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Effect of Penicillin and Streptomycin on Bacterial Contamination of Chick Embryos Inoculated with Unfiltered Sputums.

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(Introduced by A. R. Dochez).

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In a previous communication the use of penicillin for controlling bacterial contamination in chick embryos inoculated with unfiltered secretions of the respiratory tract was described.[1] It was noted, however, that while this method had been successfully employed for the isolation of influenza and herpes viruses, embryos not infrequently died as the result of infection with bacteria which were resistant to the action of penicillin; in the main these microorganisms were Gram-negative bacilli of the Proteus or Pseudomonas groups, or coliforms that were not further identified. Since streptomycin has been shown to exercise a potent bacteriostatic and bactericidal effect on many Gram-negative bacteria[2,3] it therefore seemed of interest to determine whether this compound, either singly or in combination with penicillin, was superior to penicillin alone in decreasing bacterial contamination and promoting the survival rate of chick embryos inoculated with unfiltered sputums.

Experimental. Specimens of sputum were obtained from 60 patients suffering from various infections of the respiratory tract including bacterial pneumonia, primary atypical pneumonia, bronchiectasis, acute tracheobronchitis and the common cold. In many instances specimens were deliberately collected from patients whose antecedent sputum cultures had revealed the presence of Gram-negative bacilli.

The sputums were freshly collected and either ground in a sterile mortar with quartz sand or emulsified in a Waring blendor with an equal volume of sterile physiological saline, followed by centrifugation in a Swedish angle centrifuge at 3000 R.P.M. for 10 minutes. The supernatant fluid of each specimen was then inoculated in 0.3 ml amounts into 8 developing hen's eggs which had been incubated for 10 or 11 days at 39°C. Two of the eggs were reserved as controls, 2 received 500 units of penicillin in a volume of 0.1 ml, 2 received 500 units of streptomycin in a similar volume, and 2 received both penicillin and streptomycin. All inoculations were made into the allantoic cavity.

After inoculation the eggs were incubated at 35°C and were candled daily. Eggs in which the embryos appeared to have died were opened at once, Gram-stained smears of the allantoic fluid were examined microscopically, and cultures of the allantoic fluid were made on plates of rabbits' blood agar. When colonies appeared on these plates after an incubation period of 24 or 48 hours at 37°C the individual bacterial species were identified in so far as was possible by appropriate bacteriological and serological procedures. All eggs with living embryos were opened 5 days after inoculation and were examined in a similar manner.

In Table I the data on the incidence of survival and bacterial contamination of the control eggs and those inoculated with the antibiotics are presented. Only 7 (6%) of 120 control eggs survived for 5 days and the great majority died within the first 3 days after inoculation; furthermore all of the eggs that died were found to be heavily contaminated with various bacteria and the deaths can no doubt be largely attributed to bacterial infection. Forty-eight (39%) of

[1] Rose, H. M., Molloy, E., and O'Neill, E., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 23.

[2] Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.

[3] Robinson, H. J., Smith, D. G., and Graessle, O. E., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 226.

TABLE I.
Incidence of Survival and Bacterial Contamination Among Chick Embryos Inoculated with Unfiltered Sputums and Antibiotics.

Day after inoculation	Control				Penicillin				Streptomycin				Penicillin + Streptomycin			
	Survived		Died		Survived		Died		Survived		Died		Survived		Died	
	Contaminated	Sterile	Contaminated	Sterile	Contaminated	Sterile	Contaminated	Sterile	Contaminated	Sterile	Contaminated	Sterile	Contaminated	Sterile	Contaminated	Sterile
1			22				14	2			4	4			2	5
2			58				22	6			13	12			5	8
3			22				10	3			10	2			2	4
4			7				2	5			3	4			4	9
5	4	3	4	0	8	40	4	4	24	36	6	2	19	58	2	2
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	4	3	113	0	8	40	52	20	24	36	36	24	19	58	15	28

the eggs that received penicillin survived of which 8 were contaminated, mainly with yeasts, and of the 72 that died 52 were contaminated without exception by Gram-negative bacilli. Sixty (50%) of the eggs inoculated with streptomycin survived, but 24 of these proved to be contaminated, chiefly with yeasts, molds, or green-producing streptococci. Seventy-seven (64%) of the eggs inoculated with penicillin together with streptomycin survived of which 19 were contaminated, mainly with yeasts or molds, and it should be noted that among the 43 eggs that died in this group 28 proved to be sterile on culture.

In reference to the results with individual sputum specimens, the frequency with which either one or both chick embryos survived without bacterial contamination, of the individual pairs inoculated with the antibiotics, was as follows: penicillin 26 (43%), streptomycin 28 (47%), penicillin plus streptomycin 43 (70%). With 47 (78%) of the 60 specimens at least one chick embryo survived without bacterial contamination among the 6 inoculated with the antibiotics. Only 3 specimens caused death of all embryos due to bacterial infection and of these 2 were caused by *Ps. aeruginosa* and one by an unidentified coliform organism.

