II REVIEW OF LITERATURE

1. HISTORY

Because of the great dependence of mankind upon vision, disorders of the eye have attracted attention from earliest times. The earliest record is found in the Ebers papyrus (1539 B.C.). The ancient Egyptians described the disease as "Elepharoxysis" or "Ophthalmoxysis". The ancient Chinese also recognised trachoma as a distinct disease entity. Hippocrates (5th century, B.C.) described it and advocated the important treatment procedures of grattage, scarification of follicles and application of copper salt. Heliodorus (2nd century, B.C.) wrote a treatise on trachoma.

Celsus (A.D. 14) was the first to give a detailed account of all the symptoms and he called the symptomatic state "lippitudo" which included among other things, "aspritudo" or trachoma. Galen (A.D. 130-200) compared the granular appearance of the trachomatous eye with a fig cut open. In ancient Rome, the disease was so common that all of the then eminent personalities such as Cicero, Horace and Clemy were affected by trachoma. Ali ibu Isha of Baghdad (A.D. 940-1010) described the development of trachoma in all its different clinical stages and also advocated instrumental scarification and remedial cauterisation as measures of treatment. The Arab school of physicians has been credited with the first description of "pannus" in trachoma.
During the Napoleonic invasion of Egypt (1798-99), most of the militia from Europe contracted trachoma and many of them had to be sent back home blind; in a battalion of 700 soldiers, 636 contracted the infections, 50 became blind and 40 others lost the vision of one eye. Trachoma at that time was known as "Egyptian Ophthalmia" or "Military Ophthalmia". Some authors believe its origin to be in Mongolia, others along the four river valleys, Hoang-Ho and the Yangtze-Kiang, Indus and Ganges, Euphrates and Tigris, and the Nile in Egypt. The Moslem conquest led to the spread of the disease in Europe as early as the 8th century A.D. There is no doubt that the Napoleonic invasion of Egypt (1798-1802) was responsible in large measure for its spread among Europeans. Though quite familiar to the writers of the ancient and mediaeval periods, the disease disappeared from the writings of the early modern period. It re-appeared in an 1802 description, published by Dominique-Jean Larrey and was further mentioned by John Vetch in 1807 and by Benjamin Travers in 1820.

Although different names were given to the disease by the ancient authors, the granular appearance of the conjunctiva has always been the classical description of trachoma. The word "Trachoma" first appeared in the writings of Pedinius Dioscorides (A.D. 60). It has been derived from the Greek word "trachys" which
Even though trachoma has been ravaging the human race since antiquity and was recognized as a problem of military importance during the early crusades in Europe, it was only in 1923 that Morax, Nicolle and Cuenod founded the "Ligue Centre Le Trachoma", an organisation intended to fight trachoma, and started the publication "Revue Internationale due Trachoma", a journal solely devoted to the problem of trachoma. Subsequently, in recognition of the world wide manifestation and the extreme prevalence of the disease in under-developed countries where the economic consequence is all the more devastating, the extended "International Organisation Against Trachoma" was constituted in 1929. During the past decade, the W.H.O. has undertaken an active part in the trachoma control programme, instituted an "Expert Committee on Trachoma" and sponsored mass treatment campaigns in different regions of the world where the disease is endemic. Y.K.C. Pandit elaborated on the history of trachoma from Ancient India. The history of research on the aetiology of trachoma has been well covered in monograph form by Axenfeld, Morax and Petit, Julianelle, Ishinara, Virgili, Mitsui, Cavara and Bietti, Nataf and Thygeson, and Nataf.

2. CLINICOPATHOLOGICAL FEATURES

Onset: According to Bietti trachoma may begin in
any one of three different ways: 1) an insidious onset, 2) an acute abacterial onset, 3) an apparently acute onset which is actually due to a concomitant bacterial conjunctivitis (usually Koch-Weeks bacillus infection) or viral conjunctivitis (for example, measles conjunctivitis). A 'pseudoacute' trachoma which is nothing but pre-existent chronic trachoma with an acute exacerbation, has also been observed during an epidemic of bacterial conjunctivitis. Investigations conducted in Jordan, Erythrea and Saudi Arabia indicated that in the majority of cases the disease had an insidious onset. Mitsui, on the other hand, reported high frequency of acute onset type of the disease in Japan. However, by "acute onset" he meant "sudden onset" even without important acute clinical symptoms. Tsutsui also holds that insidious onset does not refer to the true medical onset of trachoma; according to him such onset may be subjectively true in people or infants of low hygienic standard.

Of all the factors influencing the type of onset, a leading role may be assigned to the amount of infecting material coming into contact with the conjunctiva. This has been borne out by the fact that it is chiefly experimental trachoma, or the self-inflicted or professional contaminations, that as a rule display acute initial phenomena. The type of onset as well as
the clinical course of the disease are considerably modified by concomitant infection with pathogenic bacteria. Whatever the mode of onset, after a few weeks the disease invariably passes into a chronic stage and runs a long protracted course. The cases which are mild and insidious in onset show little at first, apart from some swelling of the upper eyelids with a granular condition of the tarsal conjunctiva, whereas, in those rare cases starting abruptly, inflammation of the conjunctiva spreading to the cornea is a prominent feature. The lids are swollen, the pre-auricular glands may show slight enlargement and there is a varying amount of discharge. Trachoma has been clinically classified into four stages by MacCallan and this classification has gained wide acceptance among the trachomatologists.

Clinical picture: It is difficult to make an accurate estimate of the length of incubation period in trachoma since the naturally acquired disease has an insidious onset. In those cases, where the disease has been induced in man experimentally the incubation period has varied from 4 to 7 days, but it is probably longer in naturally acquired trachoma; the difference being due to the considerably larger inoculum used in experimental transmission.

The clinical picture of trachoma consists of
keratoconjunctivitis, usually of chronic evolution, characterised by the formation of follicles, papillary hyperplasia and pannus, and typical cicatrisation. The involvement of cornea is a specific part of the disease and is an invariable primary phenomenon. The cardinal clinical criterion is the development of pannus consisting in extension of vascular loops over the cornea. Cicatrisation of follicles appearing at a very early stage may be regarded as almost diagnostic of trachoma. The minute star-shaped scars are visible with slit-lamp. Superficial keratitis involving the upper part of the cornea when seen in the early stage is also another characteristic of the disease. The corneal ulcers are most commonly seen at the advancing edge of the pannus and are associated with profuse mucopurulent discharge and photophobia.

Cattaneo observed that when trachoma affects several members of a family, its clinical aspects tend to be typical for the entire family, that is, the type and localization of the conjunctival hypertrophy, the type of corneal complication, the course of the disease, and so forth, are usually the same for all the affected members. Similarities of clinical aspect in trachoma may possibly also involve more extensive areas so that there may actually be geographic difference in the clinical picture. Alimuddin reported high frequency
of pannus formation in trachoma cases of West Pakistan. Smith et al.\textsuperscript{43} also observed that pannus is more common in Gambia than in Jordan. Severe bacterial contaminations arise more especially in the Arab countries and East Africa, are comparatively infrequent in West Europe and America and appear still less in Japan. The so-called "Herbert's Pits" occurs very frequently among the American Indians and in Egypt, whereas, in Italy and Indonesia, it is extremely rare.

That there is a tendency to spontaneous cure in trachoma is now an established fact. Contrary to the earlier belief, spontaneous cure occurs in a fairly large number of cases of trachoma, but with a noticeable difference between one country and another. According to Tsutsui\textsuperscript{44} 30 to 50 per cent of trachoma patients heal spontaneously in Japan. This is equally true of the extent of sequelae.

While the presence of typical trachomatous pannus puts one on solid ground in making a diagnosis of trachoma, its absence does not always necessarily rule out the clinical diagnosis. Several decades ago Boldt\textsuperscript{45} stated that pannus was not a constant finding and occurred only in about 36\% of the cases studied by him in the trachomatous district of Germany. According to Neese\textsuperscript{45}, pannus occurred in about 56\% of cases in the trachoma districts of Russia. Tabone\textsuperscript{47} states that he
has seen some cases of undoubted trachoma in whom pannus could not be detected, even with the slit-lamp. Taborisky\textsuperscript{48} observes that pannus tenuis (early or recent) occurs in nearly all (91-98\%) the cases of advanced trachoma; that in cases of recent trachoma it occurs less frequently, and that with children of minor age it is still less frequent. A survey by Rice and Smith\textsuperscript{49} showed that of 1,154 cases studied by them, 88\% showed the presence of pannus. Julianelle and Smith\textsuperscript{50} observed that approximately 37\% of early trachoma cases do not show any pannus.

It may be that some of the authors mentioned above had made their observations before the slit-lamp corneal microscope came into general use, but the hand loupes, although no substitute for the biomicroscope, when used with a good pencil of light are quite good for a trained observer to detect pannus. Mitsui\textsuperscript{51} states that even a microscopic pannus can be absent in the early stage in some cases of trachoma and in Japan, it is seen in about 50\% of trachoma cases during the acute stage. Stressing the slit-lamp biomicroscopy Cuenod and Nataf\textsuperscript{52} expressed the opinion that although pannus occurs almost always during the late stages, it is possible for some cases of true trachoma to occur without pannus, especially in the early stage.
Pathology: Development of Halberstaedter-Prowazek inclusion bodies in the cytoplasm of conjunctival and corneal epithelial cells is the first pathological sign of trachoma. Wilson recognized that the inclusion bodies and their component elements are regularly present at the onset of the disease. And Thygeson discovered the inclusions during the incubation period of experimental trachoma of human volunteers. The frequency with which they can be demonstrated varies directly with the severity of the disease.

The lymphoid follicles of trachoma are histologically similar to the follicles of nontrachomatous ocular infections, but differ in their size, predilection for the upper tarsus and upper fornix, and in the degenerative changes that characterise them and lead to scar formation. These degenerative changes are reflected in the large number of Leber cells, filled with engulfed cellular debris, that are seen in follicular expressions. The principal lesion in trachoma is primary subepithelial infiltration followed by degenerative cicatricial change in the supporting tissue. The infiltrating cells of the papillary hypertrophy are in large part plasma cells, and it is these cells, along with a lesser number of lymphocytes, which form the subepithelial infiltrate between trachomatous follicles. The neutrophils are abundant in the exudate of early and acute cases. The plasma cells do not seem to share the ability to migrate.
through the intact epithelium.

The essential pathological features of trachoma consist in the cicatrization and pseudogland formation, the papillary hypertrophy, the initial hypertrophy and subsequent atrophy of the epithelium and such late sequelae as hyaline degeneration. Owing to the difficulty of obtaining biopsy or autopsy specimens, little could be known of the pathology of trachomatous limbus and cornea. However, Busacca's studies have contributed to the knowledge of the pathology of limbal follicle and its cicatricial changes, as well as to the understanding of such other corneal changes as superficial keratitis, so characteristic of trachoma.

Dacryocystitis is a common complication of trachoma and it is possible to demonstrate inclusion bodies in the epithelial cells of excised sacs. The ptosis is a characteristic feature of the trachoma facies. As to its nature there is some difference of opinion. It is certainly due in part to increased weight of the lid from cellular infiltration, but in certain cases, it is also due, at least in part, to cellular infiltration of Muller's muscle.

In regard to the cardinal pathology of trachoma, Taborisky has stressed on the epithelial changes as the most significant, while other authors seem to have given more weight to the changes in the follicles that reflect the necrotizing propensities of the disease. The necrotic changes include cytoplasmic debris, cells
with bare nucleated and numerous macrophages which act as scavengers to take up cellular debris.

