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Etiology of inclusion blennorrhea

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ETIOLOGY

OF

INCLUSION BLENNORRHEA

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INTRODUCTION

It is the author's purpose in this paper to present a short general discussion of the disease, inclusion blennorrhea, of the new-born, and a more detailed study of etiologic factors associated with the disease. No reference will be made concerning the treatment of the disease in this paper, since introduction of the sulfonamide group of drugs appears, at present, almost, if not entirely, specific for the disease. (Personal communication with Dr. J.L. Gedgoud, Dr. Harold Gifford, and Dr. W.H. Morrison, all members of the Medical Staff at The University of Nebraska, College of Medicine, Omaha, Nebraska)

Since there are three inclusion diseases of the conjunctiva that are often confusing, definitions of these will follow.

Inclusion Blennorrhea is a benign form of conjunctivitis in the new-born infant, not associated with any pathogenic bacteria, but most likely due to a filterable virus. The disease makes it appearance five to ten days after birth. The source of the infection is undoubtedly the mother's genital tract. The early stage of the disease is usually acute, be-

coming chronic and running such a course for several months, and healing without panus or scars. It is transmitted to monkeys, baboons, and man, both adult and new-born. (28)

Inclusion blennorrhea is widely disseminated, as reports from many countries indicate.

The disease is characterized by onset between the fifth and tenth day after birth, The onset of symptoms is usually between the seventh and tenth days of life. At first there may be only a reddening of the conjunctiva with the appearance of a sm small amount of purulent secretion at the inner canthus of theeye. It may be unilateral at the onset, but usually spreads to involve the other eye. Many cases remain relatively mild, the inflammation involving the conjunctiva of the lower tarsal plate. In more severe cases the condition takes on the appearance of an acute ophthalmia, with swelling and redness of the eyelids, intense, beefy redness of the palpebral conjunctiva, and a profuse purulent discharge. The palpebral fissures are tightly closed. There is occasionally noted atendency to the formation on the conjunctiva of a pseudomembrane. Clinic-

ally, it may be impossible to differentiate this from a gonorrheal ophthalmia. True follicles are not seen, but a papillary conjunctivitis is common. Corneal ulceration does not occur in inclusion blennorrhea, nor are there anyvascular changes at th e limbus such as found in trachoma. No scar or panus formation has ever developed from inclusion blennorrhea. Systemic reactions are unknown. (28, 59)

Diagnosis can only be definitely established by the discovery of typical inclusion bodies in the Giemsa-stained preparations of the conjunctival secretion or,better still, the scrapings. These inclusion bodies will be discussed more in detail under the title, The nature of the inclusion bodies".

Little has been done in the study of the pathology of inclusion blennorrhea, but the work of Lumbroso (1933) and Thygeson (1934) will be mentioned here. Lumbroso (43) has presented evidence to show that follicles may form in very long standing cases. He also noted the presence of a mild, diffuse, subconjunctival fibrous-tissue development. "A biopsy from the upper fornix was made in a single case on the forty-third day of the disease. Sections revealed a diseased subepithelial infiltration

with inflammatory cells, plasma cells being the more numerous. No follicle formation was present. The epithelium was infiltrated with polymorphonuclear cells, and in the superficial layers an occasional cytoplasmic inclusion body was seen". (59) Follicle formation is characteristic only in the adult type of the disease. (59)

Inclusion Conjunctivitis in the Adult, also referred to as swimming-bath conjunctivitis, inclusion blennorrhea of the adult,"genital trachoma", is th e adult type of inclusion blennorrhea. It is characterized by diffuse inflammation of the conjunctiva with the formation of numerous follicles in the folds. Secretion is less profuse than in the infants, while the follicles are larger. It is more often unilateral than in infants. The follicular hypertrophy and inflammation persists for a period of one to four months, though the initial swelling and secretion often subside after two or three weeks. Little or no visible scarring remains after healing has occurred and the cornea is never involved. (59)

