Additional worms will be seen in the perfusate exiting from the cut edges of the liver and from the portal vein. When no further hepatic vein perfusion yields any worms, the liver is divided into its separate lobes and these cut into smaller pieces about 3 to 4 inches square. The larger portal radicals may be perfused and additional worms obtained. By a gentle squeezing and shaking motion of the pieces of liver additional worms can be recovered. (The intact liver may be kept in saline overnight safely and the above procedure carried out with the same results as with livers done shortly after removal from the animal's body.)

The mesentery of the large and small bowel is stripped from its attachments, cut into small pieces and placed in saline. This is allowed to stand at ice-box temperature overnight, at which time all the worms remaining in the mesenteric venous system will have escaped and seen lying on the bottom of the container.

Experiments are being carried on to determine fully the advantages of this method over other methods described.

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Subtilin—Antibiotic Produced by *Bacillus subtilis*.* II. Toxicity of Subtilin to Living Embryonic Tissue.†

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In a previous communication[1] it was shown that subtilin, an extract prepared from *Bacillus subtilis,* was antagonistic chiefly against Gram-positive bacteria. Two notable exceptions were *Neisseria catarrhalis* and *N. gonorrhocae,* both Gram-negative but also antagonized by subtilin. Acid-fast organisms, including *Mycobacterium tuberculosis,* and a number of pathogenic higher fungi were also found to be susceptible to the antibiotic. The agent produced a bacteriostatic action in high dilution and a germicidal effect in greater concentration.

The present communication is concerned with the toxicity of subtilin to living embryonic chick heart tissue fragments cultivated *in vitro*. The procedures followed in performing the tests were the same as those previously reported.[2,3]

Experimental. The highest dilution of subtilin that killed embryonic chick heart tissue fragments in 10 minutes at 37°C was found to be 1:500 (Fig. 1). The highest dilution of subtilin that killed *Staphylococcus aureus* (F. D. A. strain) under the same conditions was 1:9750 (Table I). From these results a toxicity index may be calculated.

The toxicity index may be defined as the ratio of the highest dilution of subtilin killing embryonic chick heart tissue after an exposure period of 10 minutes at 37°C to the highest dilution killing *Staphylococcus aureus* under the same conditions. The results give a toxicity index of 500/9750 = 0.05 (Table II). Under the conditions of the test, subtilin was found to be 20 times more toxic to *Staphylococcus aureus* than to embryonic chick heart tissue, a remarkably low figure. Theoretically, the smaller the toxicity index the more nearly perfect the germicide.

Unitage. The methods used for expressing the potencies of the various antibiotics are, in general, quite complicated and confusing. In order to simplify the method of designating

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2 Salle, A. J., McOmie, W. A., Shechmeister, I. L., and Foord, D. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 694.

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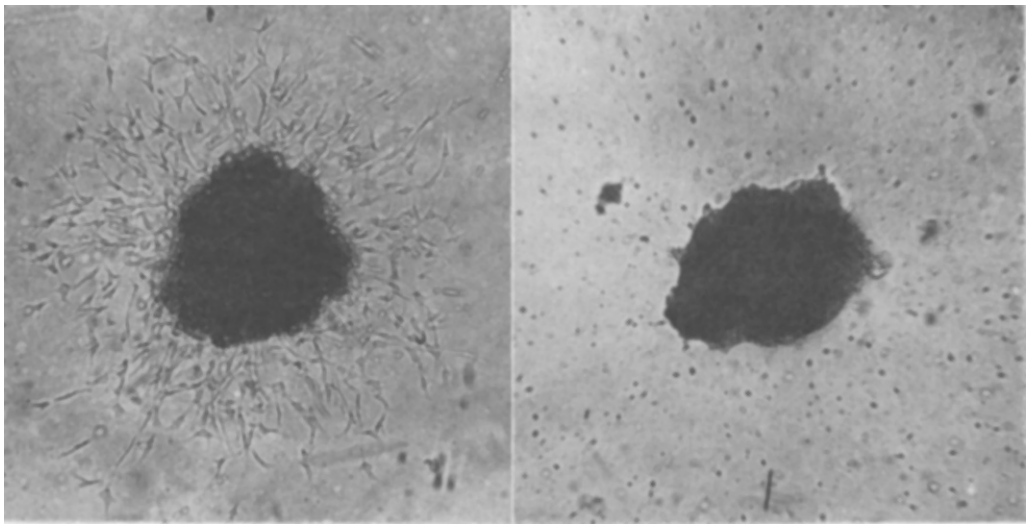

FIG. 1.
Left, fragment of chick heart tissue exposed to a sublethal concentration of subtilin. Note new growth of fibroblasts. Right, fragment exposed to a lethal concentration of subtilin.

TABLE I.
Killing Dilutions of Subtilin for Chick Heart Tissue Fragments and *Staphylococcus aureus*.

Tissue fragments		*Staphylococcus aureus*	
Dilution	Growth	Dilution	Growth
1:100	—	1:9000	—
1:200	—	1:9250	—
1:300	—	1:9500	—
1:400	—	1:9750	—
1:500	—	1:10,000	+
1:600	+	1:10,250	+
1:700	+	1:10,500	+
1:800	+	1:10,750	+
Control	+	Control	+

TABLE II.
Toxicity Index of Subtilin.

Killing dilution		
Tissue (A)	*Staphylococcus aureus* (B)	Toxicity index A/B
1:500	1:9750	0.05

the strength of subtilin as much as possible, a unit is defined as that amount present in 1 cc of the highest dilution (expressed in mg) capable of killing *Staphylococcus aureus* in 10 minutes as 37°C (F.D.A. phenol coefficient method). For example, 5 cc of a 1:9750 dilution of the preparation used, when mixed with 0.5 cc of a nutrient broth culture of *Staphylococcus aureus*, completely destroyed all organisms in 10 minutes at 37°C. One cc of this dilution contains approximately 0.1 mg of this preparation of subtilin. Therefore, 0.1 mg contains 1 unit of subtilin. The weight of subtilin containing 1 unit will vary depending upon the purity of the product.

The 24-hour culture of *Staphylococcus aureus* was standardized in a photoelectric colorimeter to contain the same density as a No. 7 McFarland Nephelometer Standard.

Summary. Subtilin, an antibiotic extracted from *Bacillus subtilis*, was found to be antagonistic chiefly against Gram-positive organisms, including *Mycobacterium tuberculosis* and other acid-fast bacteria. Two notable exceptions were *Neisseria catarrhalis* and *N. gonorrhoeae*, both Gram-negative but also antagonized by subtilin. The antibiotic showed an extremely low toxicity for embryonic chick heart tissue fragments cultivated *in vitro*. Under the conditions of the test subtilin was approximately 20 times more toxic to *Staphylococcus aureus* than to chick heart tissue, a remarkably low tissue toxicity. A unit of subtilin is defined as the amount contained in 1 cc of the highest dilution capable of killing *Staphylococcus aureus* in 10 minutes at 37°C (F.D.A. phenol coefficient method).