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Trachoma Viruses Isolated in United States.* 1. Growth in Embryonated Eggs. (25758)

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It is believed that more than 400 million people are infected with trachoma throughout the world. Diagnosis of this disease rests largely on clinical impression (conjunctivitis, with follicles particularly on upper tarsus, papillary hypertrophy, vascular invasion of cornea, pannus, scarring) and typical cytology of conjunctival scrapings with intracytoplasmic inclusion bodies. These inclusions, described over 50 years ago(1) consist of elementary and initial bodies in a matrix of glycogen. Because of the morphologic appearance of elementary bodies and their developmental stages trachoma virus was accepted as a member of the psittacosis-LGV group(2) although the virus had not been unequivocally grown in the laboratory. Tang *et al.*(3) succeeded for the first time in propagating serially trachoma viruses from patients in China, by inoculating streptomycin-treated conjunctival scrapings into the yolk sacs of embryonated eggs. Subsequently similar viruses were isolated from patients in West Africa(4), Saudi Arabia(5), and Israel(7). Trachoma is no longer a prevalent infection in the United States, except on certain Indian reservations in the Southwest. Sporadic, active cases, are occasionally seen elsewhere. We wish to report the first isolation of "trachoma viruses" from patients in the United States. To date 6 virus strains have been isolated from trachoma patients presenting typical pictures of a) acute initial adult infection, b) relapse in adult infection of 20 years' standing, and c) early infection in Apache Indian children. A preliminary report on the first of these virus strains has appeared(8).

Materials and methods. Patients: Clinical diagnoses of trachoma were made by ophthalmological examination of patients referred to us or encountered in systematic surveys of Indian reservations. *Specimens.* From active cases of trachoma conjunctival scrapings were collected in 1 ml broth-saline containing streptomycin sulfate 1-10 mg/ml and at times also polymyxin B sulfate 0.1 mg/ml. Most specimens were collected from single patients, but some field specimens were pooled scrapings from 2-6 active cases. Field specimens from Arizona were kept at −20°C to −70°C during shipment for varying periods before processing. Specimens collected in San Francisco were kept for 1 hour at 4°C before inoculation. *Embryonated eggs*: Specimens were mixed thoroughly, then 0.5 ml was inoculated into the yolk sac of 6-8-day-old embryonated eggs obtained from a commercial source. Eggs were incubated at 35°C and candled twice daily. Deaths within 48 hours of inoculation were discarded. Blind passage of surviving eggs by the yolk sac route was carried out at first between 7 and 11 days after inoculation, later regularly at 7-day in-

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TABLE I. Sources and Characteristics of "Trachoma Viruses" Isolated in U. S.

Strain	Age, yr	Patient's description: Disease	Inclusions present	Serum CF titer to psittacosis antigen	Specimen collected	No. egg passages needed for isolation: Elementary bodies	Deaths	Max egg infectivity: Passage level	LD_{50}/ml
Bour	36	Acute florid trachoma	+++	1:64	San Francisco	2	3	10	6.4*
Asgh	29	Relapse of cicatricial trachoma, 20 yr duration	++	1:4	"	1	1	7	7.4
Apache									
#1	7	Early active trachoma	—	1:2	San Carlos, Ariz.	4	5	7	6.7
#2	7	*Idem*	—	ND	*Idem*	2	2	ND	ND
#3	4	"	—	"	"	3	3	"	"
#4	10	"	—	"	Bylas, Ariz.	6	6	"	"

* Negative log of dilution resulting in 50% death of eggs. ND = Not done.

terval. Harvested yolk sacs were weighed, shaken with glass beads for 1 minute and made into a 20% suspension with streptomycin-containing broth-saline. The egg LD_{50} of established strains was estimated by inoculation of groups of 6-12 eggs with 10-fold dilutions of yolk sac suspension, recording deaths which occurred between 3rd and 12th day of incubation, and checking presence of elementary bodies in stained smears of yolk sacs from dead eggs. The egg LD_{50} was calculated by the method of Reed and Muench(9). *Microscopy*: Smears of conjunctival scrapings from patients were stained by Giemsa's method and examined for cytological picture(2), virus particles, and inclusion bodies. Impression smears of yolk sacs were stained with Giemsa's and Macchiavello's methods to detect initial or elementary bodies at a magnification of 1800 ×.