**Clinical pathology:** A number of investigators have studied the blood changes in trachoma. These blood studies were summarized by Nataf in 1952 and by Beitti in 1953. François found that in chronic cases a neutropenia existed accompanied by a relative lymphocytosis. Mihail made a similar observation; according to him, monocytes were more numerous than lymphocytes, and there was no increase in the number of eosinophilic leucocytes without concomitant parasitic infection. Nakajima and Otacke observed a slight leucocytosis of short duration at the early stage of onset, and an increase in the number of eosinophilic and lymphocytic leucocytes, which continued up to stage III. They also observed the predominant polymorphonuclear reaction at the onset of the disease.

The paper electrophoretic study of serum proteins of the IIInd and IIIrd stage of trachoma revealed slight decrease in albumin fraction and also a slight increase in beta- and gamma-globulin fraction.

Mourzium and Souchkowa investigated the lysozyme content of the trachomatous eye and showed that the titre of lysozyme was considerably diminished during the active stages, particularly so in trachoma cases with corneal ulcer. There was a tendency for the lysozyme content gradually to return to normal as healing occurred.
Licheri\textsuperscript{65} determined the vitamin-A level of the blood in a series of trachomatous patients and found it within normal limits.

Moncino\textsuperscript{66} found the sedimentation rate of trachomatous individuals normal except in cases complicated by corneal ulcers and acute dacryocystitis.

In view of the localized character of the disease, one would not expect blood changes specifically related to trachoma.

3. AETIOLOGY

According to Koch's postulates, the aetiological role of a micro-organism can be proved by its isolation from the patient suffering from the disease, its serial passage in the laboratory, the subsequent demonstration of its ability to reproduce the disease and the reisolation of the micro-organism. Until the recent success in the laboratory isolation of the virus from trachomatous patients, it was not possible to establish firmly the aetiology of trachoma. In the past various micro-organisms including protozoa, bacteria, fungi and rickettsiae were incriminated as the aetiological agent of trachoma.

While working in Egypt Robert Koch discovered in 1883 a minute rod shaped organism in the discharge from the eye of acute summer ophthalmia cases. The organism rediscovered 5 years later by John Weeks in New York was known as Koch-Weeks bacillus. The Greek surgeon Kartulis
who was then working at Alexandria thought Koch-Weeks bacillus was the cause of trachoma, the most common ophthalmia in Egypt.

Halberstaedter and Prowazek\textsuperscript{67} while in Java, discovered intracellular clumps of minute bodies in conjunctival scrapings derived from trachomatous patients and experimentally infected apes. They claimed that the structures they had found were the aetiological agents of trachoma. This important observation supplemented with the significant interpretation inspired a host of studies on trachoma and confusion supervened. Many inexperienced workers failed to distinguish inclusion bodies as described by Halberstaedter and Prowazek from pseudoinclusions of various kinds occurring in both normal and diseased conjunctiva and they claimed to have seen similar bodies in such nontrachomatous diseases as vernal catarrh and ophthalmia neonatorum, as well as in genital epithelium. The discovery of inclusion bodies by Stargardt\textsuperscript{68} and Schmeichler\textsuperscript{69} in cases of nonbacterial ophthalmia neonatorum complicated the situation all the more. Lindner\textsuperscript{70} recognized this nonbacterial ophthalmia neonatorum as a distinct disease entity called "Inclusion blemorrhoea" and thus cleared the confusion so far as the significance of inclusions in the genital epithelium of mothers and in conjunctival diseases of the new born was concerned.

Later on, in 1928, came Noguchi's\textsuperscript{71} sensational
theory on the aetiology of trachoma. He had isolated a gram negative bacillus called *B. granulosis* from cases of trachoma and firmly held that trachoma is a bacterial disease caused by *B. granulosis*. Noguchi's view was supported by many trachomatologists including Thygeson and all of them claimed to have reproduced follicular conjunctivitis in monkeys by inoculation of *B. granulosis* isolated from trachoma cases. During the following years (1929-34) several investigators contradicted Noguchi's claim. Thygeson in particular, raised the objection that the disease produced in monkeys was not associated with pannus and scar formation, so characteristic of human trachoma. Lindner who went to see the monkeys which had been inoculated at the Rockefeller Institute remarked "There is no doubt in my mind what-so-ever that Noguchi's experimentally produced conditions have nothing in common with trachoma. It can scarcely be doubted that Noguchi has discovered, not the cause of trachoma, but one of the causes of the folliculosis of the conjunctiva." Lindner also inoculated his own conjunctiva with a culture of *B. granulosis*, obtained from America, with the result that after three weeks a folliculosis developed which entirely disappeared after two and a half months. Finally it was proved that Noguchi had been mistaken and the organism *B. granulosis* was unrelated to trachoma.

Guenod and Nataf reported that the infectious agent of trachoma obtained from conjunctival scrapings could be
passaged in the intestine of louse. That some of the morphological forms of the trachoma virus have a superficial resemblance to rickettsiae led Busacca\textsuperscript{75} to propose the terms "Rickettsia trachoma". The positive Weil-Felix reaction with trachomatous sera was also advanced by some workers as an evidence in support of the rickettsial theory. Roth\textsuperscript{77} and Braley\textsuperscript{78} contradicted the findings of Cuenod and Nataf; moreover, Julianelle and Smith\textsuperscript{78} showed that both trachomatous and nontrachomatous sera gave positive Weil Felix reaction. MacCallan\textsuperscript{79} rejected outright the rickettsial theory on grounds that there is no known anthropod vector in trachoma, the course of trachoma is quite unlike any other rickettsial disease, there is no permanent or long lasting immunity following trachoma and the Weil Felix reaction is not positive in all cases of trachoma.

Thygeson and his colleagues\textsuperscript{80} performed a very significant experiment, they demonstrated the presence of elementary bodies in the gradocol filtrate of human trachomatous material and with the filtrate reproduced typical trachoma in African baboons. This experiment conclusively proved that the inclusion bodies of trachoma represent a living virus and are not inanimate cell products. Furthermore, infectivity of trachomatous material is always correlated with the presence and number of inclusion bodies. The filtrability, infectivity
of the filtrate and the presence of definite cycle of intracellular morphological variation are all suggestive of the viral nature of the agent of trachoma. Jun Tsutsui advanced some more evidences in support of the viral nature. In a review of the aetiologic problems in trachoma, Thygeson and Natar remarked "Although the virus of trachoma has not yet been cultivated in series or in quantity, there is a general agreement that the disease is caused by a virus like agent of large particle size (the elementary body of Halberstaedter and Prowazek) belonging or closely related to the psittacosis-lymphogranuloma group of atypical viruses."

The aetiology of trachoma is now conclusively proved by the successful isolation of the virus from patients with trachoma, its serial passages in the laboratory, demonstration of its ability to reproduce classical trachoma with typical inclusion bodies in human volunteers, and by its re-isolation from the experimental disease. Furthermore, the cultured virus used as complement fixation antigen gives positive reaction with sera from trachoma, psittacosis and lymphogranuloma venereum.

Classification and nomenclature: The agents of trachoma, inclusion blenorrhoea, psittacosis and lymphogranuloma venereum have all been grouped together in virtue of the possession of certain common characters. They are large, measuring between 250 and 450 μ in diameter and when multiplying they pass through a regular sequence of
of developmental forms. Like the rickettsiae, they are Castaneda-positive and the cytoplasmic inclusions which they produce are basophilic. Moreover the matrix of the inclusions is easily discernible under ordinary microscope. Unlike true viruses, they are susceptible to sulphonamides and broad-spectrum antibiotics.

They have the properties of the viruses, such as filterability, inclusion body formation and obligate cell parasitism. Thus possessing character in common with both viruses and rickettsiae they occupy an intermediate position between the orders of Virales and Rickettsiales. They appear to be more nearly related to the rickettsiae than to the viruses. Their size, their affinity for basic dyes, their mode of multiplication (probably by binary fission) and their susceptibility to chemotherapy mark them off from the viruses and suggest a closer relationship to the rickettsiae. Recognition of this relationship was probably responsible for the proposal of Moshkovsky that the agents of trachoma, inclusion blenorrhoea, psittacosis and lymphogranuloma venereum should form one of the families of the order Rickettsiales. He suggested the name of "Chlamydozoaceae" for the family. Under the generic term "Chlamydozoan", the agent of trachoma has been designated "chlamydozoan trachomatis" and the agent of inclusion blenorrhoea, "Chlamydozoan oculogenitale".

The term "chlamydozoa" was originally used by van Prowazek to describe the extra and intra-cellular minute
bodies of the trachomatous conjunctiva, but it was soon realized by him that the designation - mantle (chlamys) animals (zoa) - was a mistake. Moskovsky also admitted the mistake and said "Already in the life time of Prowazek it was elucidated that the products of the reaction of the cell, the obligatory presence of which around the parasite was thought as a characteristic peculiarity of this group of microbes, can also be absent. Lipschutz (1919) pointed out the paradoxicality of that circumstance that just in trachoma, which served to Prowazek as a prototype of Chlamydozoic infections, no cloak (chlamys) of protoplasmic origin is formed and that on the basis of Lindner's work the inclusions of Halberstaedter and Prowazek should be considered as naked aggregates of bodies of the agents, which was acknowledged also by Prowazek". Notwithstanding such definite conclusion the name "Chlamydozoa" was retained by him and perpetuated by others. Moskovsky's classification received what almost amounted to an official recognition when it was adopted by "Bergy's Manual of Determinative Bacteriology". Both K.P. Meyer and S.P. Bedson opposed this classification. According to Meyer, the agent of trachoma differs from the psittacosis-lymphogranuloma group of viruses in host range, ease of cultivation, antigenic relationship, and chemical reaction products induced in host cells. In view of the major effort currently being made to improve virus classification in general and particularly towards
simplifying terminology, it seems likely that the group under consideration will ultimately be reclassified by international agreement. Recently the name of the family has been changed to "Chlamydiaceae".

Relationship to other viruses: The viruses of trachoma and inclusion blenorrhoea are very closely related to each other. In fact, the two conjunctival viruses are microscopically indistinguishable. According to Lindner, they stemmed from the same virus originally and their relationship is like that between variola and vaccinia viruses.

The viruses of trachoma and inclusion blenorrhoea resemble fairly closely members of the P-LGV group. They are alike in size, and staining properties. The only difference between inclusion bodies of conjunctival viruses and those of the P-LGV group is that in the former the matrix is composed of carbohydrate whereas, in the latter, of protein. In all these viruses the duration of intracellular life cycle appears to be the same, approximately.

Rake and his colleagues showed that the viruses of trachoma and inclusion blenorrhoea present the same sequence of morphological change as do the psittacosis viruses when they multiply. Furthermore, they showed that the serum from cases of trachoma and inclusion blenorrhoea might contain psittacosis group antibody. This was the first serological investigation indicating
some sort of antigenic relationship among these viruses.

Nature of the agent:

Morphology The morphology and intracellular development of the trachoma virus have been classically described by Halberstaedter and Prowazek in the following account:

"In the Giemsa preparations dark blue-stained nonhomogenous irregular inclusions were visible beside the nucleus in the pale blue protoplasm of the epithelial cells. The inclusions were at first small, round or oval in shape but they gradually grew in size and finally assumed the appearance of a mulberry. During this process the inclusion, commencing from its center became less dense in character. Gradually the inclusions tended to clasp the nucleus like a cap and later fine red-stained corpuscles appeared within these inclusions, and as they rapidly multiplied the blue masses disappeared simultaneously. Finally the granules or corpuscles (elementary bodies) occupied the great part of the protoplasm and then the blue-stained substance only became visible as small islands between the mass of granules. In smear preparations, free granules were also found extracellularly.

The changes described in the human conjunctival epithelium could be transferred from man to the orangutan and also from one ape to another by experimental inoculation. The blue amorphous masses of the inclusions had the same affinity for the blue component of the Giemsa dye as the nucleoli and were probably identical with plastin. The circumscribed granules, on the contrary, which were distinctly red and approximately 0.25μ in size probably represented the virus itself, for they multiplied rapidly by growing and then dividing into two colon-like granules."

Mori studied the morphology of the inclusion bodies by means of the Giemsa method and various other histochemical methods. He found azurophilic reticular inclusions in the early stage of acute trachoma, they
are demonstrable before the appearance of the Halberstaedter-Prowazek type of inclusions and disappear after the development of the mature elementary body inclusions. Employing the PAS (periodic acid-schiff) reaction as a method for histochemical demonstration of the polysaccharide matrix he found that the mature and developing forms of the inclusions containing a large quantity of polysaccharide give a strongly positive PAS reaction, while the immature forms containing a small amount of polysaccharide give a slightly positive or negative PAS reaction.

Gordon et al.\textsuperscript{12} studied the morphology of the trachoma virus in monolayers of chick entodermal cells on coverslips. When the developing inclusions of trachoma were compared to those of other members of the psittacosis group, considerable similarity to mouse pneumonitis was seen but there was a marked difference from 3 other mammalian strains (cat, goat, cow) and from 2 avian strains (Parakeet, turkey). The basic viral elements appeared to be similar in all cases and the difference in appearance of the inclusions could be ascribed largely to difference in rigidities of the vesicles; trachoma and mouse pneumonitis viruses produce a relatively rigid vesicle that tends to retain a spherical or ovoid shape in all situations.

The initial bodies of trachoma were first
described by Lindner. They vary greatly in shape and size. The most characteristic form is an extracellular coccobacillary body with a long diameter of 1.2 μ or more, but well defined division forms and a variety of other forms are also recognized. The following different shapes have been recognized:

"ring forms" appearing as little discs stained at their edge, "diplococcus-like varieties" exhibiting polar staining, "oblong types", being either rod-shaped or with a lateral curvature, and also "sickle-shaped forms". Lindner described the role of the initial body in trachoma as follows:

"First, inside the protoplasm of the epithelial cells there are to be found forms like cocci in small numbers. These initial bodies lie inside distinctly outlined cavities in the protoplasm of the epithelial cell. They are stained blue by Giemsa's method. They apparently multiply, judging from the variety of numbers in the different inclusions and of the many double forms. Later on, inside the heap of the initial bodies, a granulated mass develops in which minute fine granules of a slightly reddish or violet colour are embedded. At the end of the development no initial bodies are left but only the fine granules in this granular mass. The initial bodies are always located peripherally adhering to the wall of the cavity. My explanation of this morphology is as follows: The initial bodies multiply within the epithelial cells so long as they have nourishment. If the surface of the nourishing wall of the cavity is insufficient, because some have been crowded toward the center of the cavity, the second stage of the virus is produced, that is, the fine granules called elementary bodies. Hence the granulated mass in the cavity surrounded by the layer of normal initial bodies consists of degenerated initial bodies. From these the fine elementary bodies originate."
It is generally agreed that the initial body represents the young or immature form of the virus. But Lindner's concept of the origin of the elementary bodies from degenerated initial bodies has been questioned by Thygeson who thinks that the initial bodies multiply by binary fission with subsequent division forms decreasing in size progressively until the elementary body form is reached.

From Giemsa stained preparations, the dimensions of the trachoma virus appear to be of the same order as those of the psittacosis virus. The particle size of an elementary body has been estimated as 0.25 μ approximately. Until recently the virus was not available in quantity, so neither ultracentrifuge nor graded collodion filter could be used for obtaining an accurate measurement.

The light microscopic measurement has been confirmed by electron microscopy. In recent years a number of Japanese investigators have made electron microscopic studies of trachoma virus. Mitsui and Susuki studied the inclusion bodies of trachoma in ultra-thin sections and found that there was an external double membrane, obscure in places, and the margin of the body was sometimes prolonged into pseudopodia; the interior of some bodies contained minute granules, in others the central area was of low
density, or else contained a dense central body. The elementary bodies are more dense to electrons than initial body forms and often contain a central granule more dense to the electron beam than the periphery. The density appears to vary inversely with the size. Some of the larger initial bodies have a reticular or alveolar structure and occasionally contain a number of distinct granules. The smaller initial bodies, however, appear as solid granules like the elementary bodies.

According to Thygeson, the elementary bodies of trachoma are remarkably uniform in size. Nataf and Sietti, on the other hand, are inclined to believe in the existence of smaller ultra-microscopic forms. It is well known that in inadequately treated cases of trachoma, inclusion bodies disappear temporarily and then reappear with relapse of the clinical disease. Furthermore, in experimental trachoma of monkeys and in mild forms of the disease in men, the virus, even though undoubtedly present in the infected conjunctiva, can not be demonstrated by light microscopic examinations. All these can be explained by the hypothesis of ultra-microscopic forms. However, there is no definite evidence in support of the hypothesis except some observations under electron microscope, which are not always sufficiently reliable. Thygeson argues that if smaller forms really existed, then it would not have been difficult to obtain positive filtrates. Besides,
inadequate sampling might well explain the common failure in finding inclusions when they are present in small numbers in cases of an ultra-microscopic phase has not been raised in connection with other members of P-LGV group - not even in relation to lymphogranuloma virus which is usually difficult to demonstrate morphologically in chronic phase of the disease.

**Staining character:** Like the rickettsiae, the trachoma virus and the other members of the P-LGV group stain well with basic dyes and they are all Castaneda positive. With Castaneda stain, the elementary bodies of trachoma stain blue and by Macchiavello's method, they stain red.

The stain most commonly employed is Giemsa's stain. Stained with Giemsa, the elementary bodies show a considerable range of colours, from blue to red, depending apparently on their maturity, since they tend to be bluish in small, immature inclusions and reddish in large, mature inclusions. The colour of the elementary bodies is also dependent on the $p^H$ of the distilled water used in the preparation of the stain. With $p^H$ on the acid side, they stain a pure red and with $p^H$ on the alkaline side, they tend to be bluish. A halo or non-staining zone around the individual elementary bodies is clearly visible in Giemsa-stained film preparations. The clear zone is probably formed by the gelatinous material which clings to the elementary bodies after
their liberation from freshly ruptured inclusions. In Gram-stained preparations, the elementary bodies are uniformly negative, but usually do not take the counterstain unless it is prolonged beyond the usual one minute.

The initial bodies stain bipolarly and because of their bipolar staining they have often been mistaken for diplococci. With Giemsa's stain, they are consistently pure blue. In smear preparations stained with Lindner's contrast stain, the basophilic initial bodies react variously to the Gram stain; some of the larger forms are Gram-positive and the smaller, transitional forms are Gram negative. The initial body inclusions stained with Gram stain take up a crystal violet colour.

**Filterability:** Unlike the typical viruses, the virus of trachoma resists filtration through bacterial filters. In the past, all such attempts employing the ordinary methods of filtrations, have yielded negative results in the majority of cases. In view of the large size of the virus and its low concentration in the material to be filtered, it was really difficult to obtain a positive result. The filtration experiments up to 1935 have been well summarized by van Rooyen and Rhodes, and up to 1950 by Bedson et al.

The first successful filtration experiment with trachoma virus in conjunctival material was performed by Nicolle and his colleagues in 1912. They used
buttons of Berkefeld V substance cemented into glass tubes and thus controlled absorption losses. With the filtrate they could produce experimental trachoma in Barbary apes and were also able to pass the infection to human volunteers. Macchiavello17 also claimed that the agent which he isolated from a trachomatous child, after being passaged on the egg yolksac would pass Seitz EX and Berkefeld V filters; however, it is still uncertain whether the agent was the virus of trachoma.

After the introduction of Elford's collodion membrane filters, a number of successful filtration experiments were carried out. Thygeson and his colleagues80 showed for the first time that the virus of trachoma was readily filtrable through collodion membranes with an APD of 0.75 u. With the filtrate thus obtained, they could reproduce typical trachoma in 5 African Sphinx baboons.

The difficulties which have to be overcome in order to obtain positive results in filtration of trachomatous material have been amply stressed by Julianelle et al.97, Stewart98, and Macchiavello17. It was shown that positive filtrations are possible only when the factors of virus concentration and absorption losses in the filter are adequately controlled and the membranes are of adequate pore size so as to allow free passage of the elementary bodies. In order to have an adequate virus concentration, the filtration
material should consist of epithelial scrapings rather than follicular expressions, and it should be collected from active cases of trachoma. The material should also be centrifuged at a low speed to remove cellular debris which tends to clog the filters.

Response to centrifugation: Stewart\textsuperscript{98} reported that repeated centrifugation failed to deprive the virus of its infectivity. Thygeson\textsuperscript{99} was able to obtain elementary body suspensions of reasonable purity by differential centrifugation of material from cases with abundant inclusions. Julianelle and Harrison\textsuperscript{100} showed that low speed centrifugation for a short period of time had no effect in depriving the supernatant fluid of its infectivity, but 30 minutes at 5,000 r.p.m. was sufficient to render the supernatant fluid non-infective for monkeys.

Response to physical agents: The virus dies rapidly outside the human body. How long it survives is not exactly known. The virus can survive mechanical grinding and emulsification of trachomatous tissue in saline. Drying appears to inactivate it fairly rapidly. It can withstand freezing for at least a week, but not repeated freezing and thawing. Minimal dilutions appear to affect the infectivity of the virus.

Data on the effect of temperature on the virus
have been summarized by van Rooyen and Rhodes. Julianelle observed marked temperature liability of infective trachoma virus in the form of fresh conjunctival scrapings. Tang et al. reported that in yolk sac suspensions the virus was inactivated at 50°C after 30 minutes or at 37°C after 24 hours or at 21°C after 7 days and at 4°C after 23 days. Recently Jawetz observed that the virus loses infectivity rapidly at 37°C but there is surprisingly little loss of infectivity at room and refrigerator temperatures.

Response to chemical agents: Trachoma virus is inactivated by the commonly used disinfectants. Julianelle and Smith found that disinfectants such as silver nitrate (2%), phenol (0.25%), and gentian violet (1 in 100,000) rapidly destroyed the virus. 25 to 35 per cent solution of bile also inactivated the virus. But the recent report of Jawetz and Hanna indicates that sodium deoxycholate interferes with staining properties of the virus far more than with infectivity. Observations on the effect of ether are contradictory; according to Tang, ether has no effect on the virus, whereas, according to Jawetz, the virus stored in ether overnight at 4°C becomes non-infective.

Although the sulphonamides seems to have no lethal effect on the virus in vitro, the therapeutic action of these drugs in the treatment of the disease
is well established. Moreover, Bietti \textsuperscript{102} clearly demonstrated that within the first few days of treatment with sulphonamides, definite degenerative changes appear in the inclusion bodies, which ultimately disappear altogether. Jawetz and Hanna \textsuperscript{103} recently studied the ability of varying amounts of penicillin and tetracycline to interfere with the growth of trachoma virus in eggs and found that the three strains exhibited a very large range of difference in susceptibility to the antibiotics. All recent reports of virus isolation clearly indicate that streptomycin even in a concentration of 20 mg/ml does not affect infectivity.

**Preservation:** In view of the rapid inactivation of the virus outside the body, attempts were made to preserve the infectivity by various methods including use of glycerol. The use of glycerol as a preservative for the trachomatous material was originally suggested by Nicolle and his colleagues. But Stewart \textsuperscript{104} found that the virus was inactivated by glycerol within 24 hours. Julianelle \textsuperscript{31}, on the other hand, observed that glycerol had neither any deleterious nor any preservative effect. Tang \textsuperscript{1} reports that the virus loses infectivity when stored in glycerol for 14 days.

Dereani et al. \textsuperscript{105} have shown that the trachoma virus grown on yolk sac could be well preserved at \(-60^\circ\text{C}\) without any appreciable loss of infectivity. Hanna et al. \textsuperscript{5} also reported that infectivity could be maintained at \(-40^\circ\text{C}\).
Tang showed that the virus in the yolk sac membranes could survive for 190 days after freeze-drying. Jawetz also found that the virus in the freeze-dried state maintained viability when stored at 4°C for at least 5 months, or when mailed without refrigeration to other parts of the world. During lyophilization a large proportion of the virus was, no doubt, lost and one blind passage of such material was usually required before it killed eggs with abundant virus in smears.

Precise knowledge about the biochemistry of trachoma virus is still lacking. Some information on the chemical nature of the virus and its inclusion body has been gained from their reactions to biological stains and from electron microscopic studies. Rice showed that the matrix of the trachoma inclusion is composed of a carbohydrate with the properties of glycogen. The absence of a protein component in the matrix seems to be indicated by its failure to stain with biological dyes. Grossfeld showed that the trachoma inclusion is Feulgen-positive, indicating the presence of desoxyribonucleic acid (DNA).

A chemical difference between the denser elementary body and the less dense initial body is clearly indicated by the marked tinctorial difference between the two forms in the multiplication cycle.

The bile-solubility of trachoma virus suggests a possible chemical similarity to the pneumococcus.
Trachoma virus resembles the rickettsiae in staining character and in susceptibility to chemotherapy and thus there is a strong possibility that it may have the same complex biochemical composition and enzymatic apparatus like the rickettsiae. According to Kirisawa and other Japanese investigators (as mentioned by Bietti, 1956) the virus of trachoma possesses an enzymatic system of its own, however, rudimentary.

As judged by epidemiological data, trachoma virus appears to have a low degree of infectivity for man. The severity of the disease largely depends on the age of onset and the secondary infection. However, the possibility of differences in virulence of virus strains cannot be excluded altogether. Of late, such evidence has been reported by Hanna et al. Of the two strains isolated by them, the one known as BOUR strain, diluted $10^{-3}$ produced intense follicular conjunctivitis with abundant inclusions in cynomolgus monkeys, whereas the other known as ASGH, diluted $10^{-1}$ produced only mild follicular conjunctivitis with rare inclusions.

Trachoma virus manifests an extraordinary degree of selectivity for specific ocular tissues in specific areas. This highly specialized epitheliotropism well explains the lack of systemic manifestation in trachoma. The inclusion bodies of Halberstaedter and Prowazek are seen only in conjunctival and
corneal epithelial cells, never in cells beneath the epithelium. A number of trachomatologists have stressed on the strict localisation of the virus to epithelial cells. This concept has also been supported by the fact that the virus containing material, infectious for the conjunctiva does not produce trachoma in man or monkeys when injected into the subepithelial layer of the conjunctiva through the skin of the lids. Opposing this concept Cuenod and Nataf\(^7\) and Chams et al.\(^8\) have pointed to the subepithelial localization of the principal lesion of trachoma.

The changes in the subepithelial tissue might be explained as a reaction to the soluble toxin liberated by the virus localized in the epithelium. Mitsui et al.\(^9\) could produce follicular hypertrophy in human volunteers by inoculation of filtrates from non-infective trachomatous material. Recently Murray et al.\(^10\) reported that a strain of trachoma virus isolated in Saudi Arabia proved highly toxic for white mice and jerbils when inoculated intravenously in concentrated suspension. Death occurred within 2 to 8 hours of inoculation; animals which received comparable amounts of the same material which had been heated for 30-45 minutes at 56°C to 60°C, survived without evidence of toxicity. Animals which succumbed showed gross lesions in the small intestine somewhat
similar to animals reacting to the typhus rickettsial toxin.

Host range: The virus of trachoma has a very limited host range. The natural infection occurs only in man. The experimental infection of man with trachoma by means of direct transfer of conjunctival material (scraping or secretion) has been effected without difficulty by many investigators. Julianelle recorded that of 104 volunteers inoculated, 73 developed the disease. Of the laboratory animals, only the primates are susceptible to trachoma virus. Experimental transmission has succeeded in monkeys, baboons and apes, all of whose susceptibility has been of a low order, with monkeys the least and apes the most susceptible. In none, can typical trachoma be reproduced, and only in the ape can the cytoplasmic inclusions be demonstrated. The clinical diagnostic features of the human disease such as pannus and cicatrization are extremely rare in monkeys or apes, the experimental disease being a self-limited chronic follicular conjunctivitis with minimal exudate lasting a matter of months only.

The virus of trachoma is believed to have no pathogenicity for the mouse; Weiss was unable to infect mice by intracerebral inoculation. However,
Arakawa et al.\textsuperscript{9} cultivated trachoma virus in mouse brain and they still uphold their claim. Recent reports\textsuperscript{110} indicate that the Tang's strain can be adapted to grow in the mouse brain. It is noteworthy that Tang himself failed to grow his strain on the mouse.

According to Renoux et al.\textsuperscript{11} the virus is capable of proliferating in the tissues of the Muridae; but the trachomatous nature of their strain is not yet well established.

Claims for the susceptibility of rabbit testicle to the virus have been made, but its transient survival in this tissue is all that has been established.

The claim for the multiplication of trachoma virus in the intestine of louse has not been confirmed and according to Lepine\textsuperscript{112}, the micro-organisms detected were in reality the normal reckettsiae of the louse, which has nothing to do with trachoma.

Laboratory cultivation: The recent reports of successful cultivation indicate that the virus of trachoma can be grown on the yolksac of developing chick embryo; multiplication occurs in the layer of entodermal cells forming the lining of the yolksac. From Tang et al.'\textsuperscript{11}'s report it appears that the yolk fluid of infected sac does not contain elementary bodies. Choricallantoic and amniotic membranes do not support the growth of the virus. Smears of the infected yolk sac stained by Macchiavello or Giemsa reveal the elementary bodies arranged singly, in
in pairs, or in small aggregates. Two (G16 and G17) of the strains isolated by Collier induced formation of compact aggregates of virus particles arranged around a central vacuole; the aggregates appeared in every passage. In the human inoculation experiment, they were again found in virus isolated from the conjunctiva. The vacuoles contained glycogen as shown by iodine staining. Regarding the growth character in eggs Collier stated "Growth in the yolk sac is often erratic but, characteristically, the embryos die about 7 days after inoculation with a large dose of the virus, and show haemorrhages into the feather shafts, and intense congestion of the liver and kidneys".

The virus does not always kill the embryos. Not infrequently, embryos of the same age receiving similar inocula die within 3 or 4 days of inoculation, whereas others survive to the time of hatching. In some cases, only those dying 5-7 days after inoculation contain the virus and those dying later are sterile. No multiplication of the virus can be evinced in embryos dying within 3 days of inoculation. 5-6 day embryos usually survive infection longer than do 3- or 9-day embryos. The freshly isolated virus seems to be less virulent than the same strain after a few passages. Variability of growth is not confined to any particular strain of trachoma virus and thus it is difficult to make an
curate titration. Sowa & Collier confirmed Tang et al.'s findings that the yolk of infected egg was less viscous than in uninfected eggs of the same age and that the membrane was thinner and more friable, but they did not consider such changes as specific responses to trachoma virus.

In view of the large number of claims for successful cultivation of trachoma virus, many of which are in fact grossly inadequate, Thygeson and Nataf proposed the following criteria for the confirmation of such claims:

1. That typical Halberstaedter-Prowazek inclusions be demonstrable in serial cultures. By a typical inclusion is meant an intracellular cytoplasmic body containing elementary bodies and a carbohydrate matrix demonstrable with iodine or other glycogen stain such as Best's carmine stain.

2. That experimental trachoma be produced in a human volunteer by the inoculation of tissue subculture material after at least ten passages in series. Material from the experimental disease must contain typical inclusion bodies (elementary bodies in a carbohydrate matrix), and before being treated the disease must be allowed to develop typical biomicroscopic pannus consisting in extension of vascular loops, epithelial keratitis and subepithelial infiltrates.

3. The cultivated virus must be available for transmission to other laboratories throughout the world.
for confirmatory study.

Since the discovery of the inclusion bodies typical of trachoma was made on experimentally infected orangutans, the primates such as monkeys, baboons and apes have continued to be the laboratory animals of choice for trachoma research. However, susceptibility of these animals to the virus of trachoma is undoubtedly of the low order. Stewart investigated on the problem of monkey susceptibility and observed that monkeys belonging to the two genera of Papyo (baboon) and Lasyopyga (grivet) were most susceptible. In view of the low grade susceptibility and the difficulty in diagnosis of the experimental disease, all such experiments with these animals yielded only dubious results. The experimental disease in monkeys is so elusive that the ophthalmologists who examined the hundred monkeys inoculated with B. granulosis of Noguchi expressed the opinion that the disease closely resembled human trachoma.

Numerous investigators have attempted to cultivate the virus of trachoma. Rumyantsev and Levkoeva and Schmidt attempted on tissue culture but failed to obtain any growth. Pandit et al. inoculated trachomatous material on the chorio-allantoic membrane of the developing chick embryo and observed development of lesions on the membrane, but the lesions proved to be nonspecific with no relevance to trachomatous infection. Poleff
employed the yolk sac route of inoculation and claimed success in 9 of 96 attempts, but his claim remained unconfirmed. Busacca attempted to grow the virus on both chorioallantoic membrane and tissue culture system but failed. Cuenod and Nataf reported observation of some lesions on the chorioallantoic membrane and virus-like granules in the smears from the membranes. John and Hamburger also made similar observations. Julianelle employed a large number of tissue cultures of various types and also grafts of human conjunctiva on the chorioallantoic membrane, but could not find any evidence of multiplication of the virus. Rotth employed tissue culture of human foetal conjunctival epithelial cells and observed inclusion bodies in only one culture. Thygeson attempted growing human corneal and conjunctival epithelial cells from normal cases and cases of trachoma in plasma clot culture but failed to obtain virus multiplication, not even survival. Ishihara claimed to have demonstrated inclusion bodies in tissue cultures of conjunctival epithelium inoculated with trachomatous material; but he failed to obtain serial transmission of his culture. Santoni employed the same tissue culture of conjunctival epithelium but no growth of the virus was obtained. Vancea inoculated on the chorioallantoic membrane and observed some alterations of the membrane but human inoculation was negative.
Wright, Gallardo et al. and Burnet et al. employed inoculation on the chorioallantoic membrane without any success. Bland attempted growing the virus in cultures of conjunctival epithelium without success. The experiment of Macchiavello is of special interest. He inoculated follicular material obtained from a case of trachoma (Stage II, 2 years duration of illness, with redness and follicles in conjunctiva but no corneal involvement or pannus) into the yolk sac of embryonated eggs and using the 9th passage material, reproduced clinical trachoma with development of typical inclusion bodies (within 20 days after inoculation) in a child volunteer. The organism isolated was filtrable through Seiz EK and Berkefeld V filters, and was pathogenic to mice and guineapigs. The strain could not be maintained and available to other laboratories for confirmation. Macchiavello's claim was corroborated by Poleff, but the W.H.O. Expert Committee on Trachoma did not accept the claims as confirmed. Hagino and Hamada claimed successful cultivation by chorioallantoic inoculations but their claim was based only on the demonstration of virus like granules by electron microscopy. Barski et al. claimed to have grown the virus in the culture of human embryonic conjunctival epithelium, they also claimed demonstration of inclusion in the culture which
was virulent for 4 human volunteers but was unable to maintain the virus in successive cultures. Arakawa and Kitamura\(^9\) claimed isolation of the virus on chorioallantoic membrane with transmission to mouse brain.

Stewart and Badir\(^{132}\) reported that by alternating inoculation of the developing egg (yolk sac) with injection of the monkey several serial passages of the virus have been achieved. Poleff\(^{133}\) again claimed growth of the virus in cultures of cornea and iris and the claim was based on demonstration of virus like granules. Bietti and de Gaspare\(^{134}\) inoculated into the yolk sac and observed granules resembling elementary bodies. Babbar and Shukla\(^{135}\) claimed isolations on chorioallantoic membrane on basis of complement fixation tests and histological findings. Kogan-Abezgus\(^{136}\) claimed to have grown the virus in the cultures of chick embryo tissues. Shrivastava et al.\(^{137}\) claimed to have propagated the virus through 3 passages on the conjunctival and corneal grafts on chorioallantoic membrane. Sezer\(^{138}\) made various attempts at cultivation by employing yolk sac and chorioallantoic routes of inoculation and claimed 50 isolations. He also used tissue cultures of chick embryo, cornea, lung and liver. With the culture he produced self-limited conjunctivitis of short duration without any inclusion body development. Sezer's culture being tested in Thygeson's laboratory failed to show HP inclusions in Giemsa-
stained sections of the specimen. Collier failed to obtain multiplication of the virus in tissue cultures of human conjunctival epithelium and other tissues. Murray et al. made similar attempts but failed to obtain any positive result. Fujiyama claimed successful serial passages on the chorioallantoic membrane and the virus in the passage material was demonstrated by electron microscopy.

The first convincing isolation of the trachoma virus was accomplished by Tang and his associates. They made 3 isolations of a virus in 68 experiments on 93 specimens from patients with trachoma by inoculating into the yolk sacs of 6-8 day embryonated eggs. The conjunctival material was treated with streptomycin before inoculation, eggs were incubated at 35°C after inoculation, and 5 blind passages were made for all such specimens. The egg-cultured virus produced a follicular conjunctivitis in 7 rhesus monkeys, in one instance with inclusion bodies. Various animal and other tests distinguished the virus from hitherto described viruses, in particular those of psittacosis and lymphogranuloma, which it resembled morphologically.

In further studies on the isolation of the trachoma virus a comparison was made of different concentrations of and periods of incubation with streptomycin in the treatment of eye swab material for inoculation; a concentration of 1000 units per ml. and incubation of the mixture at 4°C for 4 hours were found to be
best, rendering 75% of specimens sterile. They could make 9 more isolations from 22 specimens which were rendered sterile in the similar manner. Stressing the importance of bacteriological sterility of the inoculum they affirmed that all failures in virus isolations were due to bacterial contamination. They also demonstrated the presence of inclusion bodies in stained paraffin sections of infected yolk sacs.

Reviewing the isolations made by Tang et al., Lepine comments:

"The strains isolated did not grow on the chorio-allantoic membrane, but a strain adapted to the yolk sac killed the chick embryo when injected into the egg by other routes; there is thus a toxic factor associated with it. Apart from the chick embryo, all tests made on laboratory animals were completely negative. The three strains showed strictly similar characteristics. Thus the work of these Chinese authors has provided for the first time a micro-organism whose characteristics, although in perfect agreement as regards morphology and filtration with those so far accepted for the agent of trachoma, are nevertheless unlike those of similar agents with which they might be confused. The absence of any pathogenicity, whatever the route of inoculation, for the mouse, pigeon and chicken differentiates it from the agents of psittacosis, ornithosis and lymphogranuloma venereum. The absence of reactions in the mouse and guineapig clearly distinguish it from the true rickettsiae as well as from the micro-organisms so far isolated and regarded as playing a part in the aetiology of trachoma. The clinical appearance of the patients from which the infective material was obtained and the lesions developing in monkeys after inoculation showed that the disease was not inclusion conjunctivitis. The failure of attempts at isolation in tissue culture differentiated the new virus from that of epidemic keratoconjunctivitis. The absence of a life cycle and of pathogenicity for the mouse or guineapig, distinguished the Chinese strains from
that isolated by Macchiavelloa. The inhibiting effect of penicillin, the absence of pathogenicity for the mouse clearly separated it from strains which had been isolated by Arakawa, Bietti and other workers. All in all, the paper published by the Chinese team gave a strong impression of rigorousness and pointed towards success. The sole objections which could be made derived from the fact that the isolations had only been carried out successfully—and even then not always—from cases of very early trachoma, always difficult to find, and seemed to have failed completely with material from well established cases of trachoma; also that serological studies, on which the specific definition of a virus must depend, still remained to be undertaken, since this aspect of the problem had not been considered in Peking."

A British scientist visiting the communist Chinese research laboratories in 1957 obtained the Peking virus and brought to Dr. Collier of the Lister Institutes, London, late in 1957. The Chinese results as a whole were soon confirmed by Collier and Sowa in 1958. Employing the same technique as the Chinese authors, namely isolation from recent trachoma and using only streptomycin in high dosage to combat secondary bacterial infection, they isolated several strains from trachoma patients in Gambia. They confirmed the pathogenicity of the virus by human inoculation, producing typical trachoma in a volunteer; the inclusions developed within 14–20 weeks of inoculation. The virus was also re-isolated from the volunteer. They also made serological studies and showed that the virus contained a common antigen of the psittacosis-lymphogranuloma venereum group and the sera from trachoma patients contained antibodies for this antigen.
Soon came more reports of isolation. Murray et al.\textsuperscript{3} cultivated in the same way, in Saudi Arabia, fourteen strains; they reproduced the disease in chimpanzee. Bernkopf et al.\textsuperscript{4} in Israel, isolated strains which they showed to be similar to the original strain of Tang, and reproduced the disease in man. Similarly, Hanna et al.\textsuperscript{5} isolated strains in California. Grayston et al.\textsuperscript{6} in Taiwan, isolated four strains from 32 cases of trachoma, reproduced the disease in six human volunteers and recovered the virus from these inoculated subjects in 22 out of 23 attempts. Ferret and Ida Mann\textsuperscript{7}, in Australia, have also isolated the virus and reproduced the disease in man. Quite recently, Collier\textsuperscript{141} states that he has received reports of isolations in South Africa, Ethiopia, Tunisia and Algeria.

The consistent results obtained with the technique developed by the Chinese workers (the use of streptomycin alone, incubation at 35°C and systematic practice of blind passage), the similarity in the characteristics of the strains so isolated, the reproduction of experimental trachoma in man, re-isolation of the virus from the experimental infection and finally the serological evidence leave no doubt that the virus of trachoma has now been isolated on the yolk sac of the developing chick embryo.
Renoux et al.\textsuperscript{11} have claimed 3 isolations on the chorioallantoic membrane and 6 isolations on rodent (white mice, Jerbile, meriones) lungs. Two volunteers were inoculated with the egg strain and the rodent strain; agglutinins developed in sera of the volunteers.

Gordon et al.\textsuperscript{12} reported growth of the trachoma virus in tissue culture of entodermal cells of chick embryo. They inoculated a virus grown on the yolk sac and observed its multiplication as indicated by formation of inclusion bodies. Pollard et al.\textsuperscript{13} reported serial propagation of the yolk sac grown virus in cultures of human tissues; along with passages the number of infected cells increased and the tissue culture fluid exhibited infectivity when injected into the yolk sac of the developing chick embryo.

Cuthbertson et al.\textsuperscript{14} also reported cultivation of the virus on cultures of monkey testicular cells.

Furness et al.\textsuperscript{15} described growth of certain strain of trachoma virus in cell culture. They found that the crude yolk sac suspensions were too toxic to the cells and so they used the purified virus propagated in the yolk sac as inoculum. The Fang strain which is capable of growth in mouse brain has been adapted to grow in cultures of HeLa cells, but attempts to propagate two other strains of trachoma (G1 and G17) in cell culture have thus far been unsuccessful, despite the use of purified virus as inoculum; they have also failed to adapt
these strains to mice. They noted the development of serially transmissible inclusions in cell cultures, isolated the elementary bodies from them by yolk sac inoculation and demonstrated the presence of the characteristic group antigen in their cultures.

4. IMMUNITY

All the accumulated evidence indicates that trachoma in man confers little or no immunity. Julianelle and Harrison were probably first to make a detailed study of immunity in trachoma. They did not find any evidence of resistance to re-infection in experimental monkeys. The monkeys which had recovered from an attack of the experimental disease could be readily re-infected and the re-infection did not clinically differ from the first attack. Neither the sera from the patients nor sera from infected or recovered monkeys exerted any neutralizing effect on the virus; the serum-virus mixture, even after prolonged contact, remained infective for monkeys. They also noted that the sera of rabbits receiving intravenous injections of emulsions of conjunctival scrapings from active trachoma cases contained no neutralizing antibody. And in 1939 Julianelle came to the conclusion that the agent of trachoma is antigenically impotent.

In spite of the fact that the viruses of trachoma and inclusion blennorrhea are so closely related to each other Julianelle was unable to find any evidence of
cross-protection between the two in experimental infection of monkeys. It may be noted here that Lindner hypothesized trachoma virus as irreversible mutant of inclusion blennorrhoea virus.

In 1942 Rake et al. reported that sera from trachoma and inclusion blennorrhoea gave weakly positive results to complement fixation tests with the group-specific antigen obtained from yolk sac grown virus of lymphogranuloma venereum. They tested 40 sera from trachoma (stages I - III) patients, of which 35 gave titres ranging from 1/6 to 1/60.

Macchiavello reported that neutralizing antibody for lymphogranuloma virus could be detected in 3 of 14 sera from trachoma patients.

Lepine obtained positive complement fixation with psittacosis antigen in 42% of trachoma cases.

Bietti and Sanna reported the results of a series of complement fixation tests of trachomatous sera. With psittacosis antigen (group), the tests were positive in 3 of 18 cases; and with lymphogranuloma antigen (group), they were positive in only 2 of 43 cases. In another series they used a trachoma antigen (group) derived from epithelial scrapings from active cases of trachoma to test sera from trachoma, psittacosis and lymphogranuloma venereum. With the trachoma antigen a significant percentage of positive results could be obtained.
Using lygranum antigen Sugiura tested 10 sera from trachoma patients, of which 7 gave positive reactions with a titre of $1/8$ to $1/32$.

Mitsui employed both lymphogranuloma and psittacosis antigens and obtained titres of $1/2$ to $1/8$ in 9 out of 14 cases of trachoma.

K. F. Meyer used the group antigen derived from psittacosis virus in complement fixation tests with sera from trachoma cases and obtained positive result in only 1 out of 17 cases; the positive case gave a titre of 1 in 16 during the first 4 weeks of illness. In a second series he compared the serum reactions of untreated and treated trachoma cases with those of persons living in the neighbouring area; the percentage of strongly positive cases was much more in the group of persons free of any trachomatous infection of the eye. He did not attribute any diagnostic value to complement fixation test in trachoma.

Kornblueth et al. reported that with the use of lygranum antigen complement fixing antibody could be detected in 50% of acute and 9% of chronic cases of trachoma.

Babudieri et al. used trachoma antigen derived from pooled conjunctival scrapings in complement fixation tests for sera from trachoma, ornithosis and lymphogranuloma venereum; the sera from trachoma gave positive reaction in a titre of 1 in 32 as against 1 in 16 from
the sera from ornithosis and lymphogranuloma venereum. In another series when ornithosis antigen was used the sera from ornithosis and lymphogranuloma venereum gave a titre of 1 in 128 as against 1 in 8 for the sera from trachoma.

Betti et al. investigated further on the antigenic relationship concluded that there is a certain slight general immunity reaction in trachoma and the virus of trachoma shares with the other viruses of the psittacosis lymphogranuloma group a common antigen.

Giroud et al. reported similar results using both psittacosis and trachoma antigens. They also claimed that group antibodies in low concentration could be demonstrated in extracts (3 of 13 cases) of trachomatous conjunctiva.

Babbar and Shukla reported that sera from trachoma patients gave positive reactions when tested with complement fixation antigen of trachoma virus grown on the chorioallantoic membrane.

Larmande and Orfila tested 100 trachomatous sera with both trachoma and ornithosis antigens and obtained positive results in 43 cases; the authors considered the results indicative of an antigenic relationship between trachoma virus and other members of the psittacosis-lymphogranuloma group.

Coldier found that sera from some - but not all - trachoma patients contained antibodies fixing
complement with trachoma virus, but the antibody titre rarely exceeded 1 in 32. And low antibody titres were sometimes encountered in sera from apparently healthy Gambians. He opined that in trachoma complement fixation test is not at present of much diagnostic value.

Giroud and Renoux\textsuperscript{146} reported serological findings in early cases of trachoma. They used antigens other than those of trachoma but belonging to the same group (psittacosis and neo-rickettsiosis) and observed that the local trachomatous infection stimulates an increase in general antibodies reacting against certain antigens, whether they be those of psittacosis or of certain neo-rickettsiosis.

Woolridge et al.\textsuperscript{25} have put forward further evidence for the antigenic relationship between the virus of trachoma and the virus of psittacosis and lymphogranuloma venerænum.

It is now generally recognized that trachoma virus shares with the P-LGV group of viruses a common group antigen.

Giroud\textsuperscript{147} reported new findings with trachoma antigen derived from rodent lungs, allergic reaction and rise of antibodies. In persons who had previous contact with trachoma, intradermal injection of trachoma antigen caused allergic skin reaction. An injection of this antigen also provoked an immunological booster
response, the antibodies against trachoma rose within 96 hours in positive cases (7 out of 11 past trachoma infections).

Giroud et al.\(^{11}\) performed microagglutination tests on trachomatous sera using mouse lung suspension of psittacosis virus and trachoma virus; control antigens made from various rickettsiae were included in the tests. They found that sera collected from 2 volunteers 21 days after infection with different strains of trachoma virus agglutinated with trachoma antigen at 1:20 and 1:40, but were without action on psittacosis virus. The sera of 12 of 14 patients with spontaneous trachoma were positive, only 1 agglutinated psittacosis virus; and sera from 25 nontrachomatous infection were negative.

Bernkopf\(^{148}\) employed dark field agglutination method to test trachomatous sera with trachoma antigen purified by fluorocarbon treatment and obtained positive results at a titre of 1:50. He also compared the results with those by complement fixation tests and found that the titre and the percentage of positive results were higher with the agglutination test.

The phenomenon of toxicity of trachoma virus is under investigation from the immunological viewpoint. Bell et al.\(^{149}\) successfully immunized mice
against toxic doses of trachoma elementary bodies by intravenous injection of concentrated suspension of homologous virus inactivated by formalin or phenol. This device of toxin neutralization test in mice was employed in distinguishing the different strains of virus and it was shown that at least 2 antigenically distinct types of trachoma elementary bodies occur in Saudi Arabia and Egypt.

Berkopf has developed a technique to employ intranasally inoculated suckling mice for neutralization tests. Neutralizing antibodies were demonstrated in immune rabbit sera and sera of mice after intranasal inoculation. Rabbit antisera against the Israeli and Chinese strain showed a significant and apparently equal degree of neutralization of the Chinese strain of trachoma virus. However, it has not been possible to demonstrate neutralizing antibodies in the sera of trachoma patients.

Some authors have viewed immunity in trachoma as comparable to that in tuberculosis. Recently Poleff made such a hypothesis. But the absence of eosinophilic leucocytes in smears from trachoma and the absence of contact dermatitis of lids seem to indicate against the hypothesis of allergy. It has been established that trachoma cases are Frei-negative. In the past skin tests had been done with trachoma antigen derived from conjunctival scrapings or follicular expressions from
active cases and Tricoire\textsuperscript{152} reported positive skin reactions in 61\% of 150 persons with florid trachoma. Belot\textsuperscript{153} on the other hand found that 43\% of normal subjects reacted positively to Tricoire's skin test. The preliminary work by Collier and Sowa has shown that injection of trachoma virus gives a positive skin reaction in trachoma patients, but not in clinically normal subjects. If the results are confirmed, a trachoma skin test may be used in the diagnosis of clinically doubtful cases.

Tsutsui et al.\textsuperscript{154} studied the immunological and allergic nature of trachoma by means of serial inoculation experiments on human volunteers. An immunity phenomenon could be demonstrated in the diminution of the number of inclusion bodies and in the diminution of intensity of the disease during the repeated infection experiments. The diminution of intensity was found only when the repeated inoculations were made at relatively short intervals. If the interval of two inoculations was more than two years, only hypersensitivity of the conjunctiva remained and in such a condition, the inoculation produced a rapid development of intensive allergic reaction of the conjunctiva, but the proliferation of the virus was disturbed. The instillation of trachoma immune serum showed a partial therapeutic effect in acute trachoma but the effect was not enough to cure the disease. They concluded
that the immunity of trachoma is not complete enough to prevent the disease, but it seems to be beneficial for the cure of the disease. Their experiments suggest that the severity of induced human trachoma may be slightly modified by repeated experimental inoculation. But the resistance of monkeys to the disease does not seem to be increased by recovery from experimental infection, not even after repeated infection.

Bietti\textsuperscript{38} employed Hirst's haemagglutination test for trachoma virus. He obtained a low titre of agglutination with chicken red blood cells; but the control egg fluid also gave positive results. Thus the experiment provided no significant information.

In order to demonstrate a relationship between the agent of trachoma and the Rickettsiae some investigators such as Pol-aff and Nain\textsuperscript{155} and Postic\textsuperscript{156} sought support in the Weil-Felix reaction on the sera from trachoma patients and they reported positive results with such tests. Julianelle and Smith\textsuperscript{157} brought forward evidence to indicate that the results of Weil-Felix reaction were nonspecific, because positive results were obtained with both trachomatous and non-trachomatous sera. Bietti\textsuperscript{158} investigated further on the behaviour of the Weil-Felix reaction in the blood sera of trachomatous subjects and was unable to obtain any significant result; the results were negative, the percentage of agglutination at low titre being the same
as those encountered in normal subjects.

To find out whether there is any generalized alternative reaction in trachoma some investigators performed Paul Bunnel test on trachomatous sera and Majoros\textsuperscript{59} reported positive results. Bletti\textsuperscript{160} tested 200 trachomatous sera and only 3 sera gave slight agglutination at a titre of 1 in 128. A positive result in this dilution could be considered as significant if it were a frequent instead of a rare occurrence. Sztirlich\textsuperscript{161} also reported that the percentage of cases giving positive Paul Bunnel reaction was higher in persons free from trachoma than in persons suffering from trachoma or in those with cured trachoma.

5. LABORATORY DIAGNOSIS

The diagnostic clinical features of advanced trachoma, i.e., follicular hypertrophy, cicatrization, and pannus, are so characteristic that laboratory aids are not ordinarily required. However, the diagnosis of trachoma at onset and prior to the development of cicatrization and pannus may cause confusion. Pure trachoma with acute onset is often confused with the seasonal epidemic conjunctivitis of bacterial origin. In countries with a high trachoma index cases of nontrachomatous follicular conjunctivitis are sometimes diagnosed as early trachoma, and in countries with a low trachoma index the trachomatous nature of early or atypical cases may not be recognized until late changes occur.
The laboratory diagnosis of trachoma is based mainly on the demonstration of the Halberstaedter-Prowazek type of inclusion bodies. Microscopic examination of conjunctival scrapings or follicular expressions not only reveals the nature of the conjunctival response but also affords an excellent and rapid means of distinguishing the infecting agent. All recent investigators are in general agreement that the inclusion bodies are the most striking feature of trachoma. According to Wilson, they are the first recognizable sign of trachomatous infection and can be demonstrated in the incubation period before the development of any clinical signs.

There are a number of virus diseases in which the inclusion bodies have diagnostic value only when they are found, their absence in no way ruling out the disease in question. Inclusion bodies are readily demonstrable in early cases of trachoma with acute or insidious onset, in exacerbated cases or in cases of chronically high activity. They are known to vary in number and in the ease with which they can be demonstrated, according to the severity of the disease and according to the amount of conjunctival exudate. There are many cases of trachoma particularly in stage I, which are symptomless and have minimal exudate and in which the disease is unsuspected by the patient, by the members of the family, by the child's teacher, or even by his general physician. It is this type of case and those cases on
the verge of spontaneous healing in which morphological demonstration of the virus may be extremely difficult. Even in these cases inclusion bodies can usually be found after repeated careful sampling or by means of provocative tests such as application of steroids, cauterizing agents or mechanical manipulations. The provocative tests, however, do not always yield consistent results.

There are some virus diseases affecting the eye, such as herpes simplex, herpes zoster, molluscum contagiosum and vaccinia in which inclusion bodies are developed in the conjunctival cells. For their demonstration, however, special stains other than Giemsa's stain have to be employed. In human conjunctivitis caused by New Castle disease virus (NDV) of fowl, basophilic cytoplasmic inclusion bodies evident by Giemsa's method of staining have been observed by Theodore. Although the viruses of psittacosis and lymphogranuloma venereum have basophilic cytoplasmic inclusion bodies of the same general type, no confusion with either of these diseases seems possible. Psittacosis never involves the eye, and in the rare cases of primary lymphogranuloma venereum keratoconjunctivitis, inclusions have not been found in the conjunctival epithelium. The inclusions in lymphogranuloma venereum are found most commonly in the monocytes. Moreover, trachoma inclusion can be differentiated by Rice's method of staining.

The smears for detection of inclusion bodies should
preferably be made from superficial epithelial scrapings rather than from follicular expressions and the ideal procedure for their identification would be a preliminary staining by Rice's method to demonstrate the carbohydrate matrix, followed by restaining with Giemsa to distinguish the elementary body components. In Giemsa-stained preparations, typical elementary body inclusions containing individual elementary bodies of uniform size and tinctorial hue can not be confused with any of the pseudo-inclusions. The differentiation from pseudo-inclusions is rather difficult when the inclusions are densely packed; but if individual elementary or initial bodies are present at the inclusion borders, a reasonably reliable identification is assured. It is also very difficult to recognize the small initial body inclusions which so closely resemble the engulfed bacteria and the large pigment granules.

The literature is replete with reports of studies in which non-specific bodies have been mistaken for trachoma inclusions by untrained observers. These pseudo-inclusions have been considered in an article by Braley. The most commonly seen artefacts simulating inclusions are nuclear extrusions. To the trained observers, these are readily differentiable, however, because of their obvious relationship to the nucleus so far as position, tinctorial properties, and
composition are concerned. The pigment granules so common in the dark races, are usually large and may be confused with the initial bodies; however, when stained with Giemsa, they tend to have a slate gray or greenish cast, quite unlike the pure blue of the initial bodies. Pigment granules can also be seen in an unstained slide. The phagocytosed nuclear debris may sometimes resemble trachoma inclusions when viewed under low-power magnification, but examination with the oil immersion lens quickly reveals their pure nature. Of the various granules seen in smear preparations, the granules of mast cells have sometimes caused confusion, particularly so when stained with the Poleff's stain. Stain debris in improperly washed slides should cause no difficulty for the trained observer.

The Giemsa stain has been generally accepted as the most reliable stain for the recognition of the epithelial cell inclusions of trachoma. Various stains other than Giemsa, such as Wright's, Victoria blue, the Poleff stain, the Lindner contrast stain and the Macchiavello stain have been tried by Thygeson without finding a satisfactory substitute for Giemsa. Wright's stain produces a picture similar to that produced by the Giemsa stain but causes more difficulty with stain debris. The May-Grunwald-Giemsa technique advocated by Nataf has the advantage that it reduces the staining time. The stain has been employed by British workers. The Poleff
stain has been evaluated by Sie-Boen-Lian who showed that its use yielded erroneous and inconsistent results. Thygeson also affirmed that the Poleff stain is unreliable and should be abandoned. The iodine stain (Rice's method) for the carbohydrate matrix is useful for survey work but it has the disadvantage that only the relatively mature inclusions are detectable whereas the initial body inclusions may be missed. However, it has the advantage that the iodine can be easily washed out and the preparation can be re-stained with Giemsa.

The finding of inclusion bodies limits the diagnosis to trachoma or inclusion blennorrhoea. The different tissue affinities and localizations of the two agents are highly satisfactory for differential diagnosis. The inclusion bodies of trachoma are always more numerous in scrapings from the upper fornix or upper tarsus and the reverse is true of the inclusion bodies of inclusion blennorrhoea. However, such differentiation is possible only when inclusions are reasonably abundant. In inclusion blennorrhoea there are myriads of free elementary and initial bodies in the early acute stage, whereas in trachoma the free bodies are comparatively fewer in number.

Since the inclusion bodies of trachoma and inclusion blennorrhoea are morphologically identical and microscopically indistinguishable from each other, differentiation between the two diseases requires
consideration of other cytological findings. The epithelial cell changes alone have been regarded as diagnostic by Taborisky. He noted a flattening of the normally cylindrical cells of the conjunctiva and nuclear degenerations characterised by poor staining properties and irregularities in the chromatin network. Feldman also stressed on the epithelial cell keratinization and multinucleated epithelial cells. Thygeson, Sezer, and Kimura and Thygeson noted most of these changes but did not consider them specific.

Thygeson holds that the most characteristic changes are those in expressed follicular material in which the signs of necrosis differentiate the trachoma follicle from all non-trachomatous follicles. These necrotic changes include cytoplasmic debris, cells with bare nuclei and macrophages engulfing degenerated cellular material. The plasma cell when seen in exudate smears or epithelial scrapings has also been considered as diagnostic of trachoma. These cells seem to be incapable of passing an intact epithelial barrier and are therefore seen only rarely in epithelial scrapings or exudate smears from non-trachomatous follicular disease. In trachoma, on the other hand, they are seen more frequently, especially in epithelial scrapings over the degenerating follicles. The plasma cell has been noted in approximately 40% of scrapings from cases established clinically as trachoma and in only 0.5% of scrapings from non-trachomatous cases.
The Leber cells are found in the expressed follicular material and are indicative of a necrotizing conjunctivitis; they are highly suggestive of trachoma but not specific. The neutrophilic leucocytes are the prominent exudate cells in experimental trachoma and in acute trachoma not due to secondary bacterial infection. They are valuable in differentiating early trachoma from epidemic keratoconjunctivitis, primary herpetic keratoconjunctivitis, molluscum contagiosum conjunctivitis, and other conjunctivitides due to typical viruses in which mononuclear cell exudate is the rule. Their presence is, however, valueless in differentiating trachoma from inclusion blennorrhoea.

Thygeson has put the cytologic findings in scrapings in order of their importance as: 1) the inclusion, 2) Leber cells in numbers, 3) germinal centre follicle cells with degenerative changes, 4) plasma cells, and 5) multinucleated epithelial cells. In the follicular expressions the significant findings in order of importance are: 1) Leber cells in numbers, 2) degenerated germinal centre cells, 3) cytoplasmic debris, and 4) greater numbers of lymphoblasts than of other mononuclear cells. He claimed that he was able to make a diagnosis of trachoma solely on the basis of these cellular changes. The cytologic diagnosis has proved of great value in the recognition of early and atypical cases of trachoma.
Diagnosis of trachoma by inoculation of monkeys or apes is neither practicable nor reliable. The difficulty in differentiating experimental trachoma in monkeys and apes from infection with inclusion blennorrhoea and the folliculosis of spontaneous occurrence has been recognized by all. By way of contrast, the inoculation of baboons was found useful by Thygeson and Stone in the detection of inclusion blennorrhoea virus. The factors limiting the use of these animals are: 1) the lack of uniform susceptibility, 2) the occurrence of spontaneous folliculosis, 3) the failure of the experimental disease to produce pannus or scarring, so characteristic of human trachoma, 4) the lack of a satisfactory method of diagnosis of the experimental disease, 5) the long period of observation and 6) the exorbitant cost. Trachoma can be differentiated from inclusion blennorrhoea by inoculation of apes in which the latter induces a more severe disease than does the former.

In Japan, many investigators have employed the electron microscope in the study of trachoma, even for detection of the virus. While electron microscopy has great theoretical interest in trachoma, it has as yet no practical diagnostic value.

It is not possible at the present time to distinguish between the individual members of the psittacosis-lymphogranuloma venereum group of viruses by means of simple in vitro serological tests. Serological cross-
reactions between these viruses have been widely recognized and various attempts have been made to obtain sharply specific results. The presence of distinct heat stable (group-specific) and heat labile (species-specific) antigenic components was first demonstrated in psittacosis virus by Bedson\textsuperscript{166}. It is possible by absorbing the serum with the group antigen and using the fresh unheated virus as antigen in the complement fixation test to make the test virus-specific (Bedson et al.\textsuperscript{86}), but the technical difficulties make the procedure unsuited to routine use. The low antibody content of the trachomatous sera would be another limiting factor.

Recently Ross and Gogolak\textsuperscript{167} devised a method of preparing a species-specific antigen by treatment of the sonically disrupted virus with either potassium periodate or cobra venom (lecithinase A) and the use of such antigen has been recommended by K.F. Meyer\textsuperscript{168}. But the extreme lability to heat and chemical reagents and the unpredictable reactivity of the species-specific antigen might limit its use in diagnostic tests.

The complement fixation technique is the most commonly adopted procedure for the diagnosis of these diseases, and fairly satisfactory results are obtained by using the phenolized and boiled group antigen derived from the homologous virus. But such procedure would not possibly serve the purpose of differentiating trachoma from inclusion blennorrhoa. The virus of trachoma being
now available in quantity, different laboratories are presently engaged in the preparation of a specific antigen suitable for serological diagnosis of trachoma.

Of late, the technique of isolating trachoma virus in eggs has undergone considerable improvement and Sowa and Collier succeeded in making 17 isolations from 18 inclusion-positive cases and 7 isolations from 12 inclusion-negative cases. Collier reported that in Marakissa (West African village), inclusions were found in 37.5% of cases of active trachoma in stages I and II, whereas virus was isolated from 57.5% of cases. It appears that diagnosis by virus isolation might yield better result than by inclusion body demonstration.

In their preliminary observations, Collier et al. noted significant results in skin tests with trachoma antigen. If these results are confirmed, a trachoma skin test may be used in the diagnosis of clinically doubtful cases.

6. EPIDEMIOLOGY

Few diseases show such wide variations in epidemiological patterns as does trachoma. There are marked differences between regions in incidence and distribution, in the usual age at onset and in the general severity of the diseases. Incidence and severity of trachoma probably depend more on environmental pre-
disposing factors than on either host susceptibility or pathogenicity of the agent. In endemic areas the disease is commonly acquired during the first year of life from the mother. Available evidence indicates that acute cases are highly infectious and chronic cases only slightly so. In nonendemic areas it is not always possible to trace the source of infection for the sporadic cases. However, the possibility of subclinical infection flaring up can not be ruled out. There is, indeed, no definite evidence for the existence of carrier state in trachoma.

Incidence and Distribution: Estimates of the prevalence of trachoma in the world vary. In 1936, MacCallan^ remarked "It would not be too much to say that the stigmata of the disease active or cured are borne by half the inhabitants of the globe". Moutinho^ estimated in 1951 that 15 to 20% of the world population suffered from trachoma. Current estimate is more than 400 millions.

No authoritative estimate of the overall incidence in India is available; however, Taylor et al. T.N. Ursekar, S.P. Gupta, and Singh and Grover have reported on the extreme prevalence of the disease in India. Taylor et al. found that more than 90% of school children in a Punjab village were affected by trachoma and the overall prevalence rate was as high as 73%. Ursekar made a statistical analysis of the incidence of trachoma in India and Bombay State on the basis of the data supplied
by the Directors of Health Services of the different States. He observed that the incidence gets less as one travels from north towards south in the whole country as well as in the State of Bombay; the highest incidence is in Rajasthan which may be considered the nucleus of trachoma infection from which infection seems to spread mostly towards Punjab and Kashmir along the prevailing winds in June and the Aravali ranges in Rajasthan form the demarcating line between areas of very highly and comparatively low incidence. The high incidence in Punjab has also been reported by Tulsidas et al. who opined that trachoma forms 60% of the preventible blindness in Punjab. They also hypothesised that the lower incidence of trachoma in the southern India is due to lesser penetration of Mohammadans who are supposed to have imported the disease from the highly endemic Middle East. Sample surveys carried out in several northern States have shown that 80-90% of the population have trachoma.

Sidky and Freyche have published a map showing the geographic distribution of trachoma. The disease is enormously widespread, with extreme prevalence in North Africa, Middle East and the entire Orient. It is endemic in North and Central Africa, Middle East, Central and Eastern Asia including Persia, India, China, Japan, Indonesia and Pacific islands, Eastern and Central
Europe, Central and large areas of South America. It is rare in Britain, Norway, Sweden, Denmark, Iceland, Switzerland and New Zealand. The Mediterranean countries of Europe, the Balkan countries and Russia are endemic areas and the disease remains as yet unabated. The North European countries have become almost free of trachoma since the introduction of chemotherapy. In U.S.A. there are some endemic foci, but during the past 20 years the disease has been remarkably on the decline. A recrudescence amongst the tribal population of South West region has recently been reported.

Trachoma occurs in all zones including the tropical, sub-tropical, temperate and cold zones. Both hot Egypt and cold Poland are equally affected. Obviously temperature has little effect on the incidence. Altitude as well seems to have no influence; the disease is equally prevalent at sea level and at high altitudes.

Although no country is free from trachoma, its distribution seems to be irregular. In heavily infected areas such as the Middle East, the disease affects the entire population, but in certain European countries where the disease is widespread, only a particular section of the population is affected. In countries such as Canada, the U.S.A. and Australia only certain localities are involved. It appears that trachoma has an affinity for some population groups, being common amongst the Poles, Arabs, Mongolians, Jews, Australian aborigines and
North American Indians. According to MacCallan trachoma is most common among the Mongol and Semitic races of the East, the Red Indians of the New World and the Asians. **Predisposing factors:** The "cofactors" recognized to be predisposing to the trachomatous infection have been divided into the following groups,

1) Individual factors: age, sex, race, constitution, previous or concomitant infection, nutrition, state of immunity and pre-existing condition of the eye.

2) Ecologic factors: climate and meteorologic conditions, hygienic habits, customs and religious practices, social conditions, professions followed, incidence of animate and inanimate vectors.

3) Properties of the causal agent.

In areas of extreme prevalence trachoma usually attacks children, and at the age of one almost all children contract the disease; whereas in areas with a rather low prevalence, it usually spares the children under three years of age. From the survey made in a Punjab village it was shown that almost half of children under one year of age, about 90% during childhood and 60-70% of adult population are afflicted with trachoma. Clinical and epidemiological studies indicate that trachoma contracted in infancy tends to be milder than trachoma contracted in adulthood, with respect to both types of onset and clinical course. It is also quite
often self-limited when contracted in infancy but rarely if ever so when contracted in adult life.

Opinions differ on the influence of sex. Bietti observed that in Sardinia there is a slightly more marked tendency for females to contract trachoma than males. Siniscal, on the other hand, reported on the higher incidence among males in Missouri. However, it is an established fact that in an endemic area the infected mothers are the main sources of infection to their children. Taylor et al. reported that women during childbearing age had a higher infection rate than men.

There is a difference in racial susceptibility. Howard observed that the Negroes in Missouri are less susceptible than the Whites. Scott reported on the mild nature of the disease among the West African Negroes. Guerra brilliantly documented the relation of trachoma to race, not only by statistics by also by experiments and observed that trachoma arose more readily among members of the Semitic race than among members of the Nilotic-Gamitic race. Although some authors are inclined to explain this by pigmentation, Bietti maintains that the difference in susceptibility according to race must be referable to elements deeper in the racial background; since its prevalence varies widely among groups equally highly pigmented, the Bantus (Murray) and the Australian aborigines (I. Mann), for example, are heavily pigmented but trachomatous.
Malnutrition is usually associated with susceptibility to infectious diseases. But in certain virus diseases there is a reversal of malnutrition effect and this is probably true in case of trachoma. Wilson’s\textsuperscript{178} observation in this connection is very interesting. In an Egyptian village where he was studying the development of trachoma in infants, the only children to escape the disease by the end of their first year were two marasmic infants. Nataf also observes that the inoculation of trachomatous material failed to produce the disease in cachectic human volunteers, when in the same series of experiments the inoculation of the same material yielded positive results in healthy volunteers. Heter and James\textsuperscript{179} observed that the incubation period of experimental trachoma in malnourished monkeys is longer than in those on a normal diet and the course of the disease is shorter than in normal animals. They also noted that the spread of the infection to the un-inoculated eye, a common feature in experimental trachoma in monkeys on a normal diet, was never seen in monkeys on deficient diet. They concluded that the animals on deficient diet are less reactive, if not more resistant, than those maintained on an adequate diet. There is thus more than a suggestion that malnutrition actually decreases susceptibility to trachoma.

The incidence of trachoma is certainly highest in areas of low economic development and lowest in areas of high economic development. The early workers such
as Stuckey, Royer, and Gibson who associated malnutrition with trachoma, probably did so on the basis of the well known association between poverty and the disease.

Diet seems to play no important role in trachoma. According to Rice et al., there is no evidence that a balanced diet supplemented with cod-liver oil affected the course of trachoma in the untreated eyes of eighteen patients kept under their observations for varying periods of time. Siniscal's opinion that inadequate diet and avitaminosis are contributory factors must be regarded as unproved. Glikson also believes that general nutrition is a decisive factor in an individual's immunity to this disease and that proper diet, including fresh milk, and fresh, green vegetables, will shorten the duration of the disease or actually heal it. Murray reports that in South Africa, adequate feeding supplemented by vitamin concentrates generally brings about a remarkable improvement within a few weeks, even if no specific medication is used. According to Bietti, if there is any importance in nutritional factors, dietetic imbalance is more likely to play a role than dietetic deficiency.

Vitamin deficiency does not seem to predispose to trachomatous infection. The vitamin A deficiency, on the contrary, creates condition unfavourable for the infection; the trachoma virus seems to be unable
to proliferate in the keratinized epithelium. Monkeys fed on diet deficient in vitamin A could not be infected with trachomatous material by Tilden and Miller.

The existence of an acute bacterial conjunctivitis has been regarded as capable of preparing the way for trachoma. But even in areas with widespread epidemic of seasonal conjunctivitis it has been found that trachoma frequently appeared without any previous bacterial infection and that acute conjunctivitis was followed by trachoma in only a modest percentage of cases. In view of the extreme frequency and early attack of both trachoma and seasonal conjunctivitis in certain countries (North Africa for example), it is difficult to prove how close a link exists between the two in their incipience. However, there is no doubt that bacterial conjunctivitis aggravates the course of trachoma, facilitates its revival and by conveying the virus causes it to spread. Toxin of Koch-Weeks bacillus has a real provocative power and can endanger the return of inclusion in cases apparently healed altogether. W.H.O. Expert Committee on Trachoma have described epidemic of bacterial conjunctivitis, the seasonal conjunctivitis in particular as the bed on which trachoma is born. In fact, any form of exudative condition of the eye greatly favours the trachomatous infection. Akagi et al. compared the bacterial flora in trachomatous and normal conjunctiva and found 14 different kinds of bacteria, the most frequent was staphylococcus and
corynebacterium xerosis next. Most of the bacteria were nonpathogenic and had little influence for the manifestation of trachoma. There was no evidence that the incidence of contamination in trachomatous eye was greater than in others. The kind of microorganism showed no specificity and there was no tendency of infection by any particular bacteria in trachomatous eyes. Thus it was shown that in Japan, bacterial conjunctivitis did not influence the trachoma symptoms as has been reported in Egypt and Tunis. Pannarale and Huet have also shown that activation of trachoma can be more often induced by a simple inflammatory state of bacterial origin independent of the type of microorganism which has provoked it. Collier reported that in Gambia, organisms of the Haemophilus group occur more frequently in the lower age group, in whom early trachoma is common, but it is not yet clear whether the correlation is with age or with the stage of trachoma.

In 1911, Lindner found that simultaneous infection with trachoma and inclusion blennorrhoea was possible experimentally. Recently Murray et al. isolated 13 viruses from 200 cases of conjunctivitis presumed to be trachoma. 12 of them were adenoviruses of various types and 1 was a Coxsackie virus, group B-1. But Thygeson was unable to isolate any such viruses from trachoma material. Grayston et al. could isolate only one adenovirus strain from 468 trachoma patients and they
rejected the idea that these viruses play any significant role in the pathogenesis of trachoma. However, to date there is no precise information about the role played by such viruses in predisposing to trachoma infection.

Trachoma is regarded as a social disease, overwhelmingly associated with unhygienic condition of living. In those countries where people live under conditions of poverty, dirt, overcrowding and deficient water-supply, the disease is almost universal.

The sun, dust and wind have irritating effect on the conjunctiva and thereby predispose to the trachomatous infection.

In Arabian countries, the people have the peculiar habit of allowing flies to settle on the eye lids and this may be the cause of such high prevalence of the disease there.

**Infectivity and communicability:** During the years following the Egyptian conquest (1798-1802) when trachoma spread widely among Europeans, authorities of the European continent, the French Government in particular, were alarmed and sought the opinion of the French surgeons about the nature of the dreadful eye disease. Although they were doubtful about the contagious nature of the disease, Power and Vetch in England firmly held that trachoma is contagious from man to man and is caused by a putrid virus (poison) of animal origin. Later on, to prove the infectious
nature of trachoma, many early workers attempted experimental reproduction of the disease by inoculation of trachomatous material into human volunteers, apes and monkeys. As early as 1823, Wernicke is reported to have inoculated trachomatous material into the eye of human volunteer\textsuperscript{26}. The first successful experiment in human volunteer was performed by Addario who thus produced the scientific proof for the infectivity of trachoma. Similar experiment on baboons was performed by Hess and Romer.

Instances of natural transmission of trachoma to ophthalmic surgeons from their patients have been reported by Cuenod and Nataf\textsuperscript{191}. Zentmayer\textsuperscript{192} described the spread of infection among wrestlers in America. The ophthalmic surgeon Roschin\textsuperscript{193} made an interesting observation that mucosa of lip grafted on the operated trachomatous lesion became subsequently invaded by trachomatous follicles.

The communicability of trachoma is still an open question, in some quarters it is believed to be minimal. MacCallan\textsuperscript{194}, however, strongly believed that trachoma is a readily communicable disease. Bisti\textsuperscript{38} though not differing with MacCallan's view, maintained that trachoma certainly does not develop automatically when infectious material reaches the eye. Since the successful cultivation of the virus, there are many reports of accidental laboratory infections.

Very little is known about the actual mechanism of transmission. The usual mode of the spread of trachoma
is by way of transference of infected conjunctival secretion through fingers or towels. Even though MacCallan viewed fly as a possible vector conveying the trachoma virus, there is no such direct evidence for the flyborne transmission of the disease. Nataf believed that the louse could be either a vector or reservoir for the trachoma virus. No animal in the nature is known to be suffering from trachoma and this fact makes it unlikely that there is any animal reservoir for trachoma. Trachoma is highly infectious when the discharge is profuse and inclusion bodies are abundant. A child contracts the infection most commonly from his infected mother. In the community life common washbasin seems to be a source of infection.

7. TREATMENT AND CONTROL

Prior to the introduction of sulphonamides there was no specific therapy for trachoma and a combination of medical and surgical means was in use. Medical treatment consisted of antiseptics and caustics. Correction of cicatricial deformities and tarsectomy were the surgical measures. Such treatments were undoubtedly inadequate.

With sulphonamides it is now possible to cure trachoma in the early stage. It is less satisfactory in the cicatricial stage. Used orally, sulphonamides are more efficacious than administered topically.

The broad spectrum antibiotics have been found to be very effective, particularly when topically administered.
There is little to choose between them; all are efficacious. Systematic clinical trials have led to many improvements, in trachoma therapy but even with the most potent drugs now in use the minimal effective course of treatment is long and this is the real handicap in mass treatment campaigns.

Treatment of acute ophthalmia by chemotherapy constitutes the chief measure of controlling trachoma in endemic countries where the two diseases are closely associated. All measures leading to improvement in economic condition of the population exert a salutary prophylactic effect. Mass treatment campaigns instituted as control measures do not materially influence the prevalence of trachoma because of the long treatment time required, with consequent lack of cooperation from the patient. Furthermore, re-infection frequently occurs in highly endemic areas.

Apart from these general measures of control, immunoprophylaxis against the disease is under trial. Grayston et al.\textsuperscript{195} have used different kinds of vaccines made from purified elementary bodies; the live vaccine was no more effective than the vaccine inactivated by formalin. The aluminium particle vaccine showed relatively better results. Snyder et al.\textsuperscript{196} reported that the person vaccinated with Saudi Arabian strain had less severe clinical course than the person vaccinated with placebo. It appears that much
ground remains to be covered before the possibilities of immunization against trachoma are fully known.