

TRANSMISSION AND CULTIVATION EXPERIMENTS WITH
HUMAN TRACHOMA AND THE EXPERIMENTAL DIS-
EASE IN MONKEYS¹

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In recent articles by Finnoff and Thygeson (1) and by Tilden and Tyler (2), an elaboration of the work of Noguchi (3) has been reported, namely, the recovery of *Bacterium granulosis* from cases of human trachoma in Denver (1) and Arizona (2), and the description of several additional cultural and biological characteristics of the microorganism.

In this paper we shall present the results of cultivation experiments with conjunctival tissue from patients with trachoma residing in New York City, and of transmission tests in monkeys with the same material. The susceptibility of monkeys to conjunctival secretions from animals experimentally infected with trachomatous tissues or with cultures of *Bacterium granulosis* has also been studied. Thus the methods of Noguchi (3) have again been followed. The results of the repetition strengthen the view, first advanced by him, that *Bacterium granulosis* is closely related to human trachoma.

Transmission of Human Trachoma to the Monkey

The apparent failure of Noguchi to induce experimental infection in monkeys and apes by the use of human trachomatous tissue, in contrast to his success with cultures, may have been due to unfavorable experimental conditions. He made only two tests, inoculating material from American Indian cases of trachoma directly into a total of four *rhesus* monkeys and two apes. In the experiment with the latter, the tissues had been kept *in vitro* for 9 days before inoculation. The following tests show that conjunctival tissue from human trachoma in a white population, in New York City, induced in *Macacus rhesus*

¹ Preliminary reports of these experiments appeared in *Science*, 1929, 70, 612; 1930, 71, 263, 564.

monkeys characteristic granular conjunctivitis—a finding similar to that of Finnoff and Thygeson (1) with materials obtained from white patients in Denver.

Methods and Materials.—There were available for study nine patients residing in New York City, all having advanced trachoma of from 1 to 11 years' standing. With two exceptions (Cases 7 and 8), the patients exhibited scar tissue and characteristic pannus formation. The materials used consisted of the affected conjunctivæ removed, for curative purposes, from the novocaine-anesthetized upper lid. We are greatly indebted to Dr. Martin Cohen² for these materials, and for other aid. The fresh tissue from each of eight of the cases was ground in saline solution and inoculated subconjunctivally into the left upper lid of two or three *Macacus rhesus* monkeys, using methods of injection already described (2, 3). In another series of tests, the conjunctival secretions from each of two patients were used as inocula. The excised conjunctiva of one of the latter patients was used for subconjunctival inoculation of monkeys, 19 days after the final collection of secretions. The secretions were taken on cotton swabs and, by gently rubbing the mucous membrane, were transferred directly to the conjunctivæ of each of three monkeys. Nine swabbings at daily intervals were made with the secretions of the first case and seven with those of the second.

Only such monkeys as had smooth conjunctivæ at the end of an isolation period of about 1 month were employed, so as to decrease the hazard of confusion with spontaneous folliculosis.

*Results of Animal Inoculation.*³—The results obtained after single subconjunctival inoculations of monkeys with suspensions of conjunctival tissue obtained from man will be considered first.

Case 1.—Male, 26 years old, Russian. Has had trachoma for 10 years. Scars and pannus present. Suspension of conjunctival tissue inoculated into the left conjunctiva of each of three *rhesus* monkeys (A, B, and C). All these animals showed the characteristic granular conjunctivitis described by Noguchi (3) as following inoculations of *Bacterium granulosis*. For example, Monkey A revealed follicles in the conjunctivæ of both eyes on the 11th day after inoculation, and Monkeys B and C reacted similarly 4 days after the injection.

Case 2.—Female, 20 years old, Italian. Has had trachoma for several years. Scar tissue and pannus present. Monkeys D and E injected with suspension of patient's conjunctival tissue. Monkey D showed characteristic granular conjunctivitis in the left eye 33 days after the injection and in both eyes 41 days thereafter. Monkey E remained unaffected.

² We are also grateful to Drs. Arnold Knapp, Ervin Torok, and Julius Wolff, all of New York City, for their cooperation.

³ All operations were done under full ether anesthesia.

Case 3.—Male, 26 years old, American. Duration of trachoma, 7 or 8 years. Pannus and scars present. Monkeys F and G injected with tissue. Monkey F revealed the first signs of the characteristic experimental conjunctivitis in the left eye 10 days, and in the right, 45 days after inoculation. Monkey G failed to show lesions.

Case 4.—Male, 29 years old, German. Trachoma of uncertain duration, probably several years. Pannus and scars in left eye, scars only in right. Tissue from both upper lids pooled and injected into monkeys H and I. Monkey H developed the characteristic experimental disease in the left eye 7 days after inoculation. It died of tuberculosis, however, 1 month later. Monkey I showed granular conjunctivitis in both eyes also after 7 days.

Case 5.—Female, 50 years old, Russian. Duration of disease 10 years. Scar tissue and pannus present. The right and left upper conjunctivae were removed and a suspension of both tissues pooled was inoculated into the left conjunctiva of each of two monkeys, J and K, neither of which was affected by the injection.

Case 6.—Male, 48 years old, Italian. Duration of trachoma, 3 years. Scar tissue and pannus present. Tissue injected into two monkeys, L and M. Neither showed lesions.

Case 7.—Male, 31 years old, American. Has had trachoma for 11 years. 10 years ago, left tarsectomy performed and 5 years ago expression of follicles on left conjunctiva. The right upper conjunctiva showed large follicles, but no scars or pannus. A few follicles were also noted in the lower membrane and in the residual tissue of the left eye. Suspension of the upper right conjunctival tissue was inoculated into the left conjunctivae of each of two monkeys, N and O. Monkey N developed characteristic experimental conjunctivitis in both eyes 13 days after inoculation. Monkey O showed follicles in the left conjunctiva 6 days after injection, and in the right, 8 days later.

Case 8.—Male, 21 years old, German. Duration of disease 1 year. Treated for 2 months prior to tarsectomy. No scars or pannus present. Two monkeys, P and Q, inoculated subconjunctivally with suspension of patient's conjunctival tissue failed to become infected.

The next series of tests concern the production of the experimental disease in *Macacus rhesus* monkeys by means of swabbing the secretions of the patients on to the conjunctivae of the animals.

The first experiment was made with secretions obtained from Case 1, already mentioned, the secretions having been collected 19 days before the tarsectomy was performed. 13 days after the ninth and last swabbing, Monkey R developed the characteristic granular conjunctivitis; Monkey S died of tuberculosis during the swabbings; and Monkey T was unaffected.

The secretions for the second test were obtained from Case 9.

Case 9.—Male, 28 years old, Italian. Had trachoma for 2 years. Scars and

pannus present. 13 days after the seventh and last swabbing with this patient's secretions, three monkeys (U, V, and W) presented typical granulomatous changes in their conjunctivae. In none of the three animals, however, was extension of the affection noted in the untreated conjunctiva.

To summarize, a characteristic infection was produced in *Macacus rhesus* monkeys, either by means of a single subconjunctival injection of suspensions of conjunctival tissue from human trachoma, or by means of conveying the patient's secretions with cotton swabs to the animal's conjunctiva. By the former method, five out of eight patients' tis-

TABLE I
Record of Transmission Experiments

Case No.	Inoculation of material*		Recovery of <i>B. granulosis</i> from patient	Inoculation of <i>B. granulosis</i> into monkeys	Recovery of <i>B. granulosis</i> from animals inoculated with human material
	Subconjunctival	Swab			
1	+++	+-	+	+	+
2	+-		-	Not recovered	-
3	+-		-	" "	+
4	++		+	+	No experiment
5	--		+	+	" "
6	--		-	Not recovered	" "
7	++		+	+	+
8	--		-	Not recovered	No experiment
9		+++	No experiment	No experiment	-

* Plus and minus signs in the first two columns represent for each sign the reaction of an individual animal.

sues yielded successful transfers to monkeys, whereas by the latter procedure, the swabbing of two patients' secretions induced granular conjunctivitis in animals.

The Experimental Disease.—The clinical appearance of the infected monkeys' conjunctivae was identical with that in the experimental disease induced by cultures of *Bacterium granulosis*. As described by Noguchi (3), the reaction consisted of a slowly progressing granular conjunctivitis, occurring first in the inoculated conjunctiva, and later spreading to the uninoculated membrane of both eyes. The outstanding features of the affection were congestion, edema, and thickening of the conjunctiva which was studded with numerous follicles,

covering the tarsal plates as well as the retrotarsal membrane. In the lower conjunctiva, similar but less marked changes occurred. In general, the disease in monkeys closely resembled the early stages of trachoma in man. In another article (4) we have alluded to the similarity of the clinical appearance of the experimental disease to Type I and IIa (MacCallan's (5) classification) of human trachoma, and we have shown that by the superimposition of secondary infections with ordinary bacteria, the condition in the monkeys can be changed so as to resemble the florid stage of human trachoma. Indeed, the monkeys employed for the latter experiments were selected from among those described in this paper.

The microscopic changes in the conjunctiva removed from the experimental animals resembled those found by Noguchi (3) and ourselves in tissues inoculated with cultures of *Bacterium granulosis*. Moreover, the histopathological lesions were similar to those of human trachoma. They consisted chiefly of numerous large, typical lymphoid follicles, scattered monocytic infiltration, and thinning out or complete denudation of the epithelial layer. In addition, there was sparse scar tissue formation, especially in the subepithelial tissue and in a narrow zone around some follicles.

Recovery of Bacterium granulosis from Human and Monkey Lesions.—The tissue obtained from one patient (Case 9) was insufficient in amount for cultivation tests, hence cultures were made with material from only eight of the patients. The material obtained from four (Cases 1, 4, 5, and 7) yielded growths of *Bacterium granulosis*. In addition, the microorganism was recovered from two other patients whose tissues were not employed for transmission experiments. All of the cultures obtained were shown to be pathogenic for monkeys: in each instance characteristic granular conjunctivitis was induced. In view of the fact that the patients had advanced trachoma with a considerable degree of secondary infection by ordinary bacteria and that the disease had been of long standing, the numerical results of cultivation are what one might expect.

Cultures of *Bacterium granulosis* were also obtained from the conjunctivae of the following monkeys which were infected with the tissues or secretions of the patients: Monkeys A and C, Case 1; Monkey F, inoculated with tissue from Case 3, and Monkey N, with that from

Case 7. Table I summarizes the results of the transmission experiments.

We have shown, therefore, that affected tissue from human trachoma, as it exists in white patients in New York City, induces in *Macacus rhesus* monkeys characteristic granular conjunctivitis. We have also found that *Bacterium granulosis* can be isolated from the same trachomatous tissues used for successful inoculation of monkeys and from animals thus infected.

These positive results with New York cases of trachoma, when taken with those with white patients in Denver and Chicago, show clearly that *Bacterium granulosis* is not peculiar to the trachoma of American Indians in Arizona. In this connection, the isolation of the same organism from natives of Russia, Italy, and Tunisia should also be stressed (6).

Transmission Experiments with Monkeys' Conjunctival Tissue and with Cultures of Bacterium granulosis

In planning the following experiments on transmission of the experimental granular conjunctivitis induced by inoculation either of human trachomatous tissues or of cultures of *Bacterium granulosis*, an effort was made to imitate the way in which the incitant of trachoma might be implanted on the conjunctiva of man, namely, by contact or by rubbing the eyelids (7). The first experiment concerned the conveyance of infection by simple contact.

Conveyance of Infection by Contact.—The source of infection in this test consisted of two monkeys (A1 and B1). Monkey A1 had been originally inoculated subconjunctivally with *Bacterium granulosis* (Albuquerque strain No. 1), isolated by Noguchi (3) in 1926, on Feb. 10, 1928. Monkey B1 had also been inoculated with the same strain derived from a monkey passage (1928), on May 17, 1929. In both cases characteristic progressive chronic granular conjunctivitis resulted and on Nov. 8, 1929, when the contact test was begun, the conjunctival lesions were well marked. Two monkeys (C1 and D1), having smooth conjunctivae, were then placed in the same cage with Monkeys A1 and B1. They were examined on Nov. 26, 1929 (18 days later) and both showed definite signs of characteristic granular conjunctivitis in both eyes. In the case of Monkey C1, the congestion, edema, and follicles persisted for 198 days. Thereafter the conjunctiva healed. The condition in Monkey D1 is still present without any sign of amelioration (at the time of writing, the disease has endured for about 15 months). On Feb. 13, 1930, 79 days after the disease was established, a culture of *Bacterium granulosis* was recovered from the conjunctiva of this monkey.

The experiment was repeated. The sources of infection were two monkeys, originally inoculated successfully by the use of another strain of *Bacterium granulosis*. Two additional monkeys with smooth conjunctivae were caged together with the affected monkeys, and after 14 days' contact, the exposed animals revealed, in both eyes, characteristic granular conjunctivitis which is still persisting, now 10 months later. One of the monkeys infected by contact served for cultivation tests and *Bacterium granulosis* was recovered from its conjunctiva when the lesions were well advanced.

That contact can play a rôle in the extension of lesions has already been shown by Noguchi (3), who pointed out that lesions ultimately appear on the uninoculated conjunctiva of the infected monkey. The foregoing experiments show that infection can be secured merely by caging together uninoculated and inoculated animals. Similar results of infection by contact were obtained by Finnoff and Thygeson (1).

Transfer of Infection by Instillation of Cultures or Tissues.—In this test an attempt was made to stimulate in monkeys other conditions which might initiate infection in man. Cultures were instilled and the eyelids rubbed.

Suspensions of three Arizona strains (2) of *Bacterium granulosis* were pooled and instilled into the conjunctival sac of each of two monkeys having smooth conjunctivae. The suspensions were dropped into the sac on each of 7 consecutive days, and after about 5 days' rest, two additional, similar series of daily instillations for 8 days were given. In all, 23 doses were administered. The monkeys remained unaffected.

In contrast with this control experiment, demonstrating the innocuousness of merely instilling suspensions of cultures, is the following test. Two monkeys, both with smooth conjunctivae, received in their conjunctival sacs about 5 drops of a suspension of the same cultures used in the foregoing experiment. After each of eight daily instillations, the eyelids were gently massaged for about a minute. 5 days after the last instillation and massage of the eyelids, both monkeys revealed characteristic granular conjunctivitis which persisted until their death from tuberculosis 6 weeks later. They had been kept in the same cage.⁴

In contrast, again, with the control experiment is the following: Into the conjunctival sac of two macaques having smooth conjunctivae was instilled a suspen-

⁴ We have not been able to find any effect of tuberculosis on experimental trachoma or *vice versa*. As many monkeys die of tuberculosis among isolated normal stock animals as amongst trachomatous monkeys. The longest duration of experimental trachoma without supervening tuberculosis is, in our experience, 4 years.

sion of affected monkey tissues. The suspension was prepared by scraping the conjunctival tissue from six monkeys showing granular conjunctivitis which had been induced by inoculation of *granulosis* cultures, and suspending the tissue particles, or scrapings, in saline solution. Seven daily instillations were given; and 24 days later, both animals revealed definite, progressive, granular conjunctivitis which, at the present time, 8 months after inoculation, still persists.

The repeated simple instillation of cultures of *Bacterium granulosis* into the conjunctival sac of normal monkeys did not appear to infect the animals. On the other hand, the daily instillation of cultures followed by gentle rubbing of the eyelids after each instillation induced characteristic granular conjunctivitis. In addition, suspensions of conjunctival tissue freshly removed from monkeys having *granulosis* conjunctivitis, instilled repeatedly into the conjunctival sac of normal animals, gave rise to the characteristic experimental disease. The findings made it seem likely that some injury, even so slight a one as that produced by rubbing the eyelids, is a requirement for infection by the microorganism.

Conveyance of Infection by Swabbing Monkey Secretions and Cultures.—The transfer of the experimental disease of monkeys to normal animals was now attempted by swabbing secretions on the conjunctivae, thus imitating the mode of conveyance of infection from man to monkey, already described. And following this, another test was made to determine if swabbing with cultures of *Bacterium granulosis* would induce the infection.

Test with Secretions.—The source of the secretions was a *Macacus rhesus* monkey inoculated subconjunctivally in Feb., 1928, with tissue from another monkey infected with Albuquerque No. 1 strain of *Bacterium granulosis*. The conjunctival lesions were advanced and had endured for 22 months when the secretions were taken for the test. The material was collected on cotton swabs and transferred directly, by rubbing, to the smooth upper and lower conjunctivae of both eyes of two *rhesus* monkeys (A2 and B2). After six such swabbings, over a period of 8 days, the animals had developed characteristic granular conjunctivitis in both eyes. In Monkey A2 the lesions were progressive and endured for 2½ months, after which the animal died from tuberculosis. In Monkey B2 the condition still persists, after 1 year. A culture of *Bacterium granulosis* was recovered from the animal 10½ months after the disease first appeared.

Test with Cultures.—The cultures employed were the three Arizona strains (2) previously used in the instillation experiment described above. Saline suspensions of pooled growths were swabbed in the manner already described, on the clear

conjunctivae of two monkeys. Monkey A3 was swabbed 30 times in three series of eight and one of six daily dosages, with a rest interval of 8 days between series. Monkey B3 received only the first series of eight daily swabbings. Both monkeys developed characteristic granular conjunctivitis 4 days after the last dosage. In both monkeys, the lesions are still present, now after 1 year. From Monkey A3 a culture of *Bacterium granulosis* was recovered in the 7th month of the disease. No cultivation test was made on Monkey B3.

Saline suspensions of uninoculated leptospira medium, such as were employed for growth of the cultures, proved innocuous when swabbed upon monkey conjunctivae in precisely the same way.

It is plain that experimental trachomatous conjunctivitis can be produced in *Macacus rhesus* monkeys by repeatedly swabbing the conjunctivae with secretions from animals having *granulosis* conjunctivitis in an advanced stage, or with cultures of *Bacterium granulosis*.

SUMMARY AND CONCLUSIONS

1. Conjunctival tissue derived from alien and native American white persons in New York City, having trachoma in an advanced stage, has been used successfully to induce in *Macacus rhesus* monkeys characteristic granular conjunctivitis. The transfer of infection was effected either by a single subconjunctival injection, or by repeated swabbing with conjunctival secretions.

2. Pathogenic strains of *Bacterium granulosis* have been recovered from the trachomatous tissues of six out of eleven patients. In addition, the organisms have been isolated from the monkeys infected with human material.

3. Repeated swabbing with secretions obtained from monkeys having experimental trachoma has given rise to characteristic granular conjunctivitis in normal animals. In addition, repeated instillations of suspensions of conjunctival tissue fragments derived from affected monkeys have led to characteristic infection of the conjunctivae of normal monkeys.

4. Contact infection occurs in monkeys, as it has long been known to occur in human beings; animals with smooth conjunctivae developing the experimental disease when merely caged with infected monkeys.

5. Repeated instillation of cultures followed by rubbing the eyelids will lead to the disease in monkeys, a method of transfer which indicates one manner in which the affection may be transmitted from

man to man. Yet another manner of producing the experimental condition is by repeated swabbing with cultures of *Bacterium granulosis*. Noguchi has already reported the successful outcome of the subconjunctival inoculation of cultures and the spread of the disease from an infected conjunctiva to the other eye of the same animal.

6. Tissues derived from cases of human trachoma or from monkeys having the experimental disease induce, on conjunctival inoculation of *Macacus rhesus* monkeys, the same clinical and pathological effects as do cultures of *Bacterium granulosis*. The conjunctival lesions closely resemble, in clinical appearance and in microscopic changes, those of the follicular stages of trachoma in man.

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