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Studies on Bacterium Granulosis in Relation to Trachoma: Pathogenicity for Various Monkeys and Apes.*

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Noguchi's discovery[1] of a bacterium which can be artificially cultivated and which produces granular lesions in the conjunctivae of monkeys has supplied a new approach in the field of trachoma investigation. Noguchi, Tilden and Tyler,[2] working with rhesus monkeys succeeded in inducing active, progressive lesions which lasted 8 or more months in the conjunctivae of 19% of a total of 159 rhesus monkeys, and only transient lesions (granulations which receded before the 8th month) in an additional 34.5%. Their results with 10 chimpanzees were as follows: Progressive lesions lasting 8 months or longer in 30%, and transient lesions in 50%. Two out of the 10 chimpanzees failed to react.

These statistics compare favorably with the findings of previous investigators working with fresh trachoma "virus"‡ obtained directly from trachomatous lesions. However, the long periods of incubation (up to 150 days), reported in the above studies, are in striking contrast to the much shorter periods (2 to 20 days) observed by other writers in experiments on both human subjects and various species of monkeys.[3, 4, 5, 6, 7]

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1 Noguchi, H., *J. Exp. Med.*, 1928, **48**, Supplement 2.

2 Tilden, E., and Tyler, J., *J. Exp. Med.*, 1930, **52**, 617.

‡ The term "virus" is here used in the French sense, signifying the infectious agent as contained in material removed from human lesions, without reference to filterability.

3 Taborisky, J., *Arch. f. Ophth.*, 1929, **123**, 140.

4 Halberstaedter, L., and Prowazek, S. von, *Deutsche med. Wchnschr.*, 1907, **33**, 1285.

5 Greeff, R., Frosch, H., and Clausen, W., *Arch. f. Augenh.*, 1907, **58**, 52; 1908, **59**, 203.

6 Nicolle, C., Cuénod, A., and Blaizot, L., *Arch. de l'Inst. Pasteur de Tunis, Fascic.*, 1913, **3**, 157. Nicolle, C., and Cuénod, A., *Arch. Inst. Pasteur de l'Afrique du Nord*, 1921, **1**, 149.

7 Hess, C., and Römer, P., *Arch. f. Augenh.*, 1906, **55**, 1.

We§ have repeated some phases of Noguchi's work with 3 strains of *B. granulosis,* furnished by the Rockefeller Institute, and later with 2 others isolated by ourselves at the Pasteur Institute of Tunis and at Washington University. We employed 28 rhesus monkeys, 2 *M. inuus,* 1 baboon, and 1 callitriche.

Noguchi's technic as amended in the notes kindly sent by Dr. Flexner was followed. Olitzky's technic[8] of massaging the lids was also used in the latter part of the work.

We cite briefly 2 protocols as examples.

Inoculation with *B. granulosis* (Strain Tunis 34). *M. inuus* (Algerian magot) injected July 4, 1929, into the right eye with a freshly isolated culture. 16th day: both eyes showed a few, fine, granulations in the cul-de-sac and on the superior tarsi. Inflammation at the internal angle and marked edema of the lids. No secretion. 33rd day: both eyes showed persisting granulations on the superior and inferior palpebral conjunctivae. These lesions began to regress on the 50th day and had disappeared on the 90th day. Another magot similarly injected produced essentially the same results.

Inoculation of *B. granulosis* (Rockefeller Culture "A"). *M. rhesus,* injected April 30, 1929, with material from the original ampoule. 10th day: edema and hyperemia at the site of the injection. The 71st day showed a ridge of 3-4 follicles on the superior margin of the tarsus, with hyperemia. 78th day: the uninjected eye had 2 small follicles on the tarsal plate. Both eyes were clear on the 167th day.

An examination of Table I shows that various strains of *B. granulosis* induced in a certain percentage of monkeys a type of granular conjunctivitis similar to that described by Noguchi. The lesions in our animals were, however, all of the mild and transient type; *i. e.,* never very extensive and never persisting more than 4 months. Varying degrees of edema and inflammation accompanied these follicles or granulations, but no thickening nor loss of transparency of the conjunctivae, nor evidence of secretion were observed.

In 2 young chimpanzees inoculated with the Rockefeller cultures only transient follicles were induced, whereas in a third, chimpanzee infected with fresh "virus" obtained from human trachomatous eyes, advanced lesions developed which contained Prowazek bodies

§ We wish to acknowledge our appreciation of the kindness of Parke-Davis & Company and of Eli Lilly & Company, who supplied the horse blood and serum used in the preparation of our culture media.

