Lewis,[2] (b) the excision of a wedge of the nerve end and suture of the flaps (Corner[3]), and (c) a combination of the two procedures as proposed by Stookey.[4] Kirk[5] described a procedure in which the end of the nerve is cauterized with the high frequency current. Boldrey[6] reported an experimental method for overcoming neuroma formation by burying the stump of the nerve in bone. While these procedures frequently reduce the size to which neuromata grow, they have not been universally effective in preventing the development of painful amputation stumps.

In an attempt to completely inhibit neuroma formation, by controlling the growth of the nerve fibers and connective tissue, the cut ends of the sciatic nerve of the dog were placed in snugly fitting, rigid tubes of various materials: silver, cellophane, vitalium, or glass.

This experimental procedure has been carried out on 24 animals. After as long as 102 days the fibers of the sciatic nerve encased in a snugly fitting glass tube are straight and orderly and there is no tendency towards neuroma formation. All control experiments in which a portion of nerve was resected showed neuroma formation.

Conclusion. When the cut end of the sciatic nerve of the dog is supported by a closely fitting, rigid tube, there is no tendency to disorderly overgrowth of the nerve fibers and connective tissue characteristic of neuroma formation.

2 Huber, G. C., and Lewis, D., *Arch. Surg.*, 1920, **1**, 85.

3 Corner, E. M., *Proc. Roy. Soc. Med.*, **11**, 7, April 9, 1918.

4 Stookey, B., *Surgical and Mechanical Treatment of Peripheral Nerves*, p. 461, chap. 21, W. B. Saunders Co., Phila., 1922.

5 Kirk, N. T., Amputations: Dean Lewis, *Practice of Surgery*, Vol. III, chap. 10, 1943.

6 Boldrey, E., *Ann. Surg.*, 1943, **118**, 1056.

14569

In vitro Action of Penicillin Alone, and in Combination with Sulfathiazole, on *Brucella* Organisms.

TSUN T'UNG. (Introduced by Martin Frobisher, Jr.)

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Information concerning the antibacterial effect of penicillin on gram-negative organisms is meager and it is a general belief that most gram-negative bacilli, including *Brucella*,[1] are relatively resistant to penicillin. This has led to possibly undue pessimism with regard to the therapeutic use of available chemotherapy in brucellosis. The present communication presents *in vitro* studies of the action of penicillin alone, and in combination with sulfathiazole, on a number of strains of *Brucella* organisms.

Materials and Methods. Penicillin was prepared from a Fleming strain of *Pen. notatum* cultivated on modified Czapek-Dok medium[2] to which 1% of casamino acid was added. The potency of the crude penicillin on the 7th day when tested by the serial dilution method was around 1-640 against a test strain of *Staphylococcus aureus* H (3R9674). The pH of the culture fluid on the 7th day was about 7.5. Extraction with amyl acetate and buffer was carried out according to the procedure of Abraham *et al.*[3] Extraction was repeated once to concentrate the penicillin. The extracted material was shaken with 2% animal charcoal

1 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **11**, 177.

2 Hobby, Gladys L., and Meyer, Karl, PROC. SOC. EXP. BIOL. AND MED., 1943, **50**, 227.

3 Abraham, E. P., and Chain, E., *Brit. J. Exp. Path.*, 1942, **23**, 103.

TABLE I.
Bacteriostatic Action of Penicillin on *Brucella* Organisms.

Organisms	Dilution of penicillin*								
	20	40	80	160	320	640	1280	2560	5120
Suis									
Zin	—	—	—	—	—	—	—	3	4
Gilley	—	—	—	—	—	—	—	4	
Harrison	—	—	—	—	—	—	1	4	
Svic	—	—	—	—	—	—	2	4	
Stock	1	4							
Abortus									
Green	—	—	—	—	—	—	—	—	4
No. 9	—	—	—	—	—	—	—	4	
Stock	—	—	—	—	—	—	4		
No. 7	—	2	4						
Croni	—	4							
Melitensis									
No. 3	—	—	—	—	—	—	—	4	
Stock	1	3	4						
Gilly	1	4							
Bent.	2	4							
No. 1	3	4							

* A dilution of 1/320 indicates a concentration of 1 Oxford unit per cc.
Minus sign indicates no growth; numerals indicate the degree of turbidity of growth.

and filtered through Seitz pads. The filtrate has a slight amber color. The partially-purified penicillin, when tested by the cup-plate and serial dilution methods,[4] was found to contain 320 Oxford units per ml. The penicillin was then put into celluloid tubes and preserved in the low-temperature (–76°C) cabinet until used.[5]

Sodium sulfathiazole, dissolved in physiological saline solution in a concentration of 50 mg per ml, was sterilized in boiling water for 20 minutes. The solution was further diluted with broth so that 0.1 ml added to 0.9 ml of medium containing various amounts of penicillin, gave a concentration of 5 mg % of sodium sulfathiazole. For sulfathiazole-susceptible strains the amount of sulfathiazole used in conjunction with penicillin was reduced to that which, alone, produced only slight bacteriostasis.

Meat infusion broth (pH 7.4) containing 0.25% dextrose was used for all *Brucella* cultures. Penicillin was diluted with this broth. In some tests one-tenth ml of the penicillin-containing medium was replaced with the same amount of sulfathiazole-containing broth so that the volume of the medium was maintained at one ml in all test cultures in serological tubes.

The inoculum in all tests consisted of one drop of a 1-10 dilution of a 24-hour broth culture, introduced into the medium from a syringe with a 26-gauge needle. Each drop contained approximately 100 million organisms. This heavy inoculum was necessary to obtain a clear-cut and uniform result in a comparatively short time without resorting to cultivation in an atmosphere containing added CO_2

Cultures were incubated at 37°C. Since a luxuriant growth could not be obtained in the sulfathiazole-containing medium within 48 hours under the conditions of the tests described, readings were taken after 72 hours' incubation. The contents of tubes showing no visible growth were plated out on tryptose agar to detect possible slight growth or bacteriostatic action.

All of the strains used had been preserved for a year or more at –76°C. A majority of them were placed at this temperature soon after they were isolated. The stock cultures, *i.e.*, those which have been kept and trans-

[4] Foster, J. W., and Woodruff, H. B., *J. Bact.*, 1944, **47**, 43.

[5] Tung, T., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 103.

TABLE II.
Action of Penicillin in Combination with Sodium Sulfathiazole on *Brucella* Organisms.

Organisms	Dilution of penicillin										Sod. sulfathiazole alone mg %		
	20	40	80	160	320	640	1280	2560	5120	10240	5	1	0.1
Suis													
Zin	—	—	—	—	—	—	—	1	3		—	3	
Gilly	—	—	—	—	—	—	—	—	1	4	4		
Harrison	—	—	—	—	—	—	—	4			4		
Svie	—	—	—	—	—	—	—	2			3		
Stock	—	—	2	2							—	—	2
Abortus													
Greene	—	—	—	—	—	—	—	—	1	3	3		
No. 9	—	—	—	—	—	—	—	—	1	3	3		
Stock	—	—	—	—	—	—	—	2	2	2	—	—	2
No. 7	—	—	—	4							4		
Croni	—	—	1	2	3						3		
Melitensis													
No. 3	—	—	—	—	—	—	—	—	1	2	2		
Stock	—	—	—	1	2						—	—	2
Gilly	—	3									3		
Bent	—	3									3		
No. 1	1	4									4		

ferred for many generations on ordinary infusion agar, though of low virulence still possess their distinctive cultural characteristics.

Results. The bacteriostatic action of penicillin on *Brucella* organisms is shown in Table I. It can be seen that the 15 strains tested fall into two groups according to their relative susceptibility to penicillin. Eight strains were susceptible to penicillin at a concentration of about 0.1-0.5 unit whereas even 10 units could not inhibit the growth of the other strains. It seems that there are strain, instead of species, differences between the susceptibilities of *Brucella* organisms to penicillin. The data suggest that the *suis* variety is more penicillin-susceptible than are the melitensis strains, but the number of strains in each group is too small to permit definite conclusions on this point.

Penicillin in the greater concentrations exerted a definitely bactericidal effect on some of the strains. The plating test with the inoculated tubes in which no growth was visible showed that a bactericidal action was exerted by concentrations of penicillin one or two dilution intervals below those producing only a bacteriostasis.

Since, in order to be effective on some strains of *Brucella,* concentrations of penicillin are required which are attainable but not maintainable under clinical conditions, it was regarded as worth while to try the combination of a representative sulfonamide drug with penicillin[6] in the hope of producing a synergistic action. It was hoped that certain strains could thereby be rendered vulnerable to lower concentrations of penicillin. Sodium sulfathiazole was chosen for this experiment, being apparently the most effective among sulfonamides against organisms of the *Brucella* group.[7,8] The action of penicillin in combination with a small amount of sulfathiazole is presented in Table II. It can be seen that penicillin, combined with sulfathiazole, exerts a considerably greater antibacterial action than penicillin alone. This synergistic action is more noticeable on certain strains.

These experiments, in which large inocula were used, incidentally demonstrated great differences between susceptibilities of *Brucella* organisms to sulfathiazole. While the stock strains and one of the *suis* strains proved extremely susceptible to sulfathiazole, most of the recently isolated cultures were so resistant to this drug that a concentration of

6 Ungar, J., *Nature,* 1943, **152**, 254.

7 Kempner, W., Wise, B., and Schlayer, C., *Am. J. Med. Sci.,* 1940, **200**, 484.

8 Wise, Bowman, *J. Pharm. and Exp. Ther.,* 1942, **76**, 156.

even 5.0 mg % was not appreciably inhibitory to them. There seems to be no parallelism between the susceptibilities of *Brucella* organisms to the two therapeutic agents tested.

It is beyond the scope of this experiment to estimate how much of the antibacterial action of the penicillin preparation used was due to penicillin itself and how much to the so-called penatin.[9,10,11] However, it may be pointed out that the penicillin was harvested at pH 7.5 and that it was extracted with an organic solvent. Moreover, neither the presence nor absence of a small amount of dextrose in the medium for the tests appreciably affected the antibacterial action of this material. These facts suggest that it was the penicillin rather than penatin that was chiefly responsible for the antibacterial action.

Conclusion. Penicillin exerted a considerable antibacterial action on 8 out of 15 strains of *Brucella in vitro*. This action was enhanced by the combination of penicillin with a small amount of sodium sulfathiazole.

9 Kocholaty, Walter, *J. Bact.*, 1942, **44**, 469.

10 Kocholaty, Walter, *Arch. Biochemistry*, 1943, **2**, 73.

11 Kocholaty, Walter, *Science*, 1943, **97**, 186.

14570

Absence of Liver Damage in Chicks Hypoprothrombinemic Due to Vitamin K Deficiency or Ingestion of 3,3'-methylenebis (4-hydroxycoumarin)*

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It is generally assumed that the hypoprothrombinemia resulting from vitamin K deficiency is due to the inability of the liver to synthesize prothrombin in the absence of an adequate amount of vitamin K. In chicks reared on an artificial vitamin K-free diet which produced clinical signs of the deficiency disease within 12 to 14 days, one group of investigators[1] observed fatty degeneration and necrosis in the liver and suggested that the absence of vitamin K may cause liver damage which may in turn be responsible for the failure of prothrombin formation. However, patients suffering from hypoprothrombinemia which can be corrected by vitamin K do not as a rule show an abnormal response in the usual tests of liver function.

The first description of the hemorrhagic disease in cattle caused by the feeding of spoiled sweet clover hay also mentioned liver damage accompanying the hypoprothrombinemia.[2] However, investigations of the sweet clover disease carried out since the isolation and synthesis of the causative agent, 3,3'-methylenebis (4-hydroxycoumarin), commonly referred to as dicumarol, have left undecided the question of the association of liver damage with this form of hypoprothrombinemia.[3,4]

The modes of action of vitamin K and dicumarol in the body remain unexplained; but in view of the possibility that their action might be expressed in morphologic as well as functional alterations in the liver, it seemed desirable to undertake a more detailed exam-

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1 Hepding, L., and Moll, T., *E. Merck's Jahresbericht*, 1939, **53**, 5.

2 Schofield, F. W., *Can. Vet. Rec.*, 1922, **3**, 74.

3 Stahmann, M. A., Huebner, C. F., and Link, K. P., *J. Biol. Chem.*, 1941, **138**, 513.

4 Overman, R. S., Field, J. B., Baumann, C. A., and Link, K. P., *J. Nutrition*, 1942, **23**, 589.