ethanol might stimulate the growth of the mutant.

The assay with the "cholineless" mutant* was carried out by the method of Horowitz and Beadle.[3] Aminoethanol and betaine were inactive and methionine had a very slight activity, which confirms a previous report.[3] It was found that dimethylaminoethanol alone or in combination with betaine or methionine had a marked effect in stimulating growth. A comparative assay showed that dimethylaminoethanol alone was approximately 1.5 times as active as choline chloride. When calculated to a molar basis this result indicates that the two compounds show about the same activity in the upper part of the response curve, but in the lower portion of the curve the response to choline was greater than the response to dimethylaminoethanol. This is illustrated in Fig. 1. Diethylaminoethanol was found to have less than 0.1% of the activity of dimethylaminoethanol.

A solution of dimethylaminoethanol, 0.5 mg per cc, was submitted to permutit adsorption and elution[3] and the eluate and filtrate were assayed with the mutant. The biological activity was found to pass quantitatively into the eluate. The filtrate was inactive.

A solution of choline chloride was submitted to reineckate precipitation.[4] The reineckate precipitate was measured colorimetrically and the filtrate was assayed with the mutant. When dimethylaminoethanol was added to the choline chloride solution, the dimethylaminoethanol appeared to be partially precipitated, as indicated by the increased colorimetric value of the precipitate and the increased assay value of the filtrate. Dimethylaminoethanol in pure solution, however, was not precipitated with reineckate under the conditions used.

The results with the mutant might indicate that it is able to synthesize a "methyl donator," such as, perhaps, methionine, but is defective in being unable to synthesize the postulated "methyl acceptor," dimethylaminoethanol, in the formation of choline.

* Cultures of the mutant were kindly supplied by Dr. N. H. Horowitz and by Dr. Earle Arnow.

4 Glick, D., *J. Biol. Chem.*, 1944, **156**, 643.

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A Method for Production of a Localized Gonococcal Infection in the Rabbit's Eye.*

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Investigation of certain problems related to gonococcal infection have been neglected because of lack of a satisfactory method of producing a localized infection experimentally in some laboratory animal. Among the many attempts reported in the literature[1] only that by Cohn[2] warrants mention here. He injected gonococci into the anterior chamber of rabbits' eyes and occasionally succeeded in recovering them from that site several days thereafter and on 2 occasions 28 days after inoculation with 2 particularly virulent strains. He, nevertheless, concluded that the longevity of the microörganisms was "variable and irregular" and that the anterior chamber of the rabbit was unsuitable for his purposes.

The method described below was developed

* The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

1 For a comprehensive bibliography see the excellent review of the literature by Justina Hill, *Am. J. Syph., Gon. and Ven. Dis.*, 1944, **28**, 334 and 471.

2 Cohn, Alfred, *Derm. Z.*, 1930, **60**, 35.

after a protracted search for an experimental infection which would lend itself to the testing of gonococcidal agents by topical application *in vivo* such as is impractical in the mouse infection previously described.[3,4]

Materials. Animals: Young adult rabbits were used for most experiments. They were easier to handle than very young rabbits and seemed to be no less susceptible to infection. Albinos possessed two advantages—the progress of the inflammatory reaction could be more easily observed and smears of the ciliary body contained no pigment.

Strains of Gonococci Employed: Two strains of gonococci were used: one had been isolated in 1942 from the blood of a patient with bacterial endocarditis and maintained in mice by continuous animal passage.[5] At the time of its 133rd mouse passage it was found to be satisfactory for infection of the anterior chamber of the rabbit's eye. The other strain was freshly isolated from the exudate of a gonococcal urethritis which had resisted sulfathiazole for many weeks.

The medium was made by digesting a 10% suspension of casein with trypsin, boiling, filtering, diluting with 3 parts of water and adding the following ingredients: 0.65% $Na_2HPO_4 \cdot 12H_2O$, 0.50% KCl, 1.00% dextrose, 1.80% agar, 0.065% cystine, heating and adjusting to pH 7.8 which should come down during sterilization to approximately 7.4. For the recovery of gonococci from infected eyes, this medium was enriched by the addition of about 10% defibrinated rabbit blood.