[8] Olitsky, P., *Rev. intern. du Trachome,* 1930, 7, 173.

TABLE I.

Results of Injection of Various Monkeys and Chimpanzees with Cultures of B. Granulosis.

Strain of *B. granulosis* Injected	Species of Animals Used	No. Injections	Animals with Negative Results	Animals with Transient Lesions Persisting to 4 mo. (Receded)
Rockefeller "A"	*M. rhesus**	3	2	1
	Baboon	1	0	1
	Callitriche	1	1	0
Rockefeller "B"	*M. rhesus*†	11	8	3
	Baboon	1	0	1
	Chimpanzee	2	0	2
Rockefeller M. C.	*M. rhesus*	5	4	1
	Chimpanzee	1	1	0
Tunis 34	*M. rhesus**	5	5	0
	Baboon	1	1	0
	M. inuus	2	0	2
	Chimpanzee	1	0	1
St. Louis	*M. rhesus*	5	2	3
13 L	Chimpanzee	1	0	1
Denver 7‡	*M. rhesus*	3	3	0
	M. rhesus	32	24	8 or 25 %§
	Baboon	3	1	2 or 66.6 "
	M. inuus	2	0	2 or 100.0 "
	Chimpanzee	5	1	4 or 80.0 "
	Callitriche	1	1	0 or 0.0 "

Total Number of Animals Used.

28 *M. rhesus* (3 reinjected); 1 Baboon (injected 3 times); 2 *M. inuus;* 2 Chimpanzees (1 injected twice, the other 3 times); 1 Callitriche.

* Infection attempted twice.

† Infection attempted 3 times.

‡ Furnished by Drs. Finnoff and Thygeson.[9]

§ Calculated on the basis of total number of attempts at infection.

and both clinically and histologically showed a striking resemblance to the lesions of an active human trachoma. Thus 18 days after infection, the mucous membranes of the inferior conjunctiva were thrown into folds, there were papillary hyperplasia and hypertrophy, secretion and ptosis of the upper lid. The infection spread spontaneously to the uninoculated eye and follicles developed in the upper and lower conjunctivae of both eyes including the tarsi and retrotarsal folds. Neither in the material removed from the conjunctival lesions of the patient nor from those of the chimpanzee were we able to isolate *B. granulosis.*

Histological sections of the conjunctivae of the inoculated eye of this champanzee taken on the 216th day after injection (when the animal died of generalized tuberculosis) showed epithelial invagina-

[9] Finoff, W., and Thygeson, P., Presessional Volume, Section on Ophthalmology, American Medical Association, Detroit Meeting, June 23-27, 1930.

tions, lymphocytic infiltration, young fibroblasts and new connective tissue containing many small capillaries. Near the upper end of the tarsus, the layer of infiltrated inflammatory cells was thicker and contained plasma cells and histiocytes. The lids of the uninoculated eye showed a similar picture. The centers of the follicles contained groups of epithelioid cells.**

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Oxidation of Lactate by Methemoglobin in Erythrocytes with Regeneration of Hemoglobin.

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In earlier communications[1] we reported the oxidation of lactic to pyruvic acid and hemoglobin to methemoglobin by normal dog erythrocytes in the presence of 0.005% methylene blue. Pyruvic acid is not further oxidized. Because the oxygen partition seemed to indicate coupled reactions, it was tentatively suggested that the mechanism was one of peroxidation. Further studies, in which the stochiometric relationships observed with sugar-free cells were not obtained in presence of glucose, led us to question the explanation first offered.

Warburg, Kubowitz and Christian[2] explain methylene blue catalysis in red blood cells by the following chain of reactions: (a) oxidation of hemoglobin to methemoglobin by methylene blue; (b) oxidation of carbohydrate (or derivative) by methemoglobin with regeneration of hemoglobin; (c) oxidation of leuco-methylene blue by O_2. (A detailed paper by Warburg *et al*[3] which reached us while writing this report appears to substantiate their earlier interpretation.)

If methemoglobin is the agent responsible for methylene blue catalysis in red blood cells, it should be possible to oxidize lactic acid with methemoglobinized cells in the absence of O_2. And if no

** We are indebted to Dr. Harvey D. Lamb for the histological report on this specimen.

[1] Wendel, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 865; 1930, **27**, 624; *J. Biol. Chem.*, 1930, **87**, p. xx.

[2] Warburg, Kubowitz and Christian, *Biochem. Z.*, 1930, **221**, 494.

[3] Warburg, Kubowitz and Christian, *Biochem. Z.*, 1930, **227**, 245.