascaris suis* were studied. In addition, physical behavior was considered in an effort to explain the biologic activity of subtilin and other antibiotics *in vitro* and *in vivo.*

Using the technic of Heilman and Herrell (*3*), subtilin,[2] 0.05 per cent in water or in 85 per cent ethyl alcohol, showed immediate surface-tension-lowering effect. Gramicidin, as noted by Heilman and Herrell (*3*), and gramicidin derivative (formaldehyde treated), prepared by Lewis, *et al.* (*6*), exhibited similar properties. Lysozyme and streptomycin[3] produced only slight effects, while penicillin[4] did not alter surface tension. Fig. 1 summarizes our findings. The Cenco-Du Noüy tensiometer was used.

The hemolytic effect of gramicidin, reported by Heilman and Herrell (*2*), was compared with that of the other antibiotics. Confirming the studies of Lewis, *et al.,* gramicidin derivative proved less hemolytic. Subtilin had no immediate effect on red cells, but after 24 hours at 4° C. hemolysis occurred. Penicillin and streptomycin caused no hemolysis, thus confirming Van Dyke (*11*) with respect to penicillin.

Brief exposure of *T. equiperdum* to subtilin dissolved in 0.45-per cent sodium chloride solution resulted in immediate cytolysis when 1:2,000 dilution was used. Streptomycin and penicillin were not lytic. Survival of trypanosome-infected mice was not prolonged when 80 to 160 mg./kg. amounts of subtilin were given intraperitoneally.

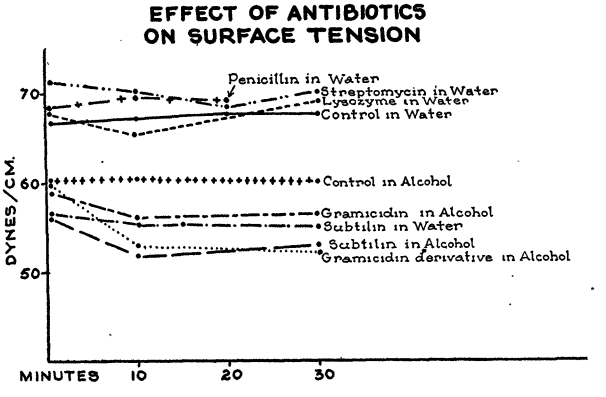

FIG. 1

Using a previously described technic (*7*), subtilin was not active *in vitro* or *in vivo* against *L. donovani.* Penicillin G in 1:1,000 dilution caused cytolysis of leishmania in 6 hours, and in 1:10,000, in 24 hours. *In vivo* it was not active. Neither streptomycin nor lysozyme was effective *in vitro.*

E. histolytica was killed *in vitro* in liquid liver medium (*1*) at 1:400,000 dilution of subtilin, as well as the associated bacterium 't'. In egg slope medium it was active within the range of emetine hydrochloride, and on autoclaving solutions for 10 minutes, subtilin's activity was markedly enhanced. The gramicidins had similar activity in egg slope medium, but were only one-fifth as active as subtilin in liquid liver medium. Streptomycin in 1:2,500 dilution killed the ameba *in vitro.* Penicillin has been reported ineffective (*8*). One monkey (*Macacus rhesus*) was cleared of *E. histolytica* for three weeks after 1.0 gram/kg. total oral doses in 10 days.

Against *L. plantarum* (342y) in liquid medium containing 1 per cent dextrose and 1 per cent Difco yeast extract (pH 6.8), a 1:80,000 dilution of subtilin inhibited growth after 48 hours at 37° C. Cholesterol did not enhance its activity, but para-aminobenzoic

[1] Part of a cooperative study with Dr. Howard D. Lightbody and associates, Western Regional Research Laboratory, U. S. Department of Agriculture, Albany, California, who produced the antibiotics reported (unless otherwise acknowledged); together with Dr. A. J. Salle, Department of Bacteriology, University of California at Los Angeles. Studies in the University were supported, in part, by Eli Lilly and Company, Indianapolis, Indiana, and the Committee on Medical Research, Office of Scientific Research and Development, under contracts with the University of California. Acknowledgment is made to Dr. Benedict E. Abreu and to Mrs. Elsa Zitcer, who performed some of the toxicity tests in mice.

[2] Amounts of antibiotics are expressed on the basis of weight of dry materials, except for penicillin (C.S.C.).

[3] Generously supplied by the Lilly Research Laboratories, Indianapolis, Indiana: streptomycin, 350 units/mg.; penicillin G, 1,650 units/mg.

[4] Sodium salt, prepared by Commercial Solvents Corporation.

acid did. The gramicidins were active at 1:40,000 dilution and streptomycin at 1:10,000.

In vitro tests against *A. suis,* using the technic of Lamson and Brown (*5*), revealed that none of the antibiotics studied was active.

The acute toxicity of subtilin in mice, on intravenous injection of 1 per cent solution, was LD_{50} (60 ± 3 mg./kg.); on subcutaneous injection, the LD_{50} was 670 ± 30 mg./kg.; when given intragastrically, 5.0 grams/kg. killed. One per cent solution instilled into the rabbit's eye was nonirritating.

Gramicidin, 1 per cent in propylene glycol, given intravenously in mice had an LD_{50} of 1.5 mg./kg. This is slightly lower than reported by Robinson and Molitor (*9*). Gramicidin derivative was less toxic, LD_{60} being 4.7 mg./kg. Lethal doses of the gramicidins killed within one minute, which precluded the possibility of delayed hemolysis being responsible.

Summary. Subtilin, a new antibiotic obtained from *B. subtilis,* proved active *in vitro* against *L. plantarum, E. histolytica* and its associated bacterium 't', and *T. equiperdum* without causing immediate hemolysis of erythrocytes. Subtilin is tensioactive, and amounts required for antibiotic effect are within the range of surface tension activity. It was relatively nontoxic for four species of mammals, especially after intragastric administration. Gramicidin is more hemolytic and more toxic than subtilin.

References

1. Hansen, E. L. *Fed. Proc.,* 1945, **4**, 122.
2. Heilman, D., and Herrell, W. E. *Proc. Soc. exp. Biol. Med.,* 1941, **46**, 182.
3. Heilman, D., and Herrell, W. E. *Proc. Soc. exp. Biol. Med.,* 1941, **47**, 480.
4. Jansen, E. F., and Hirschmann, D. J. *Arch. Biochem.,* 1944, **4**, 297.
5. Lamson, P. D., and Brown, H. W. *Amer. J. Hyg.,* 1936, **23**, 85.
6. Lewis, J. C., Dimick, K. P., Feustel, I. C., Fevold, H. L., Olcott, H. S., and Fraenkel-Conrat, H. *Science,* 1945, **102**, 274.
7. Reed, R. K., and Anderson, H. H. *Fed. Proc.,* 1945, **4**, 133.
8. Rees, C. W., and Reardon, L. V. *Trop. Med. News,* 1944, **1**, 18.
9. Robinson, H. J., and Molitor, H. *J. Pharm. exp. Therap.,* 1942, **74**, 75.
10. Salle, A. J., and Jann, G. J. *Proc. Soc. exp. Biol. Med.,* 1945, **60**, 60.
11. Van Dyke, H. B. *Proc. Soc. exp. Biol. Med.,* 1944, **56**, 212.

A Relation Between Size of the Divalent Cation and Solubility of Triple Acetate Salt of Sodium

Lockhart B. Rogers
Stanford University

The importance of the size of the alkali metal cation to the formation of the triple acetate salt, $NaM^2UO_2(OAc)_9 \cdot 6H_2O$, was first demonstrated by Caley and Baker (*2*) when they proved that potassium, unlike lithium and sodium, formed only a double salt. In their paper, they listed the divalent ions which form triple acetate salts with sodium in an order of decreasing sensitivity toward sodium. Their list is reproduced in Table 1, together with the empirical ionic radii of Pauling (*5*) and the atomic radii of Goldschmidt (*3*). It can be seen that the solubility of the triple salt increases with the radii of the ions, whereas it bears no relation to the radii of the atoms.

TABLE 1

Radii of Divalent Cations Which Form Triple Acetate Salts Arranged in Order of Decreasing Sensitivity of Their Respective Reagents Toward Sodium

Cation	Radii in Angstrom Units		
	Ionic	Atomic	
Mg	0.65	1.62	
Ni	0.70	1.24	
Co	0.72	1.26	
Zn	0.74	1.37	
Fe	0.75	...	
Mn	0.80	1.36	
Cu	...	1.28	
Cd	0.97	1.52	
Hg	1.10	1.55	(liquid)

Caley and Baker did not assign a position to the ferrous acetate reagent because the difficulties involved in handling it made its exact position in the group uncertain. However, the value of the ionic radius of the ferrous ion establishes the position of reagent between those of zinc and manganese.[1]

TABLE 2

Radii of Alkaline Earth Metals Other Than Magnesium

Cation	Radii in Angstrom Units	
	Ionic	Atomic
Be	0.31	1.05
Ca	0.99	2.21
Sr	1.13	....
Ba	1.35	....

Likewise, if the assumption is correct that the solubility varies with the ionic radius, one should be able to assign a value to the radius of the cupric ion because the sensitivity of its reagent is known. Unfortunately, the limits are very wide, so additional information must be sought. The radius of the cuprous ion is known (0.96 A.—Pauling), as is the magnitude of the change in the radius resulting from the loss of an electron by the ferrous ion (0.15 A.). Although the conditions are not exactly the same for the ferrous ion and the cuprous ion, they are sufficiently similar to enable one to guess that the radius of the cupric ion is approximately 0.81 A.

From Table 2 one might predict that the radii of the divalent ions of the alkaline earth group do not

[1] In a private communication, Dr. Caley stated that this position is consistent with his observations.