Trachoma, which is also known as chronic granular conjunctivitis or granular eyelids, is a contag-

ious granular conjunctivitis, caused by a filterable virus. It is characterized by the formation of small elevations on the conjunctiva of the lids and by atrophy, cicatricial contraction and deformity of the lids. (59, 60)

HISTORICAL SURVEY

A "benign" form conjunctivitis in the new-born infant, unassociated with pathogenic bacteria, was first recognized by Morax (50) in 1903. Halberstaedter and von Prowazek (23) discovered inclusion bodies in trachoma in 1907. Stargardt(1908) noted the presence of epithelial inclusions in conjunctivitis of the new-born(57). The findings of Heymanns in 1909 (26) of cytoplasmic inclusion bodies, similar to those in trachoma, in the conjunctival scrapings from those affected with the disease, led to the name, inclusion blennorrhea(38).

In spite of the extensive reasearches of Lindner, Botteri (6), Heymanns (26), Lohlein (41), Axenfeld (2), Morax, Lindner, and Bollack and others (51), the etiology of the disease was not established until the more recent work of Thygeson (59-63), McKee(46), Howard (28) , and others.

The inclusion bodies have been variously considered as: 1. Intracellular masses of the causal organism (38), 2. Non-specific reaction products (17), 3. Phagocytosed bacteria (58), 4. Intracellular changes resulting from the activity of a filterable virus (43).

No importance was attached to the inclusion bodies until in 1909 when Heymann (26) reported at the Congress of Medicine at Budapest the results of his researches which he had made at the request of Uhthoff. He had attempted to determine the presence or absence of inclusions in a series of preparations from trachoma and other types of conjunctivitis, with out any knowledge of their origin. The result was that inclusions were found, not only in trachoma but also in four cases of gonococcal infection of the new-born.

The findings of Heymann (26), joined with those of Leber and von Prowazek (33), who found inclusions in a conjunctivitis peculiar to the Samoan Islands, with those of Uhlenhuth and his cowworkers (65), who found inclusions in the conjunctivae of pigs ill with hog cholera, and with those of Paschelf (52) and of Flemming (13) who found inclusions in a form of conjunctivitis of the adult, tended to shake the role of inclusion bodies in trachoma.

In 1909, Lindner (38), undertook the study of conjunctivitis of the new-born and showed that inclusions were found with few exception only in that form of conjunctivitis (conjunctivite amicrobienne) which Morax (1903) had already differentiated from

gonococcic conjunctivitis and the other types of conjunctivitis of bacterial origin. In a series of one hundred twenty cases, the co-existance of inclusion bodies with gonococci was found only four times. Lindner also successfull inoculated the conjunctiva of the baboon, and noted the presence of epithelial inclusions, and thus named the disease "Enschluss Blennorrhoe", or Inclusion Blennorrhea.

In 1911, Morax, working with Lindner and Bollack (51) confirmed the existenct of inclusion blennorrhea, just referred to, and its transmissability to the monkey.

In searching for the origin of the disease of the new-born, Halberstaedter and Prowazek (23) found the typical inclusions in the scraping from the urethral apertubes of two mothers of diseased infants. On the basis of these findings, they formulated the theory of an inclusion diease of the male and female genito-urinary tracts. Lindner (38) soon supported this theory on finding inclusion, in small numbers, in several cases of non-specific urethritis, and from Heymann, who found inclusions together with gonococcuc in both parents of a child having inclusion blennorrhea.(59-62)

Later, Fritsch, Hofstaeter and Lindner (1910)

(15) inoculated the eye of a baboon with the urethral exudate from a case of non-gonococcal urethritis of fourteen days duration. The urethral exudate was scanty but contained typical inclusion bodies. Three days after inoculation, the conjunctiva of the baboon became hyperemic and on the fourth day secretion developed. After the eleventh day there was a follicular conjunctivitis which lasted for several months. Inclusions were numerous. Wolfrum is said to have concluded that the disease was identical with trachoma and Lindner also described it as "genital" trachoma (59-62).

