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THE BIOLOGY OF THE TRACHOMA AGENT

Conference Chairman
FRANCIS B. GORDON

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Conference Editor and Conference Chairman

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CONTENTS

* This series of papers is the result of a conference entitled *The Biology of the Trachoma Agent* held by The New York Academy of Sciences on May 26 and 27, 1961, and supported in part by grants, including one from the National Society for the Prevention of Blindness, New York, N.Y.

B. Morphology and Developmental Cycle of the Trachoma Agent

Part III. Primate Experimental Hosts

Part IV. Physiology and Drug Susceptibility

Part V. Immunological Studies

A. Antigens of the Trachoma-Psittacosis Group

B. Vaccines

INTRODUCTORY REMARKS

Francis B. Gordon
Division of Virology, Naval Medical Research Institute, Bethesda, Maryland

The experimental study of trachoma was severely handicapped for many years by lack of suitable tools, namely a satisfactory method of culturing the agent of the disease. In 1955, we began to overcome this difficulty and are now in an entirely new phase of trachoma investigation. The progress made in the last four to five years has been remarkable, and we can expect the advance to continue. It seemed desirable, therefore, to bring together a number of investigators who could discuss the accumulating information in this field. We have been fortunate in being able to assemble here representatives of most of the laboratories of the world in which a major effort is being made in study of the trachoma agent. Included among the formal participants are nine visitors from overseas to whom we wish to extend an especially warm welcome.

With the new tool, culture in the embryonated egg, many exploratory studies have been pursued, many of them aimed at elucidating the nature of the etiological agent of trachoma. This interest has been reflected in the choice of a title for the Conference and in the definition of its scope. We are concerned here primarily with the trachoma agent (and the agent of inclusion blennorrhea) as a microorganism; we are concerned with its morphology, biochemical nature, host range, manner of growth, pathogenicity, antigenic character, and so on. Consideration of these topics will lead to questions of practical import and, we hope, to some of the answers. Because of the close relationship between (1) the trachoma agent and the inclusion blennorrhea agent, and (2) the psittacosis and lymphogranuloma venereum organisms, we have included papers dealing all or in part with the latter agents.

Our objective in this Conference is to summarize and analyze the present status of this field of inquiry to provide a source of information and a basic guide for further investigation and interpretation—in short, to set up a milestone in the laboratory investigation of trachoma. One word of warning may be indicated. There is considerable difference of opinion about the taxonomic terms that should be used in talking of these agents. I admonish you not to use valuable time at this Conference to discuss which terms are correct or preferable. For this occasion, at least, let us accept whatever names our colleagues choose to call these creatures, as long as we know what they are talking about. For those of you who have not found a ready handle for this group of disease agents, I can suggest one that has found favor in our own laboratory. This is "PLT," for psittacosis-lymphogranuloma-trachoma. One can thus speak of the PLT group, the PLT viruses, the PLT agents, and effect a truly tremendous saving in time, energy, and printer's ink.

I take pleasure in acknowledging on behalf of The New York Academy of Sciences the generous financial support received from several sources with particular regard for assistance with travel costs for some of our foreign visitors. For this purpose, the Office of Naval Research generously made available to us over-the-ocean transportation by United States Military Air Transport

Service. Also sincerely appreciated is the financial aid received for travel and other expenses from the following: Arabian-American Oil Company; The Texaco Company; Lederle Laboratories; Eli Lilly and Company; Merck and Company; Parke Davis and Company; Charles Pfizer and Company, Inc.; The National Society for the Prevention of Blindness; Mr. Gustav Stern, and an anonymous donor.

We hope that this very valuable support will be justified by the measure of success of the Conference.

Part I. Isolation and Culture Methods

INTRODUCTORY REMARKS

L. H. Collier

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I do not think it would be proper to open this Conference, and in particular Session I, without paying tribute to the memory of the late Dr. T'ang of Pekin. Had it not been for his tenacity in repeatedly attempting to isolate the agent of trachoma by a method that others had since abandoned, it is probable that we should not be meeting here today.

