found (Fig. 3). The vacuoles have the tendency to aggregate and seem to push the spermatozoa away from the tubule wall. Three hours after the injection almost all mature spermatozoa have been released. Most of the Sertoli cells appear enormously vacuolized and with the apical border completely irregular, due to the rupture of some vacuoles (Fig. 4). During this process of vacuolization, the Golgi apparatus appears fragmented and situated close to the vacuoles.

The observations recorded here show that possibly the release of the spermatozoa of the toad, under the influence of the pituitary gland, is due to the intense changes produced in the Sertoli cells, which lead to the rupture and disintegration of the apical part of their cytoplasm.

## 15215 P

## A Method for Removal of Adult *S. mansoni* from Experimentally Infected Rabbits.

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Herein is described a method for the removal of adult *S. mansoni* worms from experimentally infected rabbits. The method has been used with good results in rabbits although there is no apparent reason why it could not be applied to other experimental animals susceptible to schistosomiasis.

The animal is tied to a board and a large dose (100 mg) of Heparin (Abbott—10 mg per cc) in 30 to 40 cc of physiological saline is injected very slowly into the heart or intravenously. Between 15 to 30 minutes are allowed to elapse at which time the animal is sacrificed in any desired fashion, the abdomen opened and the portal vein exposed. An 18-gauge needle with attached 50-cc syringe is inserted into the portal vein with the point of the needle directed towards the liver. The heparinized blood in the portal vein is aspirated while the syringe and needle are rotated to direct the bevel of the needle toward the worms which will be seen flowing into the syringe with the blood. About 15 to 20 cc can thus be aspirated before the portal vein collapses. An additional 10 to 15 cc of blood can be aspirated by exerting a gentle squeezing pressure on the lobes of the liver—this will force more blood into the portal vein, and the liver will be seen to blanch slightly. The syringe is disconnected from the needle and put aside without fear of the blood clotting. A clean dry syringe may be attached to the needle and additional aspirations carried out in the manner described.

If it is desired to keep the worms alive, the aspirated blood is poured into a solution of 0.5% saponin in physiological salt solution (0.85%)—this will lake the blood cells but have no apparent effect on the worms. If it is not necessary to keep the worms alive, the aspirated blood is poured into a large volume of tap water—this will lake the blood cells, and the dead worms will sink to the bottom of the vessel.

When no further aspirations can be done the needle is removed from the portal vein. The liver is removed as a unit by cutting and clamping the hepatic vein, portal vein, surrounding ligaments and adhesions and kept in saline for further recovery of worms. It is then placed on a dissecting board and the thin lips of the various liver lobes are cut off about ¼ inch from their edges. Using a 50-cc syringe, physiological saline is perfused through the liver by injecting the hepatic vein.

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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.

## 15216

## 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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