In 1910, Wolfrum, as reported by Thygeson in 1934 (62), described two inoculating experiments on the adult with the secretions from cases of known inclusion blennorrhea. In the first subject, an incubation period of one week was followed by conjunctival inflammation, and onthe ninth day numerous inclusions were found. No detailed description was given but he considered it to be true trachoma. In the second case, conjunctival hyperemia developed on the fifth day, and on the eighth secretion and beginning follicle formation. After the twelfth day, epithelial scrapings showed some inclusions. The case was more benign than the first case.

Lohlein (41) noted that while inclusion blenorrhea was easily transferable to the monkey, trachoma was transmissable only with difficulty. He, also, stated that he believed there existed an inclusion conjunctivitis of the adult entirely analogous to inclusion conjunctivitis (blennorrhea) of the new-born, distinct from trachoma, but corresponding in part to the cases often described as doubtful of benign trachoma.

Gebb, in 1914 (16), in repeating Wulfrum's experiments, demonstrated that the adult disease so produced could not be confused with trachoma in any way. Eight subjects, so inoculated, developed an active but self-limited disease which healed without panus or scars in less than a year.

The filterability of the disease agent was claimed by Gebb (16). He had inoculated a human subject with a Berkfeld filtrate of a suspension of virulent material in physiologic salt solution kept for one hour at room temperature. Adisease d developed on the fourth day similar to that produced by the unfiltered material. These results were confirmed by the results obtained by Botteri (6) who inoculated the eye of a baboon with a Berkfeld filtrate of a suspension of conjunctival scrapings

from an affected infant.

In the decade or so following onset of the First World War, inclusion blennorrhea received very little attention until about 1930, when James (29) reported four cases in 2446 deliveries at the Saint Louis Maternity Hospital, with the onset occurring on the fifth, eighth, eleventh, and thirteenth days after birth. Direct smears and scrapings were negative for bacteria, and cultures revealed only C. xerosis and Staphylococcus albus. On the twelfth day after inoculation, a rhesus monkey developed follicles which persisited over a period of seven months. No inclusion were demonstrable in the scrapings. He, also, mentioned the accidental infection of one of the mothers from her child on the twenty-eighth day peet-partum.

Stewart(1933) venture his conclusion in a reprot that inclusions blennorrhea had no existence as a separate disease, but was gonococcal ophthalmia in which the inclusions were nests of phagocytosed gonococci (58). But since analysis of the report discloses that Stewart had never personally seen a case of inclusion blennorrhea, his conclusions must be discarded as of no significance (59).

The monograph of Lumbroso (44), based on a study of seventeen cases, concludes that inclusion blennorrhea, which he prefers to call granular conjunctivitis (blennorrhee granuleuse), is a specific disease of the conjunctiva and almost always distinguishable clinically from gonoblennorrhea. In extensive studies he was able to eliminate the conjunctival bacteria as possible etiologic agents, but concludes that bacterial infection may complicate the disease. The inclusions, he believes, are reaction products of the epithelial cells to a filterable v virus. He was unable to determine the exact etiology.

THE ETIOLOGY OF INCLUSION BLENNORRHEA

As early as 1903, Morax recognized that this benign form of conjunctivitis in the new-born was unassociated with any pathogenic bacteria. In addition he thought the conjunctival inflammation, like snuffles, might be a manifestation of hereditary siphilis. (50)