Results. To date 41 specimens from clinical trachoma have been subjected to 3-6 egg passages and 6 strains of "trachoma viruses" have been isolated. Isolates and their sources are briefly characterized in Table I. From specimens collected in San Francisco virus was isolated in the first (fresh material) or second egg passage (frozen material) whereas from 2-6 passages were required for appearance of virus in specimens collected in Arizona and kept at −20 to −70°C for varying periods. The San Carlos Apache Indian Reservation was visited twice. In Oct. 1959 4 specimens were obtained from children with early active trachoma one of which yielded virus in 4th passage (Apache #1). In Jan. 1960 with improvements in collection and shipping procedures conjunctival scrapings from 7 children with similar disease yielded 3 isolates (in passage 2, 3, and 6). Interestingly none of these 11 children had demonstrable inclusions in their conjunctival scrapings, although in other respects the cytologic picture was typical of active trachoma.

Elementary bodies were often seen in yolk sac smears one passage prior to occurrence of deaths among inoculated eggs. Morphologically and tinctorially elementary bodies resembled those of other members of the psittacosis-LGV group. They stained bright red-purple with Macchiavello's method and purple-blue with Giemsa, and measured about 250 mμ in diameter. They occurred diffusely in impression smears of infected yolk sacs and were occasionally grouped in inclusion body-like arrangements. However true intracellular inclusions have not been observed in our egg passages. The density of elementary bodies in smears from later egg passages was very high so that each microscopic field contained hundreds or thousands.

Early egg passages of fresh isolates resulted in irregular deaths. In later passages all eggs receiving a suitable inoculum died between 5th and 9th days after inoculation. For preparation of high titer pools 6-8-day-old chick embryos were inoculated with yolk sac suspensions diluted so as to cause death of half the eggs in 6-7 days. At that point the yolk sacs of surviving embryos were harvested

and pooled for storage at –40°C. Titers of such pools (Table I) reached 6.3 to 7.4 egg LD_{50}/ml of undiluted yolk sac suspension. Pools of strain ASGH regularly had a titer 1-1.5 logs higher than pools of strain BOUR; the reason for this difference is unknown. Infective titers of established strains, and ability to isolate ANY "trachoma virus" depended to a remarkable degree on fluctuation in egg quality, as observed by others(4,6). Strain BOUR grew well from Dec. 1958 until early Aug. 1959. For the next 2 months we could not propagate any virus. In Oct. 1959 the ability of eggs to support virus growth miraculously resumed. Suspensions of uninoculated yolk sacs from "bad" eggs apparently inhibited the infectivity of "good" virus for monkey eyes and "good" eggs. Strains established in the yolk sac grew readily also in the allantoic cavity. Using harvested allantoic fluid as inoculum for 3 consecutive allantoic passages did not impair infectivity *via* yolk sac route. However, quantitative data on this are still incomplete.

Isolates prepared as antigens by exposure to flowing steam for one hour, fixed complement with antisera to psittacosis virus to full titer. Concentrated suspensions of elementary bodies were toxic to mice upon intravenous injection. Instillation of egg-grown isolates into *M. cynomolgus* monkeys resulted in follicular conjunctivitis with typical intracytoplasmic inclusions. Isolates of similar egg infectivity differed in their pathogenicity for monkeys. No other laboratory animals could be infected. Strain BOUR produced typical acute trachoma with inclusion bodies in a human volunteer. Attempts to propagate our isolates in cultures of established cell lines have thus far given inconclusive results. Details of these studies will be reported.

Discussion. In certain areas of the world where trachoma is widespread the disease is often confused with non-trachomatous follicular conjunctivitis and even bacterial conjunctivitis, especially under survey conditions. Thus the diagnosis of "trachoma" in those areas designates a clinical rather than an etiological entity(5). If the finding of typical intracytoplasmic inclusions (indicative of viruses of the psittacosis-LGV group) were made mandatory to establish the diagnosis a large majority of cases could not be classified as "trachoma." Ability to cultivate "trachoma viruses" from inclusion-negative cases now permits the etiologic diagnosis of trachoma in such patients. In addition cultivation of "trachoma viruses" from different locations or different types of disease may permit comparison of pathogenetic and antigenic characteristics of these strains(6). Our initial results indicate that virus isolation from trachoma patients in the United States is still far from easy and seems to depend on many ill-defined factors. Recovery of virus was not dependent upon presence of inclusion bodies in patient's conjunctival scrapings. None of the Apache children yielding virus had any inclusion bodies, whereas an adult with the largest number of inclusions that we have ever observed failed to yield virus. Handling of the specimen may be of great importance in view of the relative instability of the agent. Perhaps the largest factor in successful isolation of "trachoma viruses" is the fluctuating susceptibility of embryonated eggs. At times strong inhibitors are present which make virus isolation or propagation virtually impossible. These inhibitors do not appear to be antibiotics transmitted from feed. Their nature and action are under study.

Summary. From 6 active trachoma cases in the United States strains of "trachoma virus" have been isolated. Conjunctival scrapings suspended in broth-saline containing 1-10 μg/ml streptomycin were inoculated into yolk sac of embryonated eggs. From 1 to 6 blind passages were required for establishment of strains which subsequently reached titers of $10^{6.3}$-$10^{7.4}$ egg LD_{50}/ml. The essential nature of ill-defined egg "quality" for isolation of "trachoma viruses" is discussed. The isolated agents produced unequivocal eye infections in *M. cynomolgus* and in a human volunteer.

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Levels of Eosinophils, Platelets, Leukocytes and 17-Hydroxycorticosteroids During Normal Menstrual Cycle.* (25759)

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Ovulation is associated with thermal, chemical, cytologic and histologic changes in the human and affects levels of certain formed elements of blood(1-3). Eosinopenia has been observed during ovulation and menstruation, and it has been suggested that physiological stress at these periods may increase levels of adrenocortical hormones causing eosinopenia(3,4). The present study was concerned with relationship of levels of plasma 17, 21-dehydroxy-20-keto-steroids (17-hydroxycorticosteroids (17-OHCS) with levels of eosinophils, platelets and leukocytes during menstrual cycle.

Materials and methods. Twelve healthy women with normal menstrual cycles and one oophorectomized control subject were investigated. Two women were allergic. Oral temperature records were kept. Eosinophil, platelet, leukocyte and differential counts and plasma 17-OHCS determinations were performed between 12 noon and 1:00 p.m., to avoid diurnal variations. Approximately 11 to 13 counts and steroid determinations were obtained on each woman during mid-cycle and menstruation, as shown for one patient in Fig. 1. Direct platelet counts were done by phase microscopy(5) and eosinophils were counted by the Wintrobe method(6). Estimations of 17-OHCS in plasma were made by micromethod of Riondel *et al.*(7). Since it was desirable to obtain minimal amounts of blood from each woman daily, duplicate steroid determinations were not feasible. However, duplicate steroid determinations were done on 10 other normal women. The standard deviation of differences in values of the 2 determinations was 3.1. On the basis of these results, there is 95% confidence that an individual observation is no further from the true value than 7 μg%.

Results. A total of 183 eosinophil, platelet, total leukocyte and differential counts and 164 determinations of 17-OHCS levels were performed on 13 women with the results shown in Table I.

Eosinophil levels during menstrual cycle. Eosinopenia occurred during menstruation and on one day in mid-cycle in the majority of women. Menstrual eosinopenia occurred in 9 cycles (75%). However, the lowest eosinophil values of the entire cycle were usually in mid-cycle (Fig. 1). When basal

TABLE I. Results of Counts and 17-OHCS Determinations.

	Mean	Stand. error
Eosinophils	162.3	7.9
Platelets	254,955	3,907.–
W. B. C.	8,516	171.–
Polymorphonuclear leukocytes	5,483	267.–
Lymphocytes	2,573	56.–
17-OHCS	12	.71

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