I never met him personally, but during our correspondence I came to appreciate his courtesy and unfailing generosity in supplying material and information. His death is a great loss to the field of investigation that concerns this Conference, and that he did so much to pioneer.

A. The Embryonated Egg as a Culture Medium

TRACHOMA VIRUS: HISTORICAL BACKGROUND AND REVIEW OF ISOLATES

Phillips Thygeson

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Trachoma was one of the first diseases of mankind to be recognized as a distinct clinical entity. The Ebers papyrus (1500 B.C.) mentions its exudative and cicatricial features and its treatment with copper salts. It is known to have been widespread in ancient Greece and Rome and is believed to have afflicted Paul of Tarsus, Cicero, Horace, and Pliny the Younger. The name "trachoma" was first used by a Sicilian physician, Pedanius Dioscorides, in 60 A.D., and a century later the four stages of the disease were delineated by Galen.

From the Middle East, which has been heavily infected since antiquity, trachoma was spread over Europe during the Crusades by the returning knights and their followers. A fresh wave followed Napoleon's campaign in Egypt where a high percentage of French and English troops contracted "Egyptian ophthalmia." For this reason, in Europe the disease was often referred to as "military ophthalmia." Over the last century it has gradually disappeared from northern Europe but has prevailed in southern Europe and in all the countries bordering the Mediterranean.

In 1938 the first effective treatment for trachoma was discovered. First orally administered sulfonamides (introduced that year), and later topically administered medium spectrum and broad spectrum antibiotics, proved curative. In spite of intensive treatment programs, however, the disease has persisted in southern Europe, North Africa, the Middle East, and Asia, and in certain areas of South America and Mexico. In the United States and Canada it is largely confined to American Indians. Current estimates suggest that more than 400,000,000 of the world's population still suffer from trachoma. Failure of treatment programs to influence materially its prevalence can be blamed on (1) the long treatment time required, with consequent lack of patient cooperation, and (2) the frequency of reinfection in trachoma-endemic areas.

Historical Background

Halberstaedter-Prowazek inclusion bodies. Etiological studies date from 1884 when Koch[1] on an expedition to study cholera, found in Egyptian ophthalmia the small Gram-negative rod now known as *Hemophilus aegyptius* (Koch-Weeks bacillus) and the Gram-negative diplococcus now known as *Neisseria gonorrhoeae* (gonococcus). Other observers reported finding various conjunctival bacteria and fungi in trachoma, but in 1907 Halberstaedter and Prowazek[2] made the first important etiological observations when they found in epithelial scrapings the cytoplasmic inclusion bodies that bear their names. While on a

scientific expedition in Java, they found these bodies first in experimental trachoma in apes and later in human trachoma. They called them "chlamydozoa," or "mantle bodies," because when stained with Giemsa, the small reddish elementary particles, which they believed to be the agents of trachoma, seemed to be embedded in a blue matrix or mantle. Almost simultaneously, Greeff,[3] working in East Prussia, reported small granules in epithelial cells and free in exudate from trachoma. These could very well have been elementary bodies. On finding them in experimental human trachoma, Greeff at first considered them etiological but later withdrew this claim.[4]

The work of Halberstaedter and Prowazek was almost immediately challenged by Stargardt,[5] who found similar inclusions in bacteria-free ophthalmia neonatorum, and by Heymann[6] who found them in 10 of 14 cases of gonococcal ophthalmia. In rebuttal, Halberstaedter and Prowazek[7] reported failure to find inclusions in urethral gonorrhea in men or in vaginal gonorrhea in women; conversely, they found them in large numbers in three infants with gonococcus-free ophthalmia neonatorum. That the inclusions were totally independent of gonococcal infection seemed implicit.

Then in 1909 Lindner[8] showed that inclusions were to be found in most cases of gonococcus-free ophthalmia neonatorum. This bacteria-free conjunctivitis of the newborn he later defined under the name "inclusion blennorrhea." He produced disease in a macaque and a baboon with genital secretion of a mother whose baby had "inclusion blennorrhea," and found numerous inclusions in material from the diseased conjunctiva of the baboon.

In 1910 Halberstaedter and Prowazek[9] first found inclusions in female genital epithelium, and in the same year Lindner[10] found them in three cases of nongonorrheal urethritis of man. With Fritsch and Hofstätter,[11] he produced follicular conjunctivitis in monkeys with material from this type of urethritis, from the genital disease of mothers of infected babies, and from the infant disease. He postulated that trachoma and "inclusion blennorrhea" ("genital trachoma" or "paratrachoma") were caused by the same agent but suggested later that the two diseases might have a relationship like that of variola and vaccinia.

In spite of many studies, the nature of the inclusion body long remained in doubt. Lindner[12] made an important advance in 1910 by demonstrating with wet fixation methods that the blue material which Halberstaedter and Prowazek had called "plastin" was in reality a conglomerate mass of large coccoid bodies. These he called "initial bodies" because they were the first to appear in the developing inclusion. Later Rice[13] demonstrated a carbohydrate matrix, transparent in the Giemsa stain but staining a reddish brown with iodine. This study was confirmed and extended to include the inclusion bodies of inclusion blennorrhea (or more properly, *inclusion conjunctivitis*) by Thygeson.[14] In 1956 Mitsui and Suzuki,[15] and in 1958 Mitsui *et al.*[16] contributed important morphological information as a result of electron microscope study of trachoma inclusions in sections.

Filtration studies. In 1913 Nicolle *et al.*,[17] working in Tunis, passed the agent of trachoma through modified Berkefeld V filters. To avoid adsorption losses they cemented a small button of filter substance into a glass tube. With the

filtrate they inoculated a chimpanzee and a Barbary ape (*Macaca innuus*) and produced an experimental disease for which a trachomatous nature was established by transmission to the human conjunctiva. The Halberstaedter-Prowazek elementary bodies were discounted because they did not occur in the follicles which these Tunisian workers believed were the essential lesions of the disease.

The Tunisian studies were confirmed by Julianelle and Harrison[18] from 1933 to 1935; in 11 experiments they found Berkefeld V filtrates to be infectious for 3 of 33 monkeys (*Macaca rhesus*). In 1935 Thygeson and Proctor[19] performed experiments on 5 baboons with filtrates that had passed collodion membranes of 0.75-μ average pore diameter (a.p.d.). They used a specially constructed filter holder to give a small filtration area in the membrane. All 5 baboons developed disease, and the authors suggested that previous failures with Berkefeld V and Chamberland filters might be due to high adsorption losses. In the same year Thygeson *et al.*[20] obtained a positive result in a human volunteer with pooled, filtered (collodion membrane, 0.6 μ a.p.d.) conjunctival scrapings from 10 inclusion-positive Indian children. The filtrate was divided into 3 equal parts, 1 for inoculation of the volunteer, 1 for testing sterility, and 1 for centrifugation and study of the sediments. The filtrate was bacteria-free but contained elementary bodies; in the volunteer it produced an experimental trachoma that progressed to pannus and scars.

Trachoma virus as a member of the psittacosis-lymphogranuloma venereum group of viruses. In 1932 Bedson and Bland[21] reported that the elementary bodies of psittacosis passed through a developmental cycle in the cytoplasm of host cells (large coccoid bodies that stain blue in Giemsa dye), now known as "initial bodies," dividing successively to form finally the relatively small elementary bodies that stain bluish red. The resemblance of this cycle of morphological change to the cycle of change in trachoma was soon recognized. Thygeson[22] showed the same cycle in the inclusion of inclusion conjunctivitis by studying scrapings taken at intervals during the incubation of experimental human infections. Usually, three complete cycles of development could be followed in a six day incubation period.

On the basis of the morphological similarity of their developmental cycles, the agents of trachoma and inclusion conjunctivitis were accepted by Bedson,[23] and later by Blank and Rake,[24] as members of the psittacosis-lymphogranuloma venereum group of atypical viruses. The validity of this concept was strengthened by the demonstration by Rake *et al.*[25] of a serologic relationship through possession of a common group complement fixing antigen, and finally by the cultivation of the agents on the yolk sac of the embryonated egg—a group characteristic.

Relationship of trachoma and inclusion conjunctivitis. The relationship of these two diseases, one primarily a benign type of ophthalmia neonatorum but occasionally a self-limited follicular conjunctivitis of the adult, and the other a cicatrizing keratoconjunctivitis with serious visual consequences, has excited much controversy over the past 50 years or more. In 1933 Morax[26] reviewed the subject in great detail and clearly differentiated the two conditions on both clinical and epidemiological grounds. He pointed out that adult cases occurring in epidemics in public baths invariably ran a benign, self-limited

course unlike trachoma. The problem was also reviewed by Julianelle,[27] who emphasized that adult forms of conjunctivitis resulting from accidental transmission of the agent of ophthalmia neonatorum had none of the features of trachoma. Other observers have called attention repeatedly to the striking difference in the geographical distribution of the two diseases.

Chemotherapy. Until 1938 when the sulfonamides were introduced, there was no effective treatment for trachoma. Copper sulfate and other chemical cauterizing agents used by the Egyptians, and scarification and expression of follicles (surgical procedures used commonly by Greek and Roman oculists) were still the treatments of choice. Then sulfanilamide was found by Loe[28] to cure trachoma in American Indians, and simultaneously by other observers to cure trachoma in various other parts of the world.

That sulfanilamide was not virucidal was shown by Julianelle and Smith,[29] who suspended epithelial scrapings from trachoma patients in sulfanilamide for long periods and found them still infectious for monkeys. The broad spectrum and medium spectrum antibiotics, topically applied, were soon found also to be curative. The results of extensive trials with antibiotics is detailed by Reinhards *et al.*[30]

Cortisone reactivation. In 1952 Ormsby *et al.*[31] showed that subsiding inclusion conjunctivitis in which inclusions were no longer demonstrable could be reactivated by the topical use of cortisone. This phenomenon was demonstrated for trachoma by Freyche *et al.*,[32] and confirmed by Thygeson[33] and by Mohsenine and Darougar.[34] It has since become a useful provocative test for latency in old cicatricial trachoma.

Cultivation studies. The history of cultivation attempts in trachoma is a long one. In 1938 Julianelle[27] reviewed the subject, and in 1958 Thygeson and Nataf[35] covered the intervening years. Looking back on the problem of cultivation in the light of recent successful yolk sac isolations, one wonders at the scant attention given this medium compared with the great deal of attention given the chorioallantois and tissue culture. In my own case a few unsuccessful trials on the yolk sac were sufficient to discourage me in view of my mistaken idea that an epitheliotropic virus like trachoma virus would require epithelial tissues for growth. This concept was apparently a widespread one. Even experienced investigators like John Bland,[36] who was thoroughly familiar with the psittacosis group, failed to explore the yolk sac adequately. In any event, the chorioallantois and a wide variety of tissue cultures were explored and failed consistently to support cultivation in series.

The first report of growth in the yolk sac that can be taken seriously is Macchiavello's[37] in which was claimed the finding of inclusions and free elementary and initial bodies in infected eggs with passage in series, and a positive human inoculation with material from the ninth egg passage. Unfortunately, this report was not completely documented, and the inoculation experiment was incomplete. Furthermore, Macchiavello did not submit the virus to other laboratories for confirmation, and his impressive results failed to receive the attention they may very well have deserved. The same may be said of Stewart and Badir,[38] who claimed transmission of trachoma to monkeys with yolk sac material.

The confusing results of the many cultivation attempts on record prompted

the Expert Committee on Trachoma of the World Health Organization, meeting in Geneva in 1956, to formulate the following criteria for acceptance of a claim: (1) Halberstaedter-Prowazek inclusion bodies must be demonstrated in serial cultures; (2) experimental trachoma capable of transmission in series must be produced in monkeys or apes; (3) a serologic relationship between the cultured virus and trachoma must be demonstrated; and (4) final proof must be the production of typical trachoma in human volunteers after sufficient passages in culture to eliminate the dilution factor.

First to be examined in the cold light of the above criteria was the claim of T'ang *et al.*[39] who used material from untreated Stage II trachoma cases. They treated this material with streptomycin to minimize bacterial infection and then inoculated the yolk sacs of fertile 6- to 8-day-old eggs and incubated them at 35° C. Not content with the results of first egg passage, the Chinese investigators made 5 blind passages before deciding that an isolation attempt had failed. In 68 experiments with a total of 93 samples, they succeeded in

CHART 1

CRITERIA FOR IDENTIFICATION OF A STRAIN OF ELEMENTARY BODIES AS A "TRACHOMA VIRUS"*

1. Origin: conjunctival scrapings of patient with disease diagnosed clinically as trachoma.
2. Characteristic morphology and staining.
3. Multiplication in serial passage in yolk sac membrane of chick embryo.
4. Resistance to streptomycin *in vitro* (sensitivity to tetracyclines and sulfonamides).
5. Absence of pathogenicity for guinea pigs by any route, or for suckling or weanling mice intracerebrally, on primary passage.
6. Heat-stable common group antigen extractable from washed elementary bodies by ether.
7. Toxicity for white mice intravenously (heat-labile).
8. Toxicity for skin of white rabbits (heat-labile).
9. ABILITY TO INDUCE "TRACHOMA" IN MAN.

* Snyder.[48]

isolating 3 strains of an elementary body agent now recognized as the agent of trachoma. The 3 strains all showed similar biological properties. Conjunctival inoculation of *M. rhesus* gave rise to typical follicular conjunctivitis without pannus, and one of the 7 inoculated monkeys showed typical Halberstaedter-Prowazek inclusions. The Chinese workers also showed that there was a toxic factor associated with the elementary bodies since a yolk sac-adapted strain killed the eggs when injected by other routes.

This claim, which was incomplete with respect to serological relationships and human inoculation, was soon confirmed by Collier and Sowa,[40] who used a similar technique to isolate strains in the Gambia region. They completed the picture by demonstrating (1) pathogenicity of the agent for the human conjunctiva, (2) complement fixing antibodies in patients' sera, and (3) the presence in their strains of a common complement fixing antigen for the psittacosis-lymphogranuloma venereum group of viruses. They later reported the isolation of more than 60 strains, all with essentially the same characteristics.

The success of the British group was soon followed by isolations in Saudi Arabia and Egypt where Murray *et al.*[41] cultivated 14 strains. In the same year a number of strains were isolated by Bernkopf *et al.*[42] in Israel, and by

Hanna *et al.*[43] in California. In 1960 Grayson *et al.*[44] in Taiwan reported isolating 4 strains from 32 cases of trachoma. They produced experimental disease in six human volunteers from whom virus was reisolated in 22 of 23 attempts.

Since these initial isolations, trachoma virus has been cultivated in various parts of the world, including Japan, India, Tunisia,[45] Yugoslavia, Egypt, Portugal, Italy, South Africa,[46] and Australia.[47] The only continent that has not yet yielded a strain is South America. The stringent criteria formulated by the Expert Committee on Trachoma in 1956 no longer seem necessary. Those suggested by Snyder (CHART 1)[48] for future isolations would seem better suited to present needs. In view of recent controversy concerning the relation of trachoma to inclusion conjunctivitis, however, it is of first importance to document fully the clinical features of the disease from which isolate material derives and thus to eliminate an unnecessary source of confusion.

Summary

The history of etiological research on trachoma began in 1907 with the demonstration by Halberstaedter and Prowazek of the inclusion bodies of the disease, now known to be intracellular colonies of the etiological agent. It ended in 1955 when T'ang *et al.* succeeded in growing the virus in the yolk sac of developing chick embryos. Since the Chinese report, isolations have been reported from many parts of the world, including Gambia, Saudi Arabia, Europe, the United States, Taiwan, Australia, Tunisia, and Egypt.

With the question of etiology solved, ophthalmologists and virologists are now faced with problems related to the virus itself, such as strain differences, biological properties, serologic relationship to inclusion conjunctivitis and to other members of the trachoma-psittacosis-lymphogranuloma venereum group, susceptibility to antibiotics and chemotherapeutic agents, vaccination, and host susceptibilities.

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ISOLATION OF VIRUSES OF TRACHOMA FROM PATIENTS IN ETHIOPIA*

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Introduction

The historical background of the isolation of the trachoma virus has been reviewed by Thygeson.[1] The present era of isolating virus by means of the chick embryo begins with the report in 1957 of T'ang *et al.*[2] on the isolation of trachoma virus in the yolk sacs of 6- to 8-day-old embryonated eggs inoculated with conjunctival cells obtained from patients with clinical trachoma. The findings of the Peking investigators were confirmed and extended in 1958 by Collier and Sowa.[3]

Using the technique of yolk sac inoculation of conjunctival scrapings combined with the use of streptomycin to control bacterial contamination, investigators have reported the isolation of virus from trachoma patients from many areas of the world.[4-14]

This paper describes the isolation of viruses from 10 of 34 trachoma patients studied in three villages in the environs of Asmara, Eritrea, Ethiopia.

Materials and Methods

Isolation procedures. All specimens were obtained from the inhabitants of three villages within a 25-mile radius of Asmara. Several days prior to a village visit, the headman was asked to bring together a group of villagers, primarily children, to examine for evidence of trachoma. On the day of examination each volunteer villager was examined clinically; then from the lower fornix a bacterial culture was taken and streaked on a blood agar plate; from the upper tarsus a conjunctival scraping was obtained.

The technique of collecting conjunctival specimens has been described elsewhere.[5] All materials for isolation were obtained from patient examinations performed in the villages; hence, the suspensions of conjunctival scrapings in sucrose potassium glutamate (sucrose-PG)[15] were kept in an improvised refrigeration box at +4° C. for the several hours between collection and time of arrival back in the laboratory, at which time they were placed in a standard refrigerator until tested. All specimens were tested for virus between 10 and 96 hours after collection.

Where two eggs were to be inoculated, 6000 μg. of streptomycin were added to the conjunctival material; only 3000 μg. of streptomycin were added where one egg was inoculated. The mixture was held at +4° C. for 2 to 11 hours prior to inoculation into one or two 6- to 8-day-old embryonated eggs. Each inoculated egg thus received 3000 μg. of streptomycin along with either one-half or all the conjunctival specimen.

Freshly laid fertile hen eggs used in the studies were obtained from two sources in Asmara. One group of eggs was obtained from a local flock of

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Asmara chickens, the other, from a flock which originated from chickens brought to Asmara from Italy several years previously. The eggs were incubated at 39–40° C. for 6–8 days before inoculation. Thereafter, incubation was carried out at 35° C.

Eggs were candled daily. Eggs dying within 48 hours after inoculation were discarded; eggs found dead or appearing sick from the 3rd day onward were placed at +4° C. and examined within the next 24 hours. All eggs surviving on the 11th day were sacrificed that day.

Yolk sacs were removed to a sterile Petri dish; smears were made from a section of the yolk sac near the umbilical cord and stained directly with Giemsa as well as by Machiavello's method. The yolk sacs were then transferred to a thick walled 20-ml. vial containing glass beads where they were hand shaken for 5 minutes in an equal quantity of sucrose-PG solution. For serial passages, two to four eggs were inoculated with 0.4 ml. per egg of a 10 to 25 per cent yolk sac emulsion. Streptomycin was not added to the serial passage inocula except rarely when inadvertent contamination with bacteria occurred.

Selection of cases for isolation. Smears of epithelial scrapings made directly from trachoma patients were fixed in methanol and stained with Giemsa immediately upon return to the laboratory from the field. Stained slides were then examined carefully for evidences of virus. Isolation attempts were made on conjunctival specimens from any of the 179 persons surveyed in whose epithelial scrapings elementary or initial bodies could be demonstrated. In addition, isolation attempts were made on specimens from other subjects with clinically active trachoma whose Giemsa-stained epithelial scrapings showed cellular elements characteristic of trachoma as described by Thygeson[16] even though no virus particles were observed.

Results

Clinical, epidemiological, and virological observations on the 34 trachoma patients selected for isolation studies are summarized in TABLE 1. All patients surveyed as well as those selected for virus isolation attempts have been grouped in TABLE 2 according to the stage of trachoma as described by MacCallan (17). Of the total of 179 subjects surveyed, 147 were diagnosed as having Trachoma II or III. Of the 34 children who were chosen for isolation attempts, 32 were from the Trachoma II group. The two remaining patients tested for virus had Stage I and III trachoma, respectively. Virus isolations were successful from 9 patients with Stage II trachoma and 1 patient with Stage III.

The age distribution of the subjects surveyed as well as those chosen for virus isolation attempts are recorded in TABLE 3. Virus isolations were successful in 2 out of 14 children tested in the 0- to 4-year-old age group, 6 out of 16 in the 5- to 9-year-old age group, and 2 out of 4 in the 10- to 14-year-old age group.

In TABLE 4 is a summary of the virus isolation results grouped according to the village of residence and whether inclusions were or were not found in conjunctival scrapings. Inclusion bodies were found in the eye scrapings of 17 of the 34 children selected for virus isolation attempts. From 10 of the 17 inclusion-positive children, strains of virus were isolated. From 7 inclusion-

TABLE 1

CLINICAL EPIDEMIOLOGICAL AND VIROLOGICAL DATA ON 34 TRACHOMA PATIENTS SELECTED FOR ISOLATION STUDIES, ASMARA, ETHIOPIA

Patient No.	Age	Sex	Village*	Stage of disease	Date of examination 1959	Conjunctival inclusion bodies	No. of hours specimens held at +4° C before inoculation	Egg passage in which virus first:	
								Appeared	Killed eggs
A 1	5	M	S	II	7/28	+	10	1	1
					7/29	+	25	2	2
					8/10	+	31	1	2
					8/24	+		N.T.†	
A 2	10	M	S	II	7/28	+		N.T.	
					8/10	+	31	1	2
					8/24	−		N.T.	
A 3	6	F	S	II	7/29	+	13	2	2
					8/10	+	31	3	3
					8/24	+		N.T.	
A 4	12	M	S	III	7/29	+		N.T.	
					8/10	+	13	1	2
					8/24	−		N.T.	
A 5	4	F	S	II	7/29	+	13	2	3
					8/10	−	31	1	2
					8/24	+		N.T.	
A 6	8	F	Q	II	8/5	+	13	1	2
					8/18	+		N.T.	
A 7	5	M	Q	II	8/5	+	13	1	1
					8/18	+		N.T.	
A 8	8	F	Q	II	8/5	+	96	1	2
					8/18	+		N.T.	
A 9	2	M	Q	II	8/5	+	13	1	1
					8/18	+		N.T.	
A 10	6	M	Q	II	8/5	+	24	1	2
					8/18	+		N.T.	
A 11	5	M	A	II	8/13	+	16	Neg. (5)‡	
					8/21	+		N.T.	
A 12	5	M	A	II	8/13	+	16	Neg. (3)	
					8/21	+		N.T.	
A 13	4	M	A	II	8/13	+	16	Neg. (5)	
					8/21	+		N.T.	
A 14	6	M	A	II	8/13	+	16	Neg. (5)	
					8/21	+		N.Y.	
A 15	10	F	A	II	8/13	+	16	Neg. (5)	
					8/21	+		N.T.	
A 16	5	M	A	II	8/13	−	16	Neg. (6)	
					8/21	+		N.T.	
A 17	7	F	A	II	8/13	+	16	Neg. (3)	
					8/21	−		N.T.	
A 18	2	M	S	II	7/28	−		N.T.	
					8/10	−	13	Neg. (3)	
A 19	4	M	S	II	7/28	−		N.T.	
					8/10	−	13	Neg. (3)	
A 20	4	M	S	II	7/29	−		N.T.	
					8/10	−	13	Neg. (3)	
A 21	5	M	S	II	7/29	−		N.T.	
					8/10	−	13	Neg. (3)	
A 22	4	F	S	II	7/29	−		N.T.	
					8/10	−	13	Neg. (3)	
A 23	11	F	S	II	7/29	−		N.T.	
					8/10	−	13	Neg. (3)	
A 24	2	M	S	II	7/28	−	21	Neg. (4)	
A 25	7	F	S	II	7/29	−	25	Neg. (4)	
A 26	1	M	S	I	7/29	−	25	Neg. (4)	