Following the finding of epithelial inclusions, similar to those found in trachoma, in smears of conjunctival secretions in cases of the benign purulent conjunctivitis, inclusion blennorrhea), (26). theories concerning the role of these inclusion bodies in the disease were soon tobe formulated. Some of these are as follows: 1. Lindner considered the inclusion bodies to be intracellular groups of the causal organisms (38). 2. Stewart concluded that the inclusions merely clumps of phagocytosed gonococci.(58). 3. Lumbroso considered them to be reaction products of the epithelial cells to a filterable virus (43). 4. McKee's work led him to the belief that the inclusions were formed by phagocytosis of bacteria whichare not the cause of the disease, but which carry the virus (46). 5. Thygeson more explicitly concludes that they are groups of the virus which

are the cause of the disease.(59*62) That is,by a series of experimental studies, he has been led conclude that the cytoplasmic inclusion bodies constitute intracellular colonies of a specific filterable virus in the various stages of development. 6. Lohlein, Heymanym and Lumbroso (1913) believed the inclusion body was the cause of the disease, inclusion blennorrhea.(41,46) 7. Flemming (1910) considered the inclusion body as a harmless parasite (13,46). (The nature of the inclusions bodies will be more fully elsewhere in this paper.)

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The virus etiology of this non-bacterial conjunctivitis of the new-born known as inclusion blennorrhea (38) was confirmed in a report Thygeson, in 1934, in which evidence was given to indicate taht the basophilic cytoplammic inclusion colonies similar to those found in psitticosis (4), and of the same general nature as those of vaccinia variola(21).

Thygeson believes that the etiologic agent of the disease is a filterable virus having an elementary body phase and an initial body phase. In preparations of the secretion from acute cases of inclusion blennorrhea, stained with a modification

of the Giemsa technique both phases may be demonstrated and found included in the leucocytes and epithelial cells. The elementary bodies may also be found extracellularly. The initial bodies are coccobacillary in shpae, ranging from 0.3 to 0.8 micron in greatest diameter. They stain blue and usually more intensely at the poles. The elementary bodies are smaller, sharply defined granules, averaging 0.25 micron in diameter, occurring singly and in clusters of varying size. They stain a reddish-blue. All forms of the inclusion bodies may be found in an ordinary Giemsa-stained smear of the conjunctival secretions, but are much more rapidly identified in preparation of epithelial scrapings from the conjunctiva. Both the initial bodies and the elementary bodies show up well in the pale staining cytoplam of epithelial cells. The intracellular clusters are quite typical in appearance, but free form, even when numerous, are more diffie cult to identify. (59). The etiologic signiicance of these bodieswill be discussed more in detail else where in this paper.

McKee (1935) in his study of twenty-seven cases of purulent (conjunctivitis) ophthalmia in the newborn, found epithelial inclusion alone in eight

cases while in as many more instances the inclusions were present in a streptococcuc conjunctivitis. McKee, thus, differs somewhat with Thygeson concerning these inclusions by believing that they are made up of phagocytosed bacteria. Accordingly, he feels that the apparent occurrence of pure inclusion conjunctivitis is explained as being due the admitted difficulty in proving the presence of organisms in all cases. He, later, states, that while the inclusions in trachoma, swimming-bath conjunctivitis, and inclusion blennorrhea are undoubtedly due to the phagocytosed bacteria, these bacteria may not cause the disease, but may carry the virus if such is the causal agent. (46)

Howard (1938) makes the following statement, after reviewing the etiological studies: "While Thygeson's work has not beencompletely accepted, a more satisfactory explanation of the etiology of inclusion blennorrhea has not been offered,"(28)

THE NATURE OF THE INCLUSION BODIES

According to Flemming(1910), the inclusion body in inclusion blennorhea is a harmless parasite (13), but Lohlein, Heymann and Lumbroso (1913)believe it to be the cause of only one disease, inclusion blennorrhea (41). That the inclusion body is a virus of genital origin which caused inclusion diseases was the belief of Lindner and Wolfrum (1925)(46). Comberg (1920) believed that inclusion blennorrhea an d trachoma were caused by the same organism, but that the inclusions of swimming-bath conjunctivitis were different morphologically.(9) Herzog believed them to be simply gonococci with a changed biological conduct (14). Bengston(1929) is reproted as saying that the elementary and the initial bodies are modifications of the Bacillus granulosis, the change being caused by the lytic action of the conjunctiva (46).

