TABLE I.
Effect of Lanatosides A, B, and C on the Cardio-inhibition Produced by Acetylcholine in Anesthetized Dogs.

	Lanatoside A			Lanatoside B			Lanatoside C		
	I	II	III	I	II	III	I	II	III
	.25	(—)	57	.82	(—)	100	.14	84	84
	.36	(—)	67	.25	80	80	.24	83	75
	.27	(—)	77	1.17	(—)	90	.54	85	85
	.34	(—)	93	.10	(—)	100	.24	55	55
	.66	58	33	.13	(—)	67	.22	50	50
	.33	60	70	.21	(—)	76	.17	43	43
	.49	(—)	86	.22	63	54	.26	50	50
	.40	(—)	62	.25	58	58	.23	67	67
	.43	(—)	38	.22	67	55	.30	45	45
	.65	(—)	35	.29	84	38	.23	56	56
Avg	0.42		62	.37		73	.26	62	62

I Lethal dose in mg per kilo. II. % of lethal dose which abolished the cardio-inhibitory effect of acetylcholine. III. % of lethal dose which produced gross cardiac irregularities. (—) Cardio-inhibitory effect of acetylcholine not abolished at any time.

If the cardiac effects of the various glycosides are qualitatively identical there should be a similar sequence of evolution of the characteristic phenomena in each case and the phenomena produced by a certain per cent of the lethal dose of one glycoside should be produced by the same per cent of the lethal dose of another. The results indicate that such is not the case and therefore an actual qualitative dissimilarity in cardiac action appears to exist.

No explanation for this apparent qualitative dissimilarity between the glycosides is offered, and it is not clear why each glycoside did not prevent the cardio-inhibitory action of acetylcholine when evidences of idioventricular rhythms appeared. Electrocardiograms show that the dose of acetylcholine used produces auriculo-ventricular block. While digitalis principles also depress auriculo-ventricular conduction, we have seen no evidence of an enhanced response to acetylcholine in the digitalized animals.

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Activity of Penicillin Against *Hemophilus ducreyi in vitro.*

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The activity of penicillin on *Hemophilus ducreyi* was studied *in vitro* by the serial dilution method.

Both a crude filtrate of a culture of *Penicillium notatum* and a standardized penicillin* were tested against this organism. The test cultures were 5 strains of *Hemophilus ducreyi*, three JS, LB, and BF, recently isolated in our laboratory from patients with chancroid, and two, JB and S, stock cultures. All strains were morphologically typical of *H. ducreyi* and were pathogenic for rabbits

* This penicillin was obtained from Dr. G. L. Hobby of the Department of Medicine, College of Physicians and Surgeons, Columbia University. It was prepared in the laboratory of that Department under permit of the War Production Board and had been standardized against penicillin received from Dr. R. Coghill of the Northern Regional Research Laboratories, Peoria, Illinois.

TABLE I.
Activity of Crude Penicillin Filtrate Against *H. ducreyi in vitro* in Serum Broth.

	Dilutions					
	1:160	1:320	1:640	1:1280	1:2560	Control
Streptococcus hemolyticus C203Mv	—	—	—	—	—	++++
Staphylococcus aureus 209	—	—	—	++	+++	++++
Hemophilus ducreyi JB	—	—	++	++++	++++	++++
,, ,, S	—	—	++	++++	++++	++++
,, ,, JS	—	—	—	++	++++	++++
Escherichia coli	++++	++++	++++	++++	++++	++++

— No visible growth.
+ to ++++ Relative turbidity for each organism after 24 hours as compared with the respective control.

by intradermal inoculation.† In addition, strain JB proved at the time of the experiment to be virulent for man on experimental inoculation into the skin.‡

Method. Cultures of *H. ducreyi* were grown in defibrinated rabbit blood at 35° C for 24-48 hours and stored in the refrigerator. Transfers were made once a week. Good growth was obtained by inoculation of the stock cultures into 10% rabbit serum nutrient broth and subsequent daily passage in this medium. *Staphylococcus aureus,* strain 209, was used as the control test strain for determining the inhibitory titer of the penicillin preparations. *Streptococcus hemolyticus,* penicillin susceptible,[2, 3] and *Hemophilus pertussis, Hemophilus influenzae* and *Escherichia coli,* penicillin resistant,[2, 3] were included for purposes of comparison. All organisms were tested for sensitivity to penicillin in 10% rabbit serum broth, with the exception of *H. influenzæ* which was grown in Levinthal's medium. In addition, penicillin sensitivity was tested on 10% rabbit defibrinated blood agar plates.

