containing casein-digest and cystine. The organisms were washed off with saline, centrifuged, resuspended, and recentrifuged three times to free them from medium. The packed cells were finally suspended in water, adjusted to pH 8.0 and kept about 6 hours at room temperature and 14 hours at 4°C. Next the preparation was neutralized and sterilized by heating at 60°C for 30 minutes. Its concentration was adjusted to about 1.0% solids. All injections of endotoxin were made intraperitoneally in 18- to 20-g white mice. The toxicity of each preparation was determined by preliminary tests in batches of 5 or 6 mice for each trial dose.

The penicillin† used was the ordinary commercial product of several manufacturers. The mice received 8 subcutaneous injections of penicillin of 1,500 units each in 0.15 cc of water. These were usually given as follows: at 90- and 45-minute intervals before the endotoxin and at 1, 3, 5, 9, 20, and 24 hours after the endotoxin. Variations in total quantity and number of doses of penicillin were tried but no improvement was found in the amount and method of administration. Mice were used in groups of 8 to 15 for each quantity of endotoxin in the control group as well as in the group receiving penicillin. Total number of deaths was determined for a period of 90 hours after injection of the endotoxin.

In most of the experiments graded doses of endotoxin were used. Representative of some of the more striking experiments are the two summarized in Table I. In each group injected with a given dose of endotoxin, more deaths occurred among the controls than among those treated by penicillin.

Some experiments showed better protection than others, but in every one fewer of the treated mice died than of the controls. The combined results of all the series, totaling 176 control mice and 210 penicillin treated, are summarized in Fig. 1, which shows the rates of death as well as final percentages.

The difference in survivals and deaths as shown on this chart may be considered significant. Whereas 76.7% of all the control mice died, only 36.5% of those receiving the penicillin treatment died.

During the first 12 hours after endotoxin injection, 18.7% of the controls and 11.4% of the penicillin-treated mice died—some as early as 3 hours. From 12 to 20 hours the highest mortality occurred—38.1% of the controls and 18% of those treated with penicillin.

Other substances such as saline and tryptic digest of casein repeatedly injected in place of the penicillin failed to influence the lethal action of the endotoxin.

Summary and Conclusions. Large doses of penicillin repeatedly administered by subcutaneous injection protected a significant proportion of mice against the lethal action of a crude preparation of gonococcal endotoxin.

† The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for experimental investigations recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

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Action of Anterior Pituitary on Sertoli Cells and on Release of Toad Spermatozoa.

E. DE ROBERTIS, M. H. BURGOS, AND E. BREYTER. (Introduced by Vergilio G. Foglia.)
From the Institute of General Anatomy and Embryology, Faculty of Medicine, Buenos Aires, and Institute of Biology and Experimental Medicine.

Houssay and Lascano Gonzalez[1] found that in the testis of toads, in which anterior pituitary glands were implanted, spermatozoa were released into the lumen of the seminiferous tubules. Rugh[2,3] confirmed this ob-

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[2] Rugh, L. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 418.

[3] Rugh, L. R., *J. Exp. Zool.*, 1939, **80**, 81.

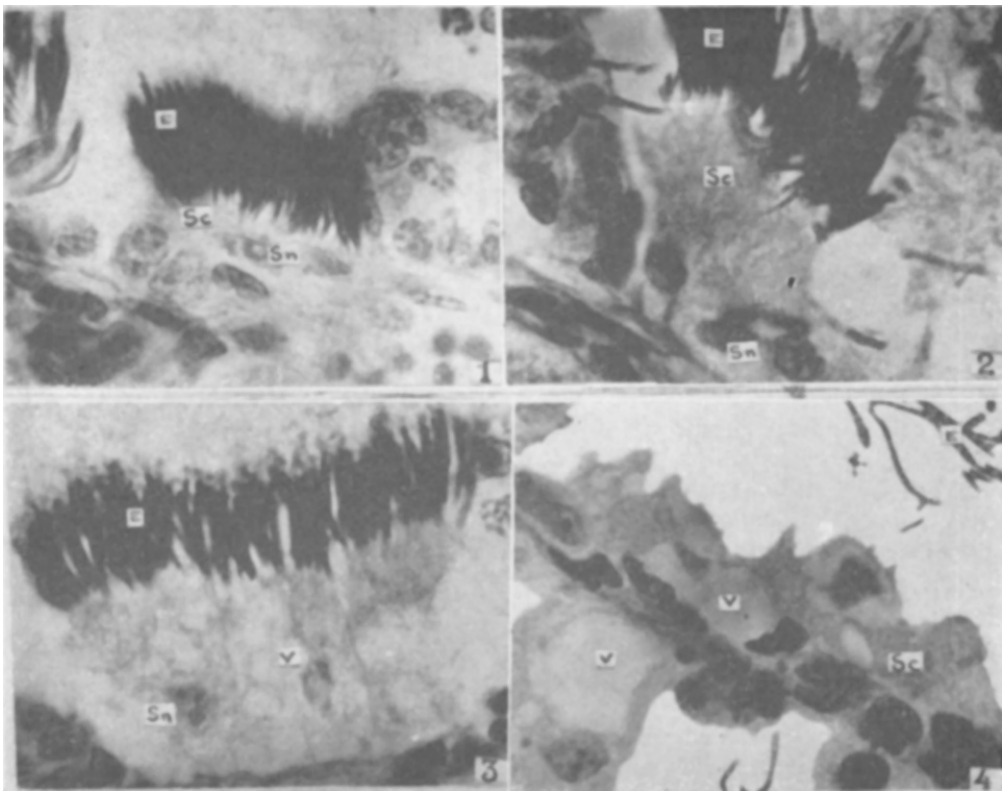

Fig. 1-4. Testis of *Bufo arenarum* Hensel. Hematoxylin-eosin. Ob. 90x, oc. 6x, Cam. length 60 cm. Se, Sertoli cell; Sn, Sertoli nucleus; E, spermatozoon; v, vacuole.

FIG. 1. Normal control. Spermatozoa tightly clustered upon Sertoli cells. Fix.: Zenker-formol.

FIG. 2. Thirty minutes after the injection of the pituitary extract. Cytoplasm of Sertoli cells is greatly increased, showing a fine vacuolization and small eosinophilic granules. Fix.: Bouin's fluid.

FIG. 3. One hour after injection. Cytoplasm of the Sertoli cells is more hypertrophic and shows big vacuoles. Fix.: Bouin's fluid.

FIG. 4. Three hours after injection. Spermatozoa are completely liberated and released into the lumen. Sertoli cells appear highly vacuolized and with the apical border torn. (Lumen slightly retouched.)

servation finding that the injection of pituitary extract produces, in the testis of hibernating frog, the release of the spermatozoa, normally attached to the Sertoli cells. The author gives no explanation of the mechanism by which the spermatozoa are released into the lumen, but suggests that: "Possibly smooth muscle fibers are involved in the expulsion of spermatozoa from the tubules" (Rugh[3]).

In order to investigate the actual mechanism of this release, toads (*Bufo arenarum* Hensel) in sexual rest (end of summer) were injected intravenously with a total extract of 3 anterior lobes of the pituitary gland of male toads. Thirty minutes, one, 2, 3, and 5 hours after the injection, the testes were fixed in Bouin or in Zenker-formol fluids; and also in the fixative of Champy, followed for 7 days by immersion in osmic acid at 38°C. Testes of normal control animals showed almost all mature spermatozoa clustered about their Sertoli cells (Fig. 1). Thirty minutes after the injection of the pituitary extract some of the spermatozoa have been liberated. The Sertoli cells showed big increase and vacuolization of cytoplasm (Fig. 2). After one hour, clear hydropic vacuoles were more conspicuous and also some acidophil droplets were

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.

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

J. LEONARD BRANDT AND E. P. FINCH. (Introduced by J. Oliver Gonzalez.)

From the Department of Medical Zoology, School of Tropical Medicine, San Juan, Puerto Rico.*

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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