Methods. Inoculation: Cultures to be used for inoculation were grown over night on the casein digest-cystine agar described above. A loopful of culture was suspended in a few ml of saline and diluted to a density which experience has shown to contain approximately 1,000,000,000 gonococci per ml. Maximal dispersion of the microörganisms was insured by repeated sucking in and out of the pipette.

Other suspending fluids were tried; *e.g.*, water, broth, gelatin, Locke's solution and mucin in concentrations of 2 and 4%,† but none possessed any advantage over saline. Mucin increased the inflammatory reaction to an undesirable degree.

The rabbits were prepared for inoculation by intravenous injection of 30 mg of morphine sulfate in 1 ml of saline and instillation of a drop of 5% cocaine into each conjunctival sac. The hair around the lid margins including lashes and whiskers was clipped to eliminate a source of contamination. The lids were opened wide and held in that position by a speculum. A fine hypodermic needle attached to a 2 cc Luer syringe was inserted at the limbus into the anterior chamber and the aqueous humor aspirated by gentle suction. This needle was withdrawn and another of the same caliber attached to a 1 ml tuberculin syringe containing a suspension of gonococci inserted through the same hole and 0.2 ml of bacterial suspension injected into the anterior chamber.

After inoculation the rabbits were kept in a room which was artificially cooled during the hot weather to a temperature between 50° and 60°F.

Recovery of gonococci from infected eyes: When aqueous humor was to be withdrawn for culture or transfer before the animals were to be sacrificed, they were prepared by general and local anesthesia as described above and the aqueous was withdrawn into a 1 or 2 ml syringe. When complete cultures were required the animal was sacrificed by an intravenous injection of air, the aqueous aspirated and cultured by spreading two drops over the surface of a freshly prepared blood-agar plate. The eye was then enucleated, rinsed in 70% alcohol, and opened by cutting the cornea with a sharp knife. A portion of the ciliary body was removed with sterile forceps, macerated and streaked over the surface of another blood agar plate. The lens was then removed and its anterior surface cultured in the same way. The vitreous humor was cultured by streaking

[3] Miller, C. Phillip, "Experimental Gonococcal Infection," in *The Gonococcus and Gonococcal Infection*, Publication No. 11, A.A.A.S., Lancaster, Pa., 1939, 20.

[4] Miller, C. Phillip, and Hawk, Walter D., *Trans. Assn. Am. Phys.*, 1940, **55**, 216.

[5] Miller, C. Phillip, *Am. J. Syph., Gon. and Ven. Dis.*, 1944, **28**, 620.

† Granular mucin, type 1701-W, supplied by the Wilson Laboratories, Chicago.

a loopful onto the surface of another plate. The cultures were all placed in a metal box containing a lighted candle and incubated at 37°.

When direct microscopic examination was desired, smears were made of the aqueous, ciliary body and lens immediately after the cultures.

The cultures were inspected carefully after 18 to 24 hours incubation and again after a second day's incubation. Colonies of gonococci were identified by smears stained by Gram's method and sugar fermentation reactions.

Results. Appearance of the infected eyes: Eyes inoculated late in the afternoon showed considerable inflammatory reaction by the following morning: injection of the bulbar conjunctiva, especially around the limbus, clouding of the cornea, and exudate in the anterior chamber. As time went on the conjunctival and ciliary injection grew more pronounced and vascularization of the cornea began. In some cases intra-ocular tension increased and even caused rupture of the bulb if it was not relieved by aspiration of fluid from the anterior chamber.

The results of cultures from different parts of each eye were not uniformly consistent, that is, the culture of one part of an eye might yield a confluent growth of gonococci although one from another part of the same eye contained few colonies or none. In general, cultures of the anterior surface of the lens most frequently contained the largest numbers of gonococci. Those of the ciliary body came next, and those of the aqueous and vitreous humors third and fourth in order of decreasing incidence. If a culture from any part of an eye contained any colonies of gonococci that eye was regarded as infected at the time of its enucleation.

TABLE I.
Infections Resulting from Inoculation of Different Numbers of Gonococci.

Approximate No. of gonococci	No. of eyes inoculated	No. of infections resulting	%
20,000,000	266	248	93
2,000,000	32	27	84
200,000	28	23	82
20,000	32	21	65
2,000	26	12	46
200	22	10	45
20	10	0	0

Size of inocula required to infect: To determine the proportion of infections resulting from inocula of varying sizes and also the minimal infective dose, eyes were injected with dilutions of the standard suspension of gonococci and cultured at the end of 24 hours.

As will be seen in Table I positive cultures were obtained at the end of 24 hours in 93% of the eyes injected with approximately 20,000,000 gonococci. The reason for the preponderant number of eyes receiving this particular inoculum was that they served as controls for a series of tests which will be reported in a subsequent communication. The percentage of infections diminished with the number of gonococci injected, but inoculation of 22 eyes with only 200 gonococci resulted in 10 infections.

Definite evidence that multiplication of the inoculated gonococci took place within the anterior chamber and its adjacent tissues was obtained from the number of colonies developing from measured portions of the aqueous and of suspensions of the macerated tissues of the eye.

Duration of the infection: To determine the length of time viable gonococci persisted in inoculated eyes the following experiment was carried out: The eyes of a series of rabbits were infected with approximately 20,000,000 gonococci each. At regular intervals, thereafter, animals were sacrificed and their eyes cultured.

Results. (See Table II) During the first week there was a progressive fall in the percentage of eyes from which viable gonococci could be cultivated. At the end of one week over a third of the eyes were still positive. Subsequent observations which were made at weekly intervals were too few to treat statistically, but the proportion of positive infections continued at about the same rate. These results indicate that this infection becomes chronic in approximately a third of the eyes and may persist for as long as 14 weeks—the maximum period of observation.

It should be noted that the data contained in Table II represent the results of cultures made at autopsy. Some of the negative cultures came from eyes which had been aspirated

TABLE II.
Duration of Infection
As determined by recovery of viable gonococci from eyes at various times after inoculation.

Time after inoculation	No. of eyes cultured	No. of eyes positive	% positive
1 day	10	10	100
2 days	11	10	91
3 "	12	9	75
4 "	12	7	58
5 "	15	7	46
6 "	24	11	45
7 "	23	8	35
2 wks	6	2	
3 "	4	2	
4 "	4	1	
5 "	4	1	
6 "	4	2	
7 "	2	1	
8 "	6	3	
9 "	4	2	
10 "	10	3	
11 "	6	2	
12 "	2	0	
14 "	2	2	

at various times during life and found to contain living gonococci.

Discussion. The lesion produced in the anterior chamber of the rabbit's eye by the introduction of gonococci is regarded as a genuine infection for the following reasons: Viable gonococci were recovered from 93% of the inoculated eyes at the end of 24 hours and from 39% at the end of 7 days. The inoculated gonococci were found to invade the neighboring tissues (lens and ciliary body) and definite evidence of multiplication was obtained. The infection could be transmitted in series by transfer of a drop or two of aqueous humor, but this method of animal passage was impracticable for routine experimental purposes, because contaminants could not be detected until after the transfer had been made. In approximately ⅓ of the inoculated animals the infection became chronic and persisted until the end of the longest period of observation which was 14 weeks.

This infection has been found to lend itself to the study of local and systemic therapy of gonococcal infection and to the testing of gonococcidal agents *in vivo*.

Summary. A method is described for the production of a localized gonococcal infection in the rabbit's eye by inoculating gonococci into the anterior chamber. Gonococci were found to invade the tissues of the eye, to multiply there and to produce severe inflammatory reaction. In approximately ⅓ of the animals the infection became chronic and viable gonococci persisted as long as 14 weeks, the maximum period of observation.

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Influence of Muscle Pain on Electrical Resistance of the Human Skin.*

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It was shown in a series of investigations from this laboratory[1-4] that muscle pain greatly influences the somatic nervous system at spinal and supra-spinal levels. Autonomic changes occur also under these conditions as evidenced by pupillary dilatation which is based on an inhibition of the tone of the third

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1 Gellhorn, E., and Thompson, L., *Am. J. Physiol.*, 1944, **142**, 231.

2 Gellhorn, E., and Thompson, L., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 209.

3 Gellhorn, E., *Journal Lancet*, 1944, **64**, 242.

4 Thompson, L., and Gellhorn, E., PROC. SOC. EXP. BIOL. AND MED., 1945, in press.