In Table II are presented data indicating the types of bacteria isolated from each of the eggs inoculated with the antibiotics. It will be seen that penicillin is much more effective than streptomycin in preventing bacterial contamination with certain Gram-positive organisms, particularly streptococci. A discrepancy would appear to exist in the case of yeasts and molds, since a larger number of these microorganisms were isolated from the eggs receiving both streptomycin and penicillin than from those inoculated with either agent alone; this may be explained by the fact that the majority of yeasts and molds were isolated from surviving eggs, and the incidence of survival was greatest when both antibiotics were employed. Streptomycin restrained the growth of Gram-negative bacilli in many instances where penicillin was ineffective, particularly with strains of *Pr. vulgaris, Es. coli, Aer. aerogenes,* and unidentified coliforms, but although the incidence

TABLE II.
Strains of Bacteria Isolated from Chick Embryos Inoculated with Unfiltered Sputums and Antibiotics.

	Strepto-cocci	Staphylo-cocci	*Pr. vulgaris*	*Ps. aeruginosa*	*Es. coli*	*Aer. aerogenes*	Coliform	Unidentified gram-negative bacilli	*Sar. lutea*	Chromogenic gram-negative bacilli	Diphtheroids	Yeasts	Molds
Penicillin	1	2	15	15	4	5	13	5	0	0	0	6	0
Streptomycin	14	2	3	10	1	2	7	3	2	0	2	13	1
Penicillin + Streptomycin	1	1	1	9	0	0	2	4	0	2	0	14	3

of infections with *Ps. aeruginosa* was somewhat lower in the eggs that received streptomycin alone or streptomycin plus penicillin, in comparison with those receiving penicillin alone the reduction was not very striking. This frequent failure of streptomycin to inhibit the growth of *Ps. aeruginosa* has been noted by others.[4] It is of considerable interest that Gram-negative bacilli were eradicated by the combined action of streptomycin and penicillin in several instances when either of these compounds alone were ineffective. A possible explanation for this phenomenon resides in the fact that penicillin in large dosage has been shown to exert an antibacterial effect on certain strains of Gram-negative bacilli,[5] and this action may possibly operate synergistically with that of streptomycin when the antibiotics are employed in combination.

Discussion. The results of this study indicate that streptomycin and penicillin together are more effective than either of these agents alone in preventing bacterial contamination and permitting the survival of chick embryos inoculated with unfiltered sputums. The relatively low survival rate of embryos inoculated with penicillin alone, when compared with the results obtained in work previously reported,[1] is probably due to the fact that many sputums were deliberately chosen because they were previously known to contain Gram-negative bacilli. Not only are such organisms ordinarily resistant to the action of penicillin, but they frequently manufacture a substance, penicillinase, which will counteract or nullify the antibacterial effect of this compound.[6] It would appear that, in studies where it is desirable to inoculate unfiltered secretions of the respiratory tract into chick embryos for the intended recovery of viral agents, a helpful procedure would be the use of both penicillin and streptomycin to combat bacterial contamination.

Conclusions. Chick embryos were inoculat-

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[5] Hobby, G. L., *Science*, 1944, **100**, 500.

[6] Bondi, A., and Dietz, C. C., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 132.

ed with unfiltered sputums treated with penicillin and streptomycin singly and in combination. The combination of agents proved to be more effective than either one alone in preventing bacterial contamination and permitting survival of the embryos. Contaminations with Gram-negative bacilli which were resistant to the action of penicillin were controlled in many instances by the addition of streptomycin.

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Role of Nitroglycerin in Accelerating Occurrence of the Histamine-Provoked Ulcer.*

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The importance of the vascular factor in the ulcer diathesis has been recently reemphasized. Relative anemia of the mucous membrane of the esophagus, stomach, or duodenum of various experimental animals produced by adrenaline-in-beeswax,[1] fat emboli,[2] and portal hypertension,[3] has been shown to render these structures much more susceptible to histamine-provoked ulcer and/or erosion. In other words, even temporary areas of anemia in the esophageal, gastric or duodenal walls render those areas prone to injury by the gastric juice. The purpose of this presentation is to indicate our observations on the acceleration of the histamine-provoked ulcer in rabbits and dogs, by a drug, nitroglycerin, which dilates the smaller splanchnic vessels.[4]

Methods. These experiments were carried out on rabbits and dogs in 4 series. In the first series, which consisted of rabbits, 0.97 mg nitroglycerin, embedded in beeswax, was implanted daily in the back muscles, in addition to 30 mg of the histamine-in-beeswax mixture prepared after the method of Code and Varco.[5] The animals were sacrificed after varying periods of time. In the second series, dogs were used. Some of these animals received 1.95 mg nitroglycerin embedded in beeswax daily, in addition to 30 mg histamine-in-beeswax intramuscularly each evening. To the remainder of the dogs 30 mg histamine-in-beeswax alone was administered each evening. In each instance the dogs' feed pans were removed and no more food given until the following morning. All the dogs were sacrificed within 2 to 6 days after the beginning of the administration of histamine.

In the third series, the effect of an aqueous solution of 1.3 mg nitroglycerin injected subcutaneously was studied upon dogs with Pavlov and Heidenhain pouches.

In the fourth series recordings of blood pressures were made on anesthetized dogs while the stomach mucosa was exposed and observed for any changes after injection of aqueous nitroglycerin 1.3 mg intramuscularly and intravenously. One series of blood pressure recordings on one animal was recorded on a kymogram by cannulation of a carotid artery. In the other animal used, the blood pressure was measured by femoral artery puncture, by

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2 Baronofsky, I., Merendino, K. A., Bratrud, J. E., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 231.

3 Baronofsky, I., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 234.

4 Goodman, L., and Gilman, A., *The Pharmacological Basis of Therapeutics*, pp. 550, MacMillan, New York, 1941.

5 Code, C. F., and Varco, R. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 475.