Thygeson presents the most logical and most w widely accepted view concerning the inclusion bodies: "The inclusion bodies constitue intracellular colonies of elementary and initial bodies in various st stages of development. The smallest are made of entirely of initial bodies and the largest ones, which may in the early stages of the disease entirely re-

place the cytoplasm of the cell, are made up entirely of elementary bodies. All intermediate stages are observable. Intracellular development from the initial body to the elementary bady take plase. For convenience, we will refer to the inclusions as 1, Initial body type, 2 Mixed type, 3 Elementary body type." (59)

"In inclusions of the intermediate type, there is evidence of multiplication of the initial bodies, which are smaller and often densely packed, forming a sort of a "mulberry mass". With wet preparations, the "mass" is seen to be with in a cytoplasmic vacuole.

"The elementary bodies are minute granules of uniform size (0.25 micron in average diameter),which stain reddish-blue with Giemsa and poorly and slowly with ordinary aniline dyes. They are gram negative. They resemble in size and staining reaction the m minute elementary bodies, characteristic of such filterable virus diseases as vaccinia, fowl-pox, and psitticosis. They are readily differentiated from the neutrophilic granules,which they somewhat resemble in size, by the fact that they are alcohol fast while the neutrophilic granules decolorize rapidly. They are differentiated from the occasional non-spe-

cific granule, met with in smear preparations, by their uniform size, frequent diplococcal form, and their peculiar reddish-blue color, Giemsa."

"Phagocytosis of the elementary bodies by leucocytes is frequent but there is no evidence of their multiplication within these cells.

"The initial bodies are coccobacillary in shape and vary from 0.3 to 0.8 micron in greatest diameter. Like the elementary bodies they are gram hegative and stain poorly with aniline dyes, a point of differe entiating them from the conjunctival bacteria. Wi With Giemsa they stain blue bipolarly. Division forms are frequent. In morphology and staining reactions they are identical with the initial bodies bodies of trachoma and strikingly similar ot the large form of psitticosis virus."(59)

"On the basis of the following findings", Thygeson states, "the initial and elementary bodies may be identified as the causal agents of inclusion blennorrhea:

1. Constant presence in the disease
2. Absence in conjunctivitis of known bacterial origin
3. Absence of pathogenic bacteria in clusion blennorrhea and failure of occasional

saprophytes to produce the disease when used for inoculation

4. Multiplication of the elementary bodies
5. Production of the disease with bacteria free suspensions of elementary bodies. (Thygeson inoculated his own lefteye and produced the disease).
6. Filtrate not containing the elementary bodies are not infective". (59)

THE LIFE CYCLE OF THE VIRUS OF INCLUSION BLENNORRHEA

According to Thygeson , "All stages in the development of the inclusion from the elementary-body stage to the initial-body stage may be floowed. So much of the cycle is beyond argument..... The remainder of the cycle is open to several interpretations. The one,which seems most probable and logical to me and which coincides with microscopic findings, considers the elementary-body to be the infective stage and is as follows:

"1. A free elementary body penetrates an epithelial cell and because of the satisfactory nutrition obtainable,

2. Develops into an initial body.

3. The initial body then divides, becoming progressingly smaller in size until,

4. The elementary-body stage is reached. The cell membrane is more weakened beaause of the exhaustion of cytoplasmic substances and

5. Ruptures, either spontaneously or because of pressure occurring during the movement of the eye lids, with subsequent scattering of the elementary bodies into the secretion". (59)

Lindner and Howard, at the time of Thygeson's writing in 1934, maintained that the initial body is the infective agent. Otherwise, they were in agreement with Thygeson. (37, 27, & 59)

A strict parallel for Thygeson's (1934) interpretation of the life cycle of the inclusion bodies in inclusion blennorrhea, is seen in psitticosis in which Bedson has demonstrated the elementary-body stage of the virus to be the infective one while the larger form of the virus (corresponding to the initial body of inclusion blennorrhea) is of low virulence. (5)

Howard (1938) now favors Thygeson's interpretation of the life cycle of the virus of inclusion blennorrhea. (28).