Crude penicillin filtrate was diluted 1:40 by adding 0.2 cc to 7.8 cc of broth. Serial twofold dilutions were then made in serum broth up to 1:2560. Each tube containing 1.8 cc of the proper dilution of penicillin was inoculated with 0.2 cc of a 10^{-1} dilution of a 20-hour culture. Control tubes without penicillin were inoculated similarly. Standardized penicillin was diluted in broth or Levinthal's medium to give the desired number of Florey units per cc. Each tube, as well as controls without penicillin, was then inoculated with 0.2 cc of a 10^{-1} dilution of culture, the final volume being 2.0 cc. Standardized penicillin was also incorporated in rabbit blood agar with duplicate plates for each concentration of penicillin used. All plates, including controls without penicillin, were divided into sections and streaked with 20-hour undiluted cultures. Observations were made after 24 to 48 hours' incubation at 35°C. The last dilution showing a clear fluid or no visible growth at the end of the 24 hours was considered the endpoint in the titration.

Results. An inhibitory action of penicillin on the growth of *H. ducreyi* was demonstrated with both the crude and purified preparations. The results of the experiment are shown in Tables I, II, and III.

The titration endpoints with both crude filtrate and standardized penicillin were in agreement with several previous tests in which the same substances had been titrated against the same organisms. All strains of *H. ducreyi* were of approximately the same order of sensitivity to penicillin as the test strain of *Staphylococcus aureus.* The greater inhibitory action of penicillin against *Streptococcus hemolyticus* than against either the Ducrey bacillus or *Staphylococcus aureus* is indicative of the different sensitivity of vari-

† This confirms the report of Maximova[1] that intradermal injection of *H. ducreyi* in rabbits is followed within 24-48 hours by a pustular lesion.

‡ Personal communication of Dr. A. J. Pereyra, Comdr. (M.C.), U. S. N.

1 Maximova, A. A., *Ann. Dermat. et Syphil.,* 1936, **7**, 840.

2 Fleming, A., *J. Path. and Bact.,* 1932, **35**, 831.

3 Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

TABLE II.
Activity of Standardized Penicillin Against *H. ducreyi in vitro* in Serum Broth.

	Units per cc									
	2.0	1.0	0.5	0.25	0.13	0.06	0.03	0.016	0.008	Control
Streptococcus hemolyticus C203Mv	—	—	—	—	—	—	—	—	+++	++++
Staphylococcus aureus 209	—	—	—	+	+	+	++	+++	++++	++++
" " " *	—	—	—	—	—	+	++	+++	+++	++++
Hemophilus ducreyi JB	—	—	—	—	+	++++	++++	++++	++++	++++
" " S	—	—	—	—	—	+++	++++	++++	++++	++++
" " JS	—	—	—	—	+	++++	++++	++++	++++	++++
" " LB	—	—	—	—	—	+++	+++	+++	+++	++++
" " BF	—	—	—	—	+	++++	++++	++++	++	++++
" *pertussis*†	—	—	+	++	+++	+++	+++	+++	++++	++++
" *influenzæ**	+	+	++	++	+++	+++	+++	+++	++++	++++
Escherichia coli	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

— No visible growth.
+ to ++++ Relative turbidity for each organism after 24 hours as compared with the respective control.
* Tested in Levinthal's medium.
† Tubes were inoculated with undiluted cultures—results were read after 28 hours.

ous organisms to the action of this substance. Similar observations have been made by other workers.[3] It may be difficult to make an accurate comparison of sensitivity since the number of organisms in the inocula used in these tests differed appreciably for different organisms. One must consider that *H. ducreyi* is a rather slowly growing organism and 24-hour cultures would therefore be expected to contain fewer organisms than similar cultures of either *Staphylococcus aureus* or *Escherichia coli*. It has been pointed out, however, that the number of organisms in the inoculum is of little significance in this regard.[4, 5]

[4] Abraham, E. P., *et al.*, *Lancet*, 1941, **2**, 177.
[5] Fisher, A. M., *Bull. Johns Hopkins Hosp.*, 1943, **73**, 343.

TABLE III.
Activity of Standardized Penicillin Against *H. ducreyi in vitro* in Blood Agar.

	Units per cc					
	2.0	0.5	0.13	0.03	0.008	Control
Streptococcus hemolyticus C203Mv	—	—	—	—	+	+
Staphylococcus aureus 209	—	—	—	+	+	+
Hemophilus ducreyi JB	—	—	—	+	+	+
" " S*	—	—	—	+	+	+
" " BF*	—	—	—	+	+	+
" *influenzæ*	+	+	+	+	+	+
Escherichia coli	+	+	+	+	+	+

— No growth.
+ Growth.
* Read after 48 hours, no visible growth being present after 24 hours.

It is of interest to note that the sensitivity of *H. ducreyi* to penicillin *in vitro* would seem to differentiate it somewhat from other organisms of the same group, such as *H. influenzae* and *H. pertussis*, which are penicillin resistant.

Summary and Conclusions. The activity of penicillin on the growth of 5 strains of *H. ducreyi* was tested *in vitro* by the serial dilution method. *H. ducreyi* was found to be penicillin sensitive. This sensitivity was less than that of *Streptococcus hemolyticus* and paralleled that of the test strain of *Staphylococcus aureus*.

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Studies on the Action of Penicillin. II. Therapeutic Action of Penicillin on Experimental Meningococcal Infection in Mice.

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The effect of penicillin on several experimental infections in mice has been investigated[1-4] but even though it has been found to be highly satisfactory in the treatment of cerebrospinal fever, its application to experimental meningococcal infection in a laboratory animal has not as yet been reported. The present study undertook to fill a gap in our knowledge about this valuable chemotherapeutic agent.

Methods. Meningococci were grown for 6 hours on agar slants, suspended in Gelatin-Locke's solution to a turbidity known to approximate 1 billion meningococci per ml and then titrated in 4% mucin* by 10-fold dilution for intraperitoneal injection in 1 ml volumes.[5] Penicillin† in aqueous or saline solution was administered in the following volumes: Subcutaneous—.2 ml, intramuscular—.1 ml, intravenous—.2 ml.

Experiment I. Relation of the Time of Administration of Penicillin to Its Effect on Experimental Meningococcal Infection. The first experiments were made with a stock strain of meningococcus which had been carried for a number of months on artificial media with frequent mouse-passages. It possessed maximal virulence for mice by ordinary standards, *i.e.* fewer than 10 meningococci in 1 ml of 4% mucin regularly initiated a lethal infection.

Mice were infected with 100,000 MLD (10^{-4} of the standard suspension which at 10^{-9} killed the controls). At intervals of ½, 2, and 4 hours thereafter, groups of 4 mice were injected intravenously or subcutaneously with penicillin in a single dose of 100 Oxford units each.

As shown in Table I, the route of administration of penicillin made practically no difference in the results. Of the 8 treated ½ hour after inoculation, only one survived; whereas among those treated at 2 and 4 hours, 8 of 12 survived.

In a series of 13 mice infected with the next lower inoculum of meningococci (10,000 MLD) and treated at the same intervals, only one, which was treated at ½ hour, failed to survive.

* Granular mucin, Type 1701-W, supplied by the Wilson Laboratories, Chicago, Ill.

† 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.

1 Hobby, G. L. Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 285.

2 McKee, C. M., and Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 275.

3 McIntosh, J., and Selbie, F. R., *Lancet*, 1943, **2**, 224.

4 Hac, L. R., *J. Infect. Dis.*, 1944, **74**, 164.

5 Miller, C. P., and Castles, Ruth, *J. Inf. Dis.*, 1936, **58**, 263.