

BACTERIUM GRANULOSIS IN RELATION TO TRACHOMA:
ITS RECOVERY FROM EXPERIMENTALLY INFECTED
MONKEYS AND FROM HUMAN TRACHOMA

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PLATE 26

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Since Noguchi's report in 1928 (1), reports have come from laboratories in various parts of the world (Stepanowa and Azarowa in Kharkov (2), Finnoff and Thygeson in Denver (3), Addario in Palermo (4), Kendall in Chicago (5), Tilden and Tyler (6) and Olitsky and Tyler (7) in New York, and Lumbroso in Tunis (8)) of the isolation of *Bacterium granulosis* from trachoma and the reproduction in monkeys of granulomatous lesions of the conjunctiva such as were originally secured by Noguchi. It has now become desirable to assemble as many strains as possible of both European and American origin, in order that a comparative study can be carried out. Since the cultures survive for many weeks, even months, on the semisolid leptospira medium at ordinary temperatures, when sealed in ampoules, such a study is entirely feasible.

Certain animals described in Noguchi's monograph are still alive, $2\frac{1}{2}$ years after inoculation, and continue to show the granulomatous lesions. The lesions in the chimpanzee "Louisa," which reached their maximum development within a period of 8 months, have since remained stationary. Of the *Macacus rhesus*, No. 80 shows, at the end of $2\frac{1}{2}$ years, complete healing, with scar tissue, in the left eye (Fig. 2), while the right lid still carries a few follicles. Two *Macacus rhesus* (Nos. 5 and 6) inoculated from *Macacus* 73 in February, 1928, continue to have pronounced lesions. In June, 1929, No. 5 showed a spontaneous exacerbation, with marked redness and lacrimation, the cornea becoming diffusely cloudy (Fig. 3).

The present report deals with the following experiments: (1) the further transmission (a) by animal passage, and (b) by inoculation of cultures, of the Albuquerque Strain 1, isolated by Noguchi from Indian trachoma in 1926; (2) the recovery of the organism from monkey lesions, in their early stages, and also after they had persisted for a year or longer; (3) the isolation of new strains from cases of Indian trachoma, and (4) comparative cultural studies of the old and new strains and viability tests of the bacterium. The isolation of new strains was made possible through the cooperation of Dr. F. I. Proctor and the U. S. Indian Service. For clinical assistance we are indebted to Drs. Polk Richards and J. F. Lane, of the Indian Service.

1. Experiments with Albuquerque Strain 1 (Noguchi)

(a) Transmission by Animal Passage.—

The transfer of the infection from monkey to monkey was accomplished by means of tissue suspensions, the entire affected tarsal conjunctiva being removed, under ether anesthesia, ground in a mortar with a minimum quantity of isotonic sodium chloride solution, and injected subconjunctivally and also applied to the scarified conjunctiva, as described by Noguchi.

The first passage from two monkeys injected with cultures by Noguchi in June, 1927, (Nos. 72 and 73) gave positive results in most of the animals inoculated (Table 1). The only exception was an experiment (9) in which the suspension from the right conjunctiva of No. 72 was distributed among 15 animals. This diluted material failed in all but one instance to produce infection. With later passages from the same series the incubation period became longer and the positive results less frequent, and further transmissions were abandoned in favor of experiments with cultures.

*(b) Transmission by Means of Cultures.—*Although the original cultures had been carried on artificial medium for a period of $2\frac{1}{2}$ to 3 years, having been transplanted every 6 to 8 weeks, inoculation tests gave positive results (Fig. 4) in about one-third of the animals (Table 2), showing that *Bacterium granulosis* may retain its pathogenicity for long periods of artificial cultivation.

TABLE 1
Transmission of Albuquerque Strain 1 by Passage

Monkey No.	Inoculated		Incubation period	Result		Remarks
	Date	Material		Left*	Right	
	1927		days			
1	Dec. 9	Conj. susp. No. 72 N**	13	++++	++++	Lesions persisted 1½ years. Died
2	"	"	13	++++	+	Lesions persisted 1 year. Right eye negative until 11 months after inoculation
	1928					
3 ¹	Feb. 10	Conj. susp. No. 73 N**		-	-	
4 ¹	"	"		-	-	
5 ¹	"	"	66	++++	++++	Lesions still present (2 years). Cultures recovered
6 ¹	"	"	45	++	++	Receded in 1 month
7 ²	"	"		-	-	
8 ²	"	"	45	++++	++++	Lesions on right still present. Cultures recovered
9 ²	"	"	33	++++	++++	Lesions persisted 20 months. Cultures negative. Died
10 ²	"	"		-	-	
11	"	"	66	+++	++++	Lesions still present after 19 months. Cultures recovered. Discarded
12	"	"	33	++++	++++	Lesions persisted 16 months. Discarded
13	"	"	139	+++	+++	Lesions persisted 8 months. Discarded

* Inoculations are always made into the left conjunctiva under novocain anesthesia (2 per cent).

** Numbers followed by N refer to monkeys inoculated by Noguchi, and protocols will be found in his report.

¹ Received 5 subcutaneous injections of heat-killed cultures of Strain 1 Noguchi on Jan. 9, 13, 17, 24, and 31.

² Received 5 intravenous injections of cultures of Strain 1 Noguchi on Jan. 9, 13, 17, 24, and 31.

TABLE 1—*Concluded*

Monkey No.	Inoculated		Incubation period	Result		Remarks
	Date	Material		Left*	Right	
	<i>1928</i>		<i>days</i>			
14	Feb. 10	Conj. susp. No. 73 N**	66	+++	+++	Lesions persisted 14 months. Discarded
15	"	"		-	-	
16	Mar. 9	Conj. susp. No. 72 N		-	-	Reinoculated May 16, 1928
17	"	"		-	-	Died of tuberculosis May 16, 1928
18	"	"	24	++	+	Lesions persisted 15 months, but did not progress further
19	Apr. 13	Conj. susp. No. 2		-	-	
20	"	"		-	-	
21	"	"		-	-	
22	"	"		-	-	
16	May 16	Conj. susp. Nos. 86 N, 79 N, and No. 1	83	-	++++	Died of tuberculosis in 3 months
23	"	"		-	-	
24	"	"		-	-	
25	"	"		-	-	
26	"	"		-	-	
"June" chimpanzee	June 20	Conj. susp. "Venus" and 81 N	66	+++	++++	Lesions persisted 1 year; disappearing
31	"	"	48	-	++	Died in 4 months
32	"	"	132	+++	-	Lesions persisted 13 months and remained unilateral. Discarded
33	"	"		-	-	
34	"	"		-	-	

TABLE 2

Inoculations with Routine Subcultures of Bacterium granulosis (Strain 1 Noguchi)

Monkey No.	Inoculated		Incubation period	Result		Remarks	
	Date	Material		Left	Right		
	1928						
27	June 12	Cultures on horse blood and horse serum agar plus carbohydrates and on semi-solid medium, 4 and 13 days old	16 days	++++	++++	Lesions persisted 6 months. Cultures negative at 176 days, transmission positive	
28	"			-	-		Discarded after 140 days
29	"			-	-		
30	"			-	-		
"Paulina" chimpanzee	Aug. 8	Similar cultures, 4 days old	16 days	+	+	Few follicles at border of tarsus. Did not progress further	
35	"	"		-	-		
36	"	"		-	-		
37	"	"		-	-		
38	"	"		-	-		
"Julia" chimpanzee	Nov. 21	Similar cultures, 4 and 15 days old	42 days	++	++	Few granules at border of tarsus, and on tarsal plate. Did not progress further	
41	"	"		-	-	Discarded after 6 months	
42	"	"		-	-	Discarded after 7 months	
43	"	"	5 mos.	++	++	Lesions receded after 2 months	
44	"	"		-	-	Discarded after 117 days	
	1929						
61	Jan. 24	Similar cultures, 6 days old	11 days	++++	++++	Lesions progressed for 8 months. Cultures positive at 4 months. Few follicles remaining after 11 months. Discarded	
62	"	"	11 days	++	++	Transient lesions, disappeared in 4 months	
63	"	"	11 days	+++	+++	Died in 3 months	

2. *Recovery of Bacterium granulosis (Albuquerque Strain 1) from the Monkey Lesions*

The material used for isolation experiments was similar to that employed for direct passage. The basic medium was 2 per cent nutrient agar, adjusted to pH

TABLE 3
Recovery of Bacterium granulosis from Monkey Lesions

Monkey No.	Inoculated		Incubation days	Result		Date cultured	Result
	Date	Material		Left	Right		
81 N	1927 Oct. 13	Conj. susp. "Louisa"	89	++++	++++	Dec. 5, 1928 (390 days)	+
5	1928 Feb. 10	Conj. susp. 73 N	66	++++	++++	Dec. 5, 1928 (299 days)	+
27	June 12	Strain 1 cultures	16	++++	++	Dec. 5, 1928 (176 days)	-
8	Feb. 10	Conj. susp. 73 N	45	++++	++++	Jan. 31, 1929 (356 days)	+
12	"	"	33	+++	+++	Jan. 31, 1929 (356 days)	-
46	Dec. 5	Conj. susp. No. 5	47	+++	+++	Jan. 31, 1929 (57 days)	+
9	Feb. 10	Conj. susp. No. 73 N	33	++++	++++	Mar. 20, 1929 (405 days)	-
11	"	"	45	++++	+++	Mar. 20, 1929 (405 days)	+
18	Mar. 9	Conj. susp. No. 72 N	24	++	+	Mar. 20, 1929 (377 days)	-
61	1929 Jan. 24	Strain 1 cultures	11	++++	++++	Apr. 5, 1929 (71 days)	+
Chimpan- zee "Louisa"	1927 May 20	Conj. susp. chimpanzee "Kitty" (3d passage)	28	++++	++++	May 8, 1930 (1083 days)	+

7.4, to each 100 cc. of which was added 12 cc. of defibrinated horse blood and 5 cc. of a Berkefeld V filtrate of a mixture of 10 per cent dextrose, 10 per cent saccharose, 2.5 per cent mannose, 2.5 per cent levulose, and 2.5 per cent inulin. The conjunctival suspensions were smeared over the freshly prepared plates, which were then strapped with adhesive tape and incubated at 28°C. for 3 to 6 days.

TABLE 4
Inoculation of Cultures Recovered from Monkeys

Monkey No.	Inoculated		Incubation period	Result		Remarks
	Date	Material		Left	Right	
	<i>1928</i>					
55	Dec. 12	No. 81 N cultures, I gen., 52 hrs. old, semisolid medium		-	-	Discarded after 177 days
56	"		147 days	++++	++++	Lesions persisted 9 months
57	"			-	-	
58	"			-	-	
51	"	No. 5 cultures, I gen. 52 hrs. old, semisolid medium	100 days	++++	++++	Marked congestion and secretion noted 65th day of disease (Fig. 5). Cultures (secretion) negative
52	"	"		-	-	
53	"	"		-	-	
54	"	"		-	-	
	<i>1929</i>					
60	Jan. 16	No. 5 cultures, 12 days old, blood agar and semisolid	142 days	++++	++++	Lesions persisted 4 months. Still marked when animal was discarded
59	"	"		-	-	
64	Jan. 24	No. 5 cultures, 6 days old, blood agar and semisolid	11 days	+++	+++	Lesions progressed for 6 months
65	"	"	11 days	+++	+++	"
66	"	"	11 days	+++	+++	"
67	"	No. 81 N cultures 6 days, blood agar and semisolid	22 days	+++	+++	Lesions persisted 6 months
68	"	"	11 days	+	+	Died of pneumonia, 4 months
69	"	"	11 days	+++	+++	Receding after 7 months. Discarded
45	Apr. 3	No. 8 cultures I, II, III gen.		-	-	
47	"	"	35 days	+	±	Negative after 5 months
48	"	"		-	-	

TABLE 4—*Concluded*

Monkey No.	Inoculated		Incubation period	Result		Remarks
	Date	Material		Left	Right	
	<i>1929</i>					
52	Apr. 3	No. 46 cultures I, II, III gen.	35 days	++++	++++	Lesions persisted 7 months
53	"	"		—	—	
54	"	"	65 days	++++	++++	Lesions persisted 7 months
70	"	No. 61 cultures III gen., blood agar and semi-solid	1-2 mos.	++	—	Transient lesions
71	"	"	21 days	++++	++++	Lesions persisted 6 months. Discarded
72	"	"		—	—	
73	"	"	21 days	++++	++++	Lesions persisting (10 months)
74	May 17	No. 11 cultures blood agar and semisolid IV gen.		—	—	Discarded after 4 months
75	"	"		—	—	"
76	"	"		—	—	"
77	"	"		—	—	"

Cultures were recovered from 6 of 10 *Macacus rhesus* cultured, and from the chimpanzee "Louisa." With one exception, these animals had all been infected by passage from other monkeys; one *rhesus* had received cultures. The periods elapsing between inoculation and culture varied from 57 days to nearly 3 years (1083 days), and the length of this period seems to have had no relation to the success of recovery. The recovery of the organism from the chimpanzee "Louisa" 3 years after inoculation shows that the organism may persist in the experimental infection, as in the human disease, for a long period of time.

The cultures recovered in some instances proved highly virulent (Fig. 5).

3. Recovery of *Bacterium granulosis* from Human Trachoma Lesions

(a) *Fort Defiance Cases.*—In May, 1929, an expedition was made to Fort Defiance, Arizona, where is situated one of the two special schools

which the United States Government maintains for the segregation of Indian children having trachoma. Five children who had been receiving daily treatment with copper sulfate or silver nitrate and two with advanced trachoma who had never received any treatment were selected for study.

Case 1. K., female, probably between 7 and 9 years old. Has been in the school 1 year; had advanced trachoma when she came. Scar tissue present. Treated with silver nitrate daily from Sept. 1928 to Jan. 1929, since then with copper sulfate (until May 16). Tissue taken May 23, 1929.

Case 2. A. L., female, about 7 years old. Has been in the school 2 years. Had advanced trachoma when she came. Scar tissue present. Treatment as in Case 1. Tissue taken May 23, 1929.

Case 3. N. T. Y., male, about 8 years old. Has not been in the school and has had no treatment. Mostly scar tissue; few follicles along edge of tarsus. Portion of tissue showing follicles taken.

Case 4. S., male, 7 years old. Conjunctivae covered with follicles, not much scar tissue. Has not been in the school and has had no treatment. Duration of disease unknown.

Case 5. A. L., female, 12 years old. From St. Michael's school. Disease probably of 3 to 4 years duration. Upper lids show mostly scar tissue, but lower lids show follicles; tissue taken from lower lids. Treatment irregular.

Case 6. K. A., female, 10 years old. Trachoma of 5 years duration. Mostly scar tissue; few follicles. Treatment as in Case 1.

Case 7. L. W., female, 9 years old. Mostly scar tissue, few small follicles. Duration of disease unknown. Treatment as in Case 1.

The eyes were washed with sterile saline, and the follicles were removed by Dr. Richards after novocain anesthesia (2 per cent). The medium used was the same as that employed for cultivation from the monkey conjunctiva, except that some plates were made with human blood. The plates, which had been brought from New York in a humidor and protected to some extent from drying by being strapped with adhesive tape, were again closed with adhesive after inoculation. They were transported to New York in the humidor and were 9 days old when examined.

Cultures of *Bacterium granulosis* were obtained from Cases 3 and 4, *i.e.*, from the two cases which had received no treatment. The human blood medium appeared to be as satisfactory as that made with horse blood. The cultures were typical of *Bacterium granulosis* morphologically and culturally and induced the characteristic conjunctival lesions in monkeys (Table 6). Subsequently their action on carbohydrates was determined and found to conform with that of the Albuquerque Strain 1 of Noguchi.

(b) *Santa Fé and Albuquerque Cases.*—A second expedition to the Indian Schools was made in September, 1929. We are indebted to the Indian Service for providing facilities for the preparation of culture medium at the U. S. Indian Hospital in Santa Fé. No advanced untreated cases of trachoma were available for cultural study either at Santa Fé or at Albuquerque, and experiments with treated cases

TABLE 5

Monkey No.	Date	Culture inoc.		Incubation period	Result		Remarks
		Age	Temp.		Left	Right	
{ P-1	June 15	57	4°-6°C.	73	+++	+++	Lesions persisting (8 months)
		"	"	"	+++	+++	
		"	"	"	+++	+++	
{ P-4	Sept. 28	162	4°-6°C.	149 days	-	-	
		"	"		-	-	
		"	"		-	-	
{ P-7	Feb. 10	297	4°-6°C.	284 days	-	-	
{ P-8	"	"	"	8	++	++++	
{ P-9	"	75	Room	69 days	-	-	
{ P-10	"	"	"		8	+	

proved negative. It is of interest in this connection that a recent report of the Indian Service (10) shows a marked decline in the incidence of trachoma among the New Mexico (Pueblo) Indians, owing apparently to the systematic treatment which is being carried out in the Pueblo villages.

(c) *Leupp Cases.*—Through the kindness of Doctors Proctor and Richards, in cooperation with Commissioner Rhoads, of the Indian

TABLE 6
Inoculation of Bacterium granulosis from Fort Defiance Cases

Monkey No.	Inoculated		Incubation period	Result		Remarks
	Date	Material		Left	Right	
	1929		days			
F. D. 1	June 10	Cultures I gen. 7 days semi-solid II gen. blood agar 4 days, Case 3	77	+++	++++	Lesions progressed for 3 months. Discarded 5 months
F. D. 2	"	"		-	-	Reinoculated with pooled strains Cases 3 and 4 after 5 months. ++ lesions in 35 days
F. D. 3	"	"	64	+++	++++	Began to recede after 2 months. Discarded 7 months after inoc.
F. D. 4	"	"		-	-	Reinoculated with pooled strains Cases 3 and 4 after 5 months. Negative
Chimpanzee "May"	"	"		-	-	
F. D. 5	"	Similar cultures. Case 4	132	+++	+++	Lesions progressed for 2 months. Discarded 1 month later
F. D. 6	"	"	64	+++	++++	Lesions progressed for 3 months
F. D. 7	"	"	117	+	+	Transient lesions
F. D. 8	"	"		-	-	Discarded after months
F. D. 9	July 27	Case 4 cultures III, IV, V gen. 7, 14, 19, 25 days semisolid med. and blood agar		-	-	
F. D. 10	"	"		-	-	
F. D. 11	"	"		-	-	"
F. D. 12	Nov. 11	Pooled cultures. Cases 3 and 4 10 days semi-solid	35	++	+++	
F. D. 13	"	"	35	+	+	Died 78 days after inoc.

TABLE 6—*Concluded*

Monkey No.	Inoculated		Incubation period	Result		Remarks
	Date	Material		Left	Right	
	1929		days			
Passage from F. D. 3						
F. D. 14	Aug. 16	Conj. susp. No. F. D. 3		—	—	
F. D. 15	"	"		—	—	
F. D. 16	"	"		—	—	
F. D. 17	"	"		—	—	

Service, a search for untreated cases of trachoma was made in the Navajo territory in Arizona, where systematic treatment has been less successfully applied because of the nomadic habits of the Navajos. It was found that at the Indian School at Leupp the treatment had not been carried out for at least 6 months, no physician having been on duty regularly. Thirteen cases were selected by Dr. Richards for study. Of these, 6 were cases of granular conjunctivitis of recent onset in which the diagnosis was not altogether certain, but in which there had been no treatment whatever, 5 were unquestionably trachoma, and 2 showed evidence of the beginning scar tissue formation, indicating that they were in all probability trachoma. The clinical data are presented in Table 7.

The follicular material used for inoculation of the culture media was removed by Dr. Richards, the eyes being first washed with sterile saline and the lids anesthetized with 0.5 per cent novocain. The usual medium was employed, *i.e.*, blood agar plates containing horse or human blood and a mixture of carbohydrates. The latter ingredient was the mixture originally used by Noguchi (1). It had been sterilized fractionally for 10 minutes on 3 successive days at 103°C. in a pressure cooker. There was some sedimentation on cooling, and only the clear supernatant fluid was added to the medium. The plates, which were prepared in Santa Fé the day previous to their use in Leupp, were strapped with adhesive tape after inoculation and brought back to New York in a humidior. They were not opened until arrival in New York, 9 days after inoculation. The weather conditions on the day of inoculation were unfortunate, in that high winds prevailed, and much sand and dust blew in through the windows and skylight of the operating room, where the cultures were made, hence the plates were in most cases nearly

overgrown with molds by the 9th day. A few, however, were less seriously contaminated, and colonies of *Bacterium granulosis* were found to be present. The follicle suspensions were brought back to New York and fresh plates inoculated.

TABLE 7

Case No.	Patient	Age	Diagnosis	Remarks	Culture
1	E. L.	7	Trachoma. Beginning scar tissue	Fairly recent case. Treated last year	—
2	M. N.	9	Doubtful (<i>i.e.</i> , no scar tissue)	May be beginning trachoma. Treated last year	—
3	P. T.	7	"	May be beginning trachoma. Treated last year	—
4	F. C.	14	Unquestionably trachoma. Scar tissue present	Old case. No treatment since last May	+
5	J. D.	10	"	Old case. Treated last year	+
6	H. W.	6	Recent case; some evidence of beginning scar tissue formation	Treated last year	+
7	M. C.	9	Recent case; some evidence of beginning scar tissue. Follicles in left eye only	Treated last year	—
8	B. H.	9	Unquestionably trachoma. Scar tissue present	No treatment since last May	—
9	H.	6?	Doubtful (<i>i.e.</i> , no scar tissue)	No treatment	+
10	I. M.	6?	"	" "	—
11	K. W.	7	"	" "	—
12	M. (Keems Canyon)	9	Unquestionably trachoma. Scar tissue present	" "	—
13	E. M.	10-11	Doubtful (<i>i.e.</i> , no scar tissue). Right eye only affected	" "	—

From the 4 cases which were regarded as unquestionably trachoma, *Bacterium granulosis* was obtained in 3. The organism was also obtained from Case 6, which showed evidence of early scar tissue forma-

tion, and from Case 9, which showed no scar tissue. The results of inoculation of the pooled cultures from Cases 5, 6, and 9 are recorded in Table 8. The lesions in Monkey L-6 are shown in Fig. 1.

4. Cultural and Biological Characteristics of the Various Strains

(a) *Viability*.—*Bacterium granulosis* remains viable for long periods on the semisolid ("leptospira") medium, and cultures 6 months old, kept at room temperature, with no other protection than the

TABLE 8
Inoculation of Bacterium granulosis from Leuþþ Cases

Mon- key No.	Inoculated		Incu- bation period <i>days</i>	Result		Remarks
	Date	Material		Left	Right	
L-1	1929 Nov. 22	Pooled cultures Cases 5, 6, and 9, I-II gen. semisolid med., blood agar plus carbohy- drates, and hor- mone agar plus carbohydrates 48- 72 hrs. old		—	—	
L-2	"	"	13	++	++++	++++ at 74 days
L-3	"	"	13	++	++	++ " " "
L-4	"	"		—	—	Died 13 days after inoc.
L-5	"	"	13	++++	++++	++++ at 74 days
L-6	"	"	13	++++	++++	++++ " " "

cotton plug, are readily transplantable. Even on a plain agar slant, on which the organism grows less readily, it has been found to remain transplantable for at least 49 days. It is probable that the bacterium lives as long as the medium contains a certain amount of moisture. Cultures kept in sealed ampoules for periods of 6 weeks to 10 months at 4° to 6°C., and for 69 days at room temperature, have proven viable and infective (Table 5).

(b) *Cultural Characteristics*.—The cultural characteristics of the strains of *Bacterium granulosis* thus far isolated have been practically

constant. In Tables 9 and 10 are shown the results of fermentation tests of the original strain and of the six strains isolated by us from Indian trachoma, as well as seven strains isolated by Finnoff and Thygeson (3) and one by Kendall (5), which these workers kindly sent us.

The tests recorded in Table 9 were made on Hiss serum water medium containing 1 per cent of the respective carbohydrates and litmus (about 0.01 per cent of a 25 per cent solution) as indicator, to which were added 10 per cent horse serum and 0.2 per cent horse hemoglobin; those shown in Table 10 were made on Durham's peptone water containing 1 per cent carbohydrate and 1 per cent Andrade indicator (0.5 per cent acid fuchsin to which normal sodium hydroxide is added until the dye is decolorized). With the latter media a definite acid reaction is evident in 3 to 5 days, the lactose being the only one of the carbohydrates affected which shows no reaction until after this period. The use of Durham tubes shows that no gas is formed in any of the carbohydrate media.

Bacterium granulosis reduces nitrates to nitrites but forms no indole. It does not liquefy gelatin.

It has been found that after a few subcultures, all the strains of *Bacterium granulosis* grow well on nutrient agar (veal infusion agar containing 1 per cent of Witte's peptone and 0.5 per cent sodium chloride). The medium must be freshly prepared. Growth is more abundant if the agar is enriched by the addition of a carbohydrate mixture and horse blood or rabbit hemoglobin. A number of carbohydrates has been added individually to the horse blood agar medium in a concentration of 2 per cent; of these, media containing dextrose gave the best growth.

(c) *Reaction to Gram's Stain.*—Since *Bacterium granulosis* failed to retain an appreciable amount of the Gram stain (Sterling's gentian violet) when decolorized for 2 minutes and counterstained with dilute fuchsin, Noguchi regarded it as Gram-negative. It may be noted that Morax (11) regards it as Gram-positive, and since he apparently does not make use of a contrast stain in his technique, his point of view is comprehensible. However, after a contrast stain, even one as weak as Bismarck brown, no Gram stain can be detected in young, actively growing cultures. In old cultures, or cultures grown under unfavorable conditions (*e.g.*, in dextrose broth), numerous bizarre forms are seen which may retain the Gram stain. A form with an equatorial

swelling, suggesting a central spore, is not uncommon in such cultures and usually stains Gram-positive, especially in the central portion.

(d) *Effect of Heat.*—*Bacterium granulosis* is rather sensitive to heating, being killed by exposure for 10 minutes to a temperature of 57°C. The thermal death point was tested by submerging sealed ampoules

TABLE 9
Fermentation Tests. Hiss Serum Water Medium

	F. D. 3	F. D. 4	L. 5	L. 6	L. 9	Kendall
Dextrose.....	+	+	+	+	+	+
Levulose.....	+	+	+	+	+	+
Mannose.....	+	+	+	+	+	+
Saccharose.....	+	+	+	+	+	+
Raffinose.....	+	+	-	+	+	-
Inulin.....	+	+	-	-	-	+
Galactose.....	+	+	+	+	+	+
Maltose.....	+	+	+	+	+	+
Salicin.....	+	+	+	+	+	+
Xylose.....	+	+	+	+	+	+
Mannitol.....	+	+	+	+	+	+
Dextrin.....	+	+	+	+	+	+
Arabinose.....	+	+	+	+	+	+
Amygdalin.....	+	+	+	+	+	+
Lactose.....	+	+	+	+	+	±
Dulcitol.....	-	-	-	-	-	-
Rhamnose.....	-	-	+	+	+	+
Trehalose.....	-	-	+	-	-	+
Sorbitol.....	-	-	-	-	-	-
Inositol.....	-	-	-	-	-	-
None.....	-	-	-	-	-	-

+, color of indicator changed to red. No coagulation.

-, no change in color of indicator.

Readings recorded were made at 9 days. No change occurred after this time, and the tubes were discarded at 14 days.

F. D. = Fort Defiance. L. = Leupp.

containing 0.5 cc. of a rich culture (5 days old on the semisolid medium) in water at temperatures varying from 52°C. to 65°C., and 5 and 10 minute exposures were made at each temperature. The whole amount of the heated culture was transferred, immediately after the heating, to fresh semisolid medium.

TABLE 10
Fermentation Tests. *Dunham's Peptone Water Medium*

	Aq. 1	D. 1	D. 2	D. 4A	D. 5	D. 6	D. 7	F. D. 4	L. 4
Dextrose.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Levulose.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Mannose.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Saccharose.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Raffinose.....	-	-	-	-	-	-	-	±	-
Inulin.....	+	±	+	±	+	±	+	-	+
Galactose.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Maltose.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Salicin.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Xylose.....	++	+++	+++	+++	+++	+++	+++	+++	+++
Mannitol.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Dextrin.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Arabinose.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Amygdalin.....	+++	+	+	+	+	+	+	+	+
Lactose.....	+	+	+	+	+	±	±	+	+
Dulcitol.....	-	-	-	-	-	-	-	-	-
Rhamnose.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Trehalose.....	+++	+++	+++	+++	+++	+++	+++	+++	+++
Sorbitol.....	-	-	-	-	-	-	-	-	-
Inositol.....	-	-	-	-	-	-	-	-	-

Aq. = Albuquerque.
D. = Strains isolated by Thygeson in Denver.
F. D. = Fort Defiance.
L. = Leupp.

(e) *Effect of Cocaine.*—The organism is also sensitive to the action of cocaine hydrochloride, being killed in 5 minutes by 4 and 5 per cent solutions, and in 15 minutes by weaker solutions (2 and 3 per cent). Similar concentrations of procaine hydrochloride (novocain) have no inhibitory effect on its growth. In obtaining material for cultivation, therefore, it is desirable to avoid long exposure to high concentrations of cocaine.

SUMMARY AND CONCLUSIONS

One of the strains of *Bacterium granulosis* isolated by Noguchi in 1926 has been maintained in culture and in monkeys and continues to be capable, after 3 years, of inducing a chronic granular conjunctivitis in monkeys. Cultures of this strain have been recovered from the monkey lesions as late as 3 years after inoculation and have been shown to reproduce the granular disease in monkeys.

Six additional strains of *Bacterium granulosis* have been isolated from cases of trachoma occurring in the Indian schools of Arizona. The cultures thus obtained are identical morphologically and culturally with those isolated by Noguchi and have induced the same chronic granular conjunctivitis in monkeys. Advanced untreated cases are more favorable for cultural study than treated cases.

Cultures of *Bacterium granulosis* kept on semisolid medium containing 10 per cent rabbit serum ("leptospira medium") remain viable for many months at room temperature, and sealed ampoules of such cultures have been found to retain their pathogenicity for the monkey conjunctiva for at least 69 days at room temperature and at least 284 days at 4° to 6°C.

Several additional cultural and biological characteristics of *Bacterium granulosis* have been described. Of outstanding importance is the fact that cocaine, in contradistinction to novocain, has a bactericidal effect on the organism. This fact, in view of the common use of cocaine for anesthesia, may explain the negative results of cultivation experiments reported by some workers.

REFERENCES

1. Noguchi, H., *J. Exper. Med.*, 1928, **48**, suppl. 2.
2. Stepanowa, H., and Azarowa, N., *J. Microbiol.*, 1929, **8**, 180; Abstr. *Arch. Ophthalm.*, 1929, **2**, 357.

3. Finnoff, W. C., and Thygeson, P., *Amer. J. Ophthalm.*, 1929, 12, 651.
4. Addario, C., Personal communication from Dr. W. C. Finnoff.
5. Kendall, A. I., Personal communication to The Rockefeller Institute.
6. Tilden, E. B., and Tyler, J. R., *Science*, 1930, 71, 186.
7. Oliitsky, P. K., and Tyler, J. R., *Science*, 1930, 71, 263.
8. Lumbroso, U., *Compt. rend. Acad. Sci.*, 1930, 190, 1026.
9. Tilden, E. B., and Miller, E. G., Jr., *J. Nutrition*, 1930, 3, 121.
10. Warner, H. J., *Publ. Health Repts.*, 1929, 44, 2913.
11. Morax, V., and Petit, J., *Le trachome; conjonctivite granuleuse*, J. Morax, Paris, 1929.

EXPLANATION OF PLATE 26

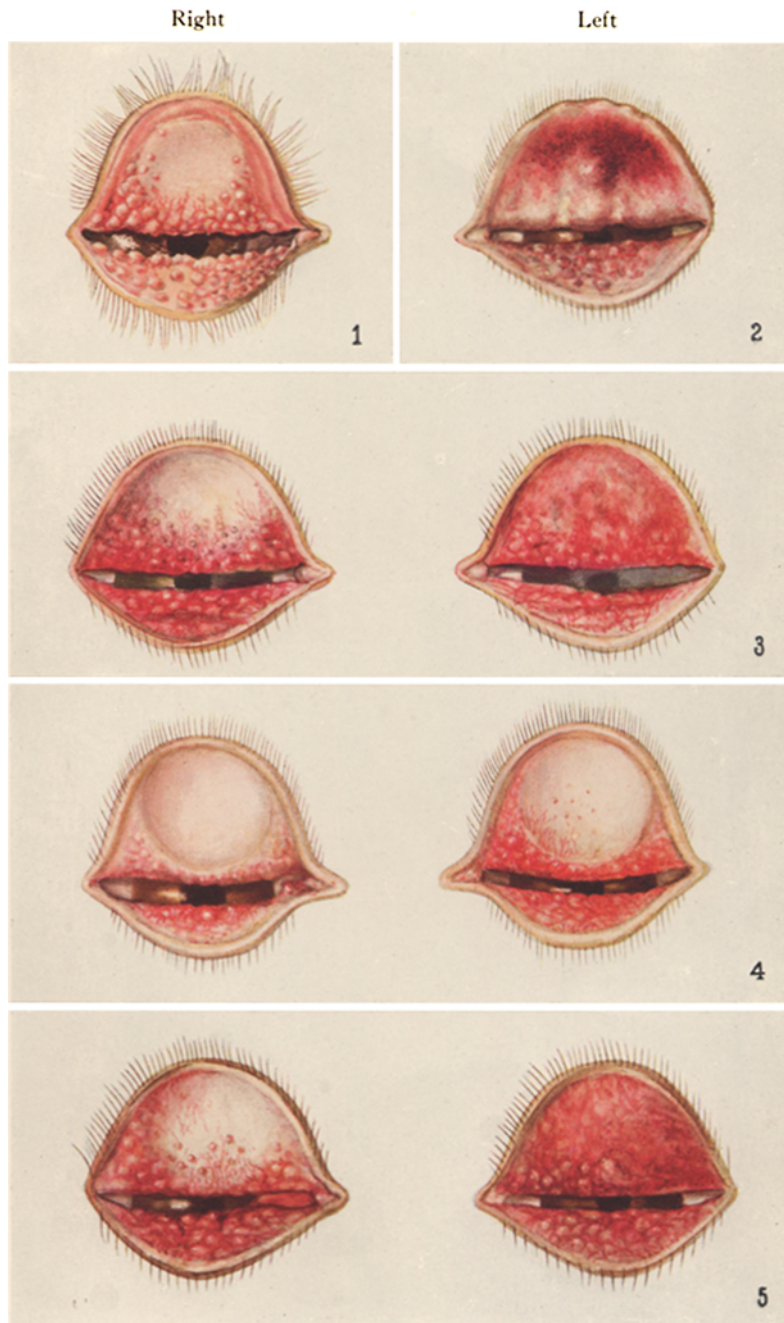
FIG. 1. *M. rhesus* L-6, 19 days after inoculation with pooled cultures from Leupp cases.

FIG. 2. *M. rhesus* 80, 2½ years after inoculation (1), showing presence of scar tissue.

FIG. 3. *M. rhesus* 5, 1½ years after inoculation, when the lid showed renewed activity of the lesions, and the left cornea became diffusely cloudy.

FIG. 4. *M. rhesus* 61, 57 days after inoculation with routine subcultures of Albuquerque Strain 1.

FIG. 5. *M. rhesus* 51, 65 days after inoculation with cultures recovered from *M. rhesus* 5.



(Tilden and Tyler: *Bacterium granulosis* and trachoma)