Studies were also made in attemps to determine the duration of the life cycle of the virus. Following inoculation of a normal non-diseased eye with secretions from a diseased eye, Thygeson gave the following results: (59)

"30 hours... Conjunctiva was mormal, inclusions were present, 99% being the elementary-body type and 1% being mixed types. No leucocytes were present.

51 hours... Same as above, plus a few polymorphonuclear neutrophiles.

...

75 hours... Numerous inclusions, mostly of mixed type, e few early initial body types, a slight leucocytosis

100 hours... Mostly elementary-body type, a few early initial-body type, leucocytosis.

122 hours ;;;Inflammation and secretion, inclusions with initial and mixed type predominating, and many polymorphs.

144 hours... Inflammation, secretion, mixed inclusions predominate, a few elementary-body type. Cycle is now somewhat indistinct.

165 hours...Elementary inclusions with only a few initial-body forms present. "(5?(

The complete cycles were thus observed during the first six days after inoculation. Therefore, the life cycle is apprximately 48 hours" (59). This corresponds with Bedson's findings in his work on the life cycle of the psitticosis virus. (5)

THE FILTERABILITY OF THE VIRUS

Like the psitticosis virus, the inclusion blennorrhea virus filters only in the elementary-body stage. Filterability is dependent upon the preparation of virulent suspension of a high elementary-body content, since it is necessary to have more than suffeceint virus to saturate the absorptive surfaces of the pores of the particular type of filter used. With the kieselguhr filters, such as the Berkfeld or Mandler, the absorptive properties are seriously complicating factors due to the necessarily extensive pore surfaces present in even the smallest models. The series of graded collodion membranes, developed by Elford (12), are now more nearly true filters since the pore size and not the absorptive surface is the more important factor in determining the size of the particles passed or retained. (59)

"Using the Elford filters of average pore size greater than 0.6 micron and filtration area of 0.64 sq. cm., it is possible consistently to pass elementary bodies when material from early acute cases is used. It is impossible to produce virulent filtrates with material from chronic stages of the disease ow-

ing to the limited material available and the scarcity of elementary bodies.

The high loss of virus which occurs even when collodion membranes are canbe demonstrated by comparing the elementary-body content of the filtered and unfiltered suspension. When the elementary-body count is low, it is frequently necessary to centrifugalize the suspension (30 minute at 18000 R.P.M.) to concentrate the bodies for staining purposes. The high speed centrifuge offer a satisfactory means of concentrating dilute elementary-body suspensions obtainable from chronic or sub-cute cases.(59)

Tilden and Gifford(1936) reported the following result and conclusion of personally conducted filtration experiments: (64)

"Two patients with inclusion blennorrhea were used for detailed study and inoculation of animals. Graded collodion membrames were used. The material for filtration was prepared as previously described by Thygeson, and smears stained with the Giemsa solution were decolorized slightly.in order to facilitate differentiation of the inclusion bodies from the leucocyte granules. The filtrations were carried out under fifty pounds pressure in the Bauer abd H Hughes filter chamber. The suspensions were first

Hughes filter chamber. The suspensions were first freed ofrom the particles of tissue by centrifugation; hence, the clogging of the membrane was reduced to the minimum, nearly the whole fluid was recovered in the filtrate.....
The experiments,reported, furnish additional evidence that the virus of inclusion blennorrhea is filterable through graded collodion membranes with an average pore diameter of 0.46 - 0.62 micron and is transmissable to the sphinx baboon, whether produced by the filtered or unfiltered material.

"Although inclusions have been observed in experimental inclusion blennorrhea produced by filtrates in man (Thygeson,1934), theyhave not been reported in the disease produced by filtrates in the baboon". (64)

The foregoing observation add further evidence of the significance of inclusions in the etiology of the disease.

INCLUSION VIRUSES and THE RICKETTSIAE

Attempts have been made to identify the agents of psitticosis and inclusion blennorrhea with the Rickettsiae (35). While similarities do exist between these agents and the Rickettsiae, such as staining reaction, and intracellular mode of existence, the Rickettsiae differ from the viruses, as Bedson, in a communic tion with Thygeson, has pointed out, in the following respects:

"1. They never form inclusion, they produce filamentous forms, and they have an anthropod as one of their hosts.

"2. Most virus inclusions are acidophilic and and homogeneous, whereas the cytoplasmic masses of inclusion blennorrhea are basophilic and heterogeneous." (59)

A similarity of the inclusions of inclusion blennorrhea to those of fowl-pox, vaccinia, molluscum contagiosum is well shown by the work of Woodruff (20), Goodpasture, Woodruff and Buddingh (21). Their findings indicate that the acidophilic, apparently homogeneous inclusion of these diseases are in reality composed of myriads on min-

ute granules, the elementary-bodies, embedded in an amorphous acidophilic material material, the matrix, which varies in composition in the different diseases. The identity of the elementary body and the virus has been demonstrated in vaccinia (10,11,34) and fowl-pox (67) and appears probable in molluscum contagiosum. (19)

The view that the cytoplasmic inclusion bodies on many of the virus diseases are in reality intracellular virus colonies seems thus to have considerable evidence to support it. (59)

"The inclusion blennorrhea virus shares with other viruses the common properties of 1.

1. Filterability
2. Cytotropism, the inability to multiply in the absence of living cells,
3. Inclusion formation, and should be classed with the other large viruses of vaccinia, fowl-pox, molluscum contagiosum, and psittacosis". (59)

THE GENITAL ORIGIN OF THE INCLUSION VIRUS

The genital origin of the virus of inclusion blennorrhea has been briefly discussed else where in this paper (p.__), but will be carried more in detail here.

In searching for the origin of this disease in the new-born, Halberstaedter and Prowazek (23) found the typical inclusions in the scrapings from the urethral apertures of two mothers of diseased infants. On this basis , they formulated the theory of an inclusion disease of the male and female genito-urinary tracts. Lindner (38) soon supported this view on finding inclusions, in small numbers, in several cases of non-specific urethritis. Heymann (26) also found inclusions together with gonococcus in both parents of a child having inclusion blennorrhea. Fritsch, Hofstaeter and Lindner (15) inoculated the eye of a baboon with the urethral exudate from a case of non-specific (non-gonococcal) urethritis of fourteen days duration. The urethral exudate was scanty, but contained the typical inclusions bodies. A follicular conjunctivitis resulted and lasted for seven months. Lindner, thus, described the disease as

"genital trachoma". (59)

The fact that inclusion blennorrhea characteristically occurs in the new-born infant naturally led to the suspicion that it was transmitted during passage through the birth canal, as in the case of gonorrheal ophthalmia. Vaginal discharge has been a frequent finding in the mothers of infected infants. Examination of cervical smears stained by the Giemsa technic has shown the presence of typical inclusions in the epithelial cells. (28) Thygeson and Mengert (63) found the inclusions in the cervical epithelium of seven of nine mothers of infants with the disease, inclusion blennorrhea. They also reported an instance in which a gynecologist became accidentally during the performance of a dilatation and curettage. The infection ran a typical course of inclusion conjunctivitis in the adult. The patient upon whom the operation was performed was examined three months later but no inclusions cound be demonstrated in the cervical smears. They also searched for evidence of inclusions in the urethra of male patients. In eleven cases of non-specific urethritis, inclusions were found in only one instance. The case healed after seven months duration. (63)

Thygeson (59) reports the following in support

of the genital origin of the inclusions: "..... by the findings of typical inclusion bodies in preparations from the vaginas of four mothers so far examined. The infectivity of the vaginal secretions in three cases was proved by transfer to the eyes of sphinx baboons, with the development in each case of a typical follicular conjunctivitis. Material in the fourth case was transferred to the eye of a Macacus rhesus which did not develop the disease."

Inclusion bodies were found in the material from the cervix and from the vagina but not in scrapings from the urethral aperture. In one case, the inclusions were numerous; in the other three cases they corresponded in number to those in a case of inclusion blennorrhea a month or more after infection. Free elementary bodies were numerous in one case, but few in the others. In one case there was a post-partum febrile reaction with temperature of 103 degrees F. There was a profuse vaginal discharge in which hemolytic streptococci were found. In the other three the post-partum histories were normal. In no one of the four was there a previous history of pelvic inflammatory disease obtainable.

"It seems obvious that the inclusion infection of the female genito-urinary tract must be mild

disease. No clinical entity which could be due to an inclusion infection is recognized either by the urologists or the gynecologists, although a large number of non-specific infection of unknown etiology are encounter. There is, however, a type of non-specific urethritis in the male in which bacteria are not found. The subacute symptoms disappear after a few weeks and the disease heals without complications#. (59)

TRANSMISSION EXPERIMENTS

As early as 1909, Lindner in his study of inclusion blennorrhea successfully inoculated the conjunctiva of the baboon, (38) Two years later, Morax, working with Lindner and Bodlack, confirmed the existence of inclusion blennorrhea and its tramamissability to the monkey. (51)

Fritsch, Hofstaedter and Lindner (15) inoculated the eye of a baboon with urethral exudate containscanty inclusion bodies and produced a follicular [illegible] conjunctivitis with some inclusions.

The same year, 1910, Wolfrum is reported to have made two inoculation experiments on the adult with the secretions fron cases known to have inclusion blennorrhea. One case developed a conjunctival inflammation with numerous inclusions. The second case resulted in a mildfollicular conjunctivitis with some inclusions. (59)

Lohlein (41) noted that inclusion blennorrhea was easily transmitted to the monkey.

Gebb(16) demonstrated that the disease was transmissable to the human adult, and also that that it produced a self-limiting follicular conjunctivitis with inclusion bodies and which healed

without panus or scars in less than a year. Otherwise, the disease resembled trachoma.

Very little research concerning inclusion blennorrhea was done from the beginning of the first World War until about 1930. The most outstanding work was performed by Thygeson in 1934-36. A brief summary of his work follows.

1. With Ordinary Laboratory Animals. "Seven attempts to transfer the disease to the conjunctivae of white rabbits were unsuccessful. Four similar attemps each with the guinea pig, white rats, and the dog also failed. Epithelial scrapings from active cases induced no inflammatory changes when inoculated into the anterior chamber of the rabbit's eye or intraperitoneally in the rat.

2. Monkeys. Ten Macacus rhesus were inoculated. These animals proved rather difficult to infect, direct transfers from early acute cases being required. If the material was allowed to stand for intervals of fifteen minutes or longer, or was diluted, no disease resulted. Six infections were p produced, however. The disease was a chronic follicular conjunctivitis, involving the fornices but leaving the upper tarsal conjunctiva relatively unaffected. It resembled the conjunctivitis produced

by fresh strains of Bacterium granulosus, in fact not clinically differentiated from trachoma, except that healing occurred in two to three month, with no scars. Transfer to the uninoculated eye occurred in two cases.

In a single Sooty Mangeby, a follicular conjunctivitis with more striking inflammatory signs developed. Here, again, the upper tarsus participated but mildly in the inflammation, whereas the remainder of the conjunctiