Eserine Potentiation of Streptomycin Activity in Bacteria-Infected Embryonated Chicken Eggs

By VERNON A. GREEN†

Increases in per cent egg-day survival of eggs treated with combined streptomycin and eserine above the summation of the per cent egg-day survival of the two used separately were interpreted as potentiation of the antibacterial activity of the antibiotic. An attempt was made to correlate the cholinesterase activity of the bacteria with the eserine potentiation of the antibiotic activity of the streptomycin.

POTENTIATIONS of drug activity by eserine have been reported by Lewis (1), Greig (2, 3), and Green, *et al.* (4). Green and Davis (5) found that eserine potentiated the toxicity as well as the antibacterial activity of chloramphenicol in embryonated chick eggs.

In this study, possible eserine potentiation of the antibacterial activity of streptomycin, in embryonated chicken eggs infected with *Salmonella typhosa* WLR1207, *Proteus vulgaris* ATCC93, *Shigella dysenteriae* WLR194, or *Pseudomonus aeruginosa* ATCC9027, was investigated using the technique of McKenna and Taylor (6) for growing bacteria in yolk sacs. The criterion for determining potentiation was an increase in the per cent egg embryo (egg-day) survival above the summation of the per cent egg embryo survival of the antibiotic and eserine administered separately. Cholinesterase activity of the bacterial cells was determined by the method of Hall and Lucas (7); each unit of esterase activity being equal to 1 ml. of 0.01 *N* acetic acid formed in 10 minutes on the hydrolysis of acetylcholine solution at pH 8.

EXPERIMENTAL

Bacterial Inhibition.—Using the method reported by Green and Davis (5), embryonated chick eggs were infected deep in the yolk sac with 0.2 ml. of bacteria suspension to be used in each investigation. See Tables I–IV for concentration of microorganisms in seed suspension. After incubating at 37° for 6 hours, the eggs with living embryos designated for the investigation with each microorganism were divided into four groups of 40 eggs each. For each microorganism the groups were as follows: (*a*) control group, received only 0.2 ml./egg of sterile saline; (*b*) received streptomycin 0.1 mg./egg; (*c*) received streptomycin, 0.1 mg., and eserine salicylate, 0.0015 mg./egg; and (*d*) received eserine salicylate 0.0015 mg./egg.

The streptomycin, eserine, and eserine-streptomycin combinations were contained in 0.2 ml. of solution and were injected beneath the shell over the blood vessels of the embryo without rupturing the yolk sac. Upon sealing the shell with paraffin, the eggs were returned to the incubator and maintained at 37°.

After 7 hours the eggs were again candled and, thereafter, candled twice daily at 9:00 a.m. and 4:00 p.m. The embryo survivals were determined for 14 days, at which time any surviving embryos were destroyed, and the total day survivals for each group were recorded. The mean egg-day survival for each group was calculated; and using the mean egg-day survival of the saline controls used as the base or 100%, the mean egg-day survival of each group was reported as a percentage of the controls (see Tables I–IV).

Cholinesterase Determination.—Bacterial cells grown in beef heart infusion at 37° were packed by centrifuging, then washed and resuspended in sterile 0.9% saline three times. When approximately 3 ml. of packed cells were obtained, the cells were ground with sterile powdered glass in a bacteria mill and the bacterial protein extracted with 5% ammonium sulfate solution after the method of Nachmansohn (8). No attempt was made to concentrate the enzyme, but an amount of the extract equivalent to 1 ml. of the packed cells was used to determine the cholinesterase activity. The esterase activity per ml. of packed cells was as follows: (*a*) *Salmonella typhosa*, 0.021 units; (*b*) *Proteus vulgaris*, 0.050 units; (*c*) *Shigella dysenteriae*, 0.000 units; and (*d*) *Pseudomonus aeruginosa*, 0.025 units

RESULTS

In this study of eserine potentiation of the activity of streptomycin in *S. typhosa*-infected eggs, it was noted that the summation of the per cent egg-day survival of streptomycin- and eserine-treated eggs was approximately 90% less than the per cent survival of eggs receiving a combination of the two agents (Table I). Further, the difference in the per cent egg-day survival of *P. aeruginosa*-infected eggs treated with combined streptomycin and eserine and the summation of the per cent survival of the two used separately was approximately 120% (Table IV). The cholinesterase activity of the packed cells of the above two organisms corresponded closely in this study.

The increase in per cent egg-day survival in combined streptomycin- and eserine-treated eggs was greatest in eggs infected with *P. vulgaris*, approximately 500% (Table II). Eggs infected with *S. dysenteriae* treated with combined streptomycin and eserine showed a per cent egg-day survival approximately 110% below the summation of the two used separately (Table III). These findings were in

Received April 28, 1961, from the Pharmacology Division, The University of Texas, College of Pharmacy, Austin.
Accepted for publication July 6, 1961.
Presented to the Scientific Section, A.PH.A., Chicago meeting, April 1961.
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TABLE I.—ESERINE POTENTIATION OF STREPTOMYCIN ACTIVITY IN CHICKEN EGGS INFECTED WITH *Salmonella typhosa*[a]

Agent Used	No. of Eggs	Egg-Day Survivals	% Egg-Day Survivals
Controls (saline)	40	56	100
Streptomycin, 0.1 mg.	40	176	300
Streptomycin, 0.1 mg., and eserine salicylate, 0.0015 mg.	40	288	500
Eserine salicylate, 0.0015 mg.	40	63	110

[a] Each egg infected with 0.2 ml. of a suspension of *Salmonella typhosa* containing 100,000 organisms per ml.

TABLE II.—ESERINE POTENTIATION OF STREPTOMYCIN ACTIVITY IN CHICKEN EGGS INFECTED WITH *Proteus vulgaris*[a]

Agent Used	No. of Eggs	Egg-Day Survivals	% Egg-Day Survivals
Controls (saline)	40	3	100
Streptomycin, 0.1 mg.	40	14	460
Streptomycin, 0.1 mg., and eserine salicylate, 0.0015 mg.	40	40	1300
Eserine salicylate, 0.0015 mg.	40	10	330

[a] Each egg infected with 0.2 ml. of a suspension of *Proteus vulgaris* containing 80,000 organisms per ml.

TABLE III.—ESERINE POTENTIATION OF STREPTOMYCIN ACTIVITY IN CHICKEN EGGS INFECTED WITH *Shigella dysenteriae*[a]

Agent Used	No. of Eggs	Egg-Day Survivals	% Egg-Day Survivals
Control (saline)	40	60	100
Streptomycin, 0.1 mg.	40	80	130
Streptomycin, 0.1 mg., and eserine salicylate, 0.0015 mg.	40	84	140
Eserine salicylate, 0.0015 mg.	40	72	120

[a] Each egg infected with 0.2 ml. of a suspension of *Shigella dysenteriae* containing 80,000 organisms per ml.

TABLE IV.—ESERINE POTENTIATION OF STREPTOMYCIN ACTIVITY IN CHICKEN EGGS INFECTED WITH *Pseudomonus aeruginosa*[a]

Agent Used	No. of Eggs	Egg-Day Survivals	% Egg-Day Survivals
Control (saline)	40	23	100
Streptomycin, 0.1 mg.	40	36	150
Streptomycin, 0.1 mg., and eserine salicylate, 0.0015 mg.	40	90	390

keeping with the cholinesterase activity of the packed cells, since *P. vulgaris* showed the highest esterase activity and *S. dysenteriae* showed the least esterase activity (no esterase activity by this method).

DISCUSSION

It appears from the data obtained in this study that eserine potentiation of streptomycin activity was greatest in eggs infected with *P. vulgaris* and least in eggs infected with *S. dysenteriae*. According to Schwartz, *et al.* (9), the minimal inhibitory concentration of streptomycin for *P. vulgaris* ATCC 93, *S. typhosa* WLRI 207, and *P. aeruginosa* ATCC 9027 is 37 mcg./ml., while the minimal inhibitory concentration of streptomycin for *S. dysenteriae* WLRI 94 is only 12.3 mcg./ml.

If the synergistic action of eserine on the antibacterial activity of streptomycin were linear, it appears that the increase in the per cent egg-day survivals of eggs infected with *P. vulgaris*, *S. typhosa*, and *P. aeruginosa* should have been of the same magnitude. Further, it appears that if the increase in embryo survivals in the above were due entirely to antibacterial action of the eserine salicylate, then the egg-day survival of the combined streptomycin- and eserine-treated eggs should have been at least equal to the summation of the two when used separately in *S. dysenteriae*-infected eggs.

Increases in the permeability of mouse brain and dog erythrocytes brought about by eserine was reported by Greig (2, 3). Further, this investigator found a 45% potentiation of the antibacterial activity of streptomycin on *E. coli* by eserine (4).

It appears probable that eserine, by some activity, may have increased the rate of absorption and, consequently, the concentration of the antibiotic in the tissues of the chick embryo. There appears, also, the probability that eserine, through its action with cholinesterase, may change the permeability of bacterial cells containing the enzyme, thus allowing the streptomycin to enter the cells more easily. At least, there appears to be some correlation between increases in per cent egg-day survivals and the cholinesterase content of the bacterial cells.

SUMMARY

In this study, the per cent egg-day survivals of eggs infected with *S. typhosa*, *P. vulgaris*, and *P. aeruginosa*, and treated with combined streptomycin and eserine were increased above the summation of the per cent egg-day survivals of streptomycin, 0.1 mg./egg, or 0.0015 mg./egg of eserine, used separately. The per cent egg-day survival of *S. dysenteriae*-infected eggs treated with combined streptomycin and eserine was less than the summation of the two agents used separately.

increase was greatest in *P. vulgaris*-infected eggs and least in *S. dysenteriae*-infected eggs. These findings appear to correlate with the esterase activity of the packed cells.

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Synthesis of Compounds Containing the Isoindoline Ring System

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In an attempt to gain further knowledge concerning the pharmacological properties inherent in the isoindoline ring system, a number of compounds containing this ring system were synthesized and screened for biological activity. These compounds are analogs of biologically active compounds. Only the amide of N-[3-(2-isoindolinyl)propyl]-beta-alanine demonstrated appreciable biological activity. The carbon-hydrogen absorption bands present in the infrared spectra of these compounds were related to their chemical structure.

ALTHOUGH isoindoline is a relatively unstable compound, N-substituted isoindolines are quite stable, easily prepared, and provide convenient intermediates for synthesis. The ganglionic blocking action of 4,5,6,7-tetrachloro-2-(dimethylaminoethyl)isoindoline dimethochloride (1) and the hypertensive action of a series of the quaternary salts of hydrogenated bis-(2-isoindolinyl)alkanes (2) have been extensively studied. Few other compounds containing the isoindoline ring system have been reported to contain biological activity. It therefore appeared desirable to synthesize a number of analogs of biologically active compounds in which the isoindoline ring system is substituted for the amino or substituted amino group.

Three esters which contain the isoindoline ring system and which are analogs of procaine, Trasentine, and eucatropine were synthesized. These esters were 2-isoindolinylethyl 4-aminobenzoate hydrobromide, 2-isoindolinylethyl diphenylacetate hydrochloride, and 2-isoindolinylethyl *dl*-mandelate hydrochloride. The first of these esters was prepared by reacting 2-(2-hydroxyethyl)isoindoline with *p*-nitrobenzoyl chloride. The resulting 2-isoindolinylethyl *p*-nitrobenzoate was catalytically reduced to 2-isoindolinylethyl *p*-aminobenzoate. 2-Isoindolinylethyl diphenylacetate hydrochloride was similarly synthesized by the reaction of 2-(2-hydroxyethyl)isoindoline with diphenylacetyl chloride. 2-Isoindolinylethyl *dl*-mandelate was prepared by reacting silver mandelate with 2-(2-chloroethyl)-isoindoline.

In order to introduce the isoindoline ring system into a molecule having the prerequisites for antihistamine activity, 2-(2-isoindolinyl)ethyl benzhydryl ether was synthesized by reacting benzhydryl bromide with 2-(2-hydroxyethyl)-isoindoline in the presence of metallic sodium. It was isolated as its hydrochloride.

1,6-Bis-(2-isoindolinyl)hexane was prepared by the lithium aluminum hydride reduction of 1,6-hexane diphthalimide.

It also appeared desirable to introduce this ring system into compounds which might possess antitumor activity. 2-[2-Bis-(2-chloroethyl)-aminoethyl]isoindoline was synthesized by first reacting 2-(2-chloroethyl)isoindoline with diethanolamine to yield crude 2-[2-bis-(2-hydroxyethyl)aminoethyl]isoindoline which was then converted to the nitrogen mustard analog by treatment with thionyl chloride. The second possible antitumor agent, 1-[3-(2-isoindolinyl)-propyl]dihydrouracil, was prepared by the following series of reactions

Received April 28, 1961, from the Department of Chemistry, University of Illinois, College of Pharmacy, Chicago.
Accepted for publication July 18, 1961.
Presented to the Scientific Section, A.PH.A., Chicago meeting, April 1961.