influenza.† Each embryo received an original total inoculum of 0.15 cc; of this 0.05 cc was a 1:1000 dilution of infected allantoic fluid, and 0.10 cc was either saline or the antibiotic reconstituted and diluted with sufficient saline to give the desired concentration. The virus and streptomycin were mixed immediately before inoculation. In those instances where more than one "dose" of streptomycin was given, the subsequent injections were made in 0.15 cc quantities within the first 24 hours. In the interim, the shellholes were sealed with a small tent of Scotch tape. The eggs were incubated at 36° to 37°C for 48 hours. After this period the allantoic fluids were collected and their hemagglutinating titers for chicken red cells were determined.[4]

† The influenza strains were furnished by Dr. M. D. Eaton.

[4] Florman, A. L., and Crawford, J. P., *Am. J. Med. Sci.*, 1944, **208**, 494.

Results of Study. The results of several experiments are summarized in Chart I.‡ From this it is apparent that:

1. A total of 12,000 units given over a period of 24 hours failed to make any significant difference in the multiplication in the chick embryo of the PR-8, Olsen, and Lee strains of influenza. This represents a total dosage of approximately 200,000 units per kilo of egg weight per 24 hours. It may be contrasted with the 150 and 300 units of streptomycin which protect chick embryos against *Shigella Gallinarum* and *Brucella abortus* infections.[3]

2. Despite the very large amounts of streptomycin administered in these experiments there was no evidence of any lethal effect on the developing embryo. The low toxicity of this new agent is noteworthy.

‡ The chart was prepared by Cpl. Fred W. Trader and photographed by the U.S.A. Signal Corps.

15213 P

Protection of Mice Against Lethal Action of Gonococcal Endotoxin by Penicillin.*

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We have reported[1] that large subcutaneous doses of penicillin repeatedly administered exert a considerable degree of protection against the toxicity of sterile meningococcal endotoxin as measured by its lethal action in mice and rabbits. Neter[2] claimed that penicillin mixed with tetanus toxin did not protect mice from death. According to Ercoli, Lewis, and Moench[3] large doses of penicillin neutralized diphtheria toxin *in vitro*, but had no effect in guinea pigs on the lethal action of incompletely neutralized diphtheria toxin. Little other investigation has been directed toward the effect of penicillin on the action of toxic bacterial substances. Carpenter and his co-workers[4,5,6] reported detoxification by sulfanilamide and sulfapyridine of the "toxins" of gonococcus and other bacteria.

The crude endotoxin used in these experiments was prepared from 5 strains of gonococcus grown for 18 hours on an agar medium[7]

* Aided by a grant from the John and Mary R. Markle Foundation.

[1] Boor, Alden K., and Miller, C. Phillip, *Science*, 1945, **102**, 427.

[2] Neter, Erwin, *J. Inf. Dis.*, 1945, **76**, 20.

[3] Ercoli, N., Lewis, M. N., and Moench, Lucille J., *J. Pharm. Exp. Ther.*, 1945, **84**, 120.

[4] Carpenter, C. M., Hawley, P. L., and Barbour, G. M., *Science*, 1938, **88**, 530.

[5] Barbour, Gerald M., and Carpenter, Charles M., Am. Assn. Adv. Sci., Pub. No. 11, 1939, 114.

[6] Carpenter, Charles M., and Barbour, Gerald M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 354.

[7] Boor, Alden K., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 22.

TABLE I.

Effect of Penicillin on the Lethal Action of Gonococcal Endotoxin in Mice.

Gonococcal endotoxin	Dose, ml	Control mice Died/injected	Penicillin treated mice Died/injected
From strain 362	0.4	11/12	6/12
	0.1	8/12	2/12
From strain 354	0.5	10/10	6/10
	0.25	8/10	2/10
	0.1	7/10	0/10

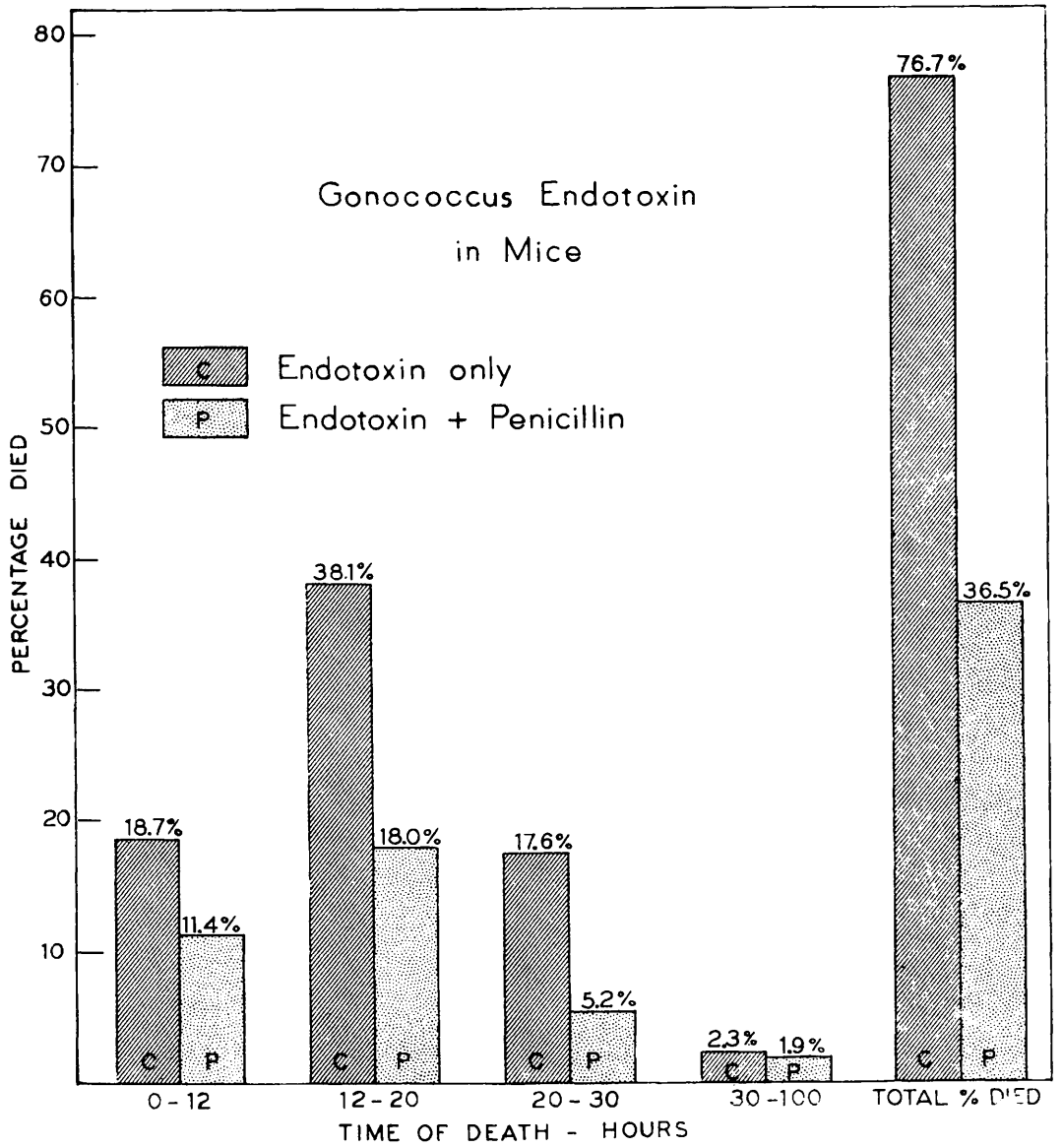

FIG. 1.

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.

15214

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

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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-

[1] Houssay, B. A., and Lascano Gonzalez, J. M., *Rev. Soc. Arg. Biol.*, 1929, **5**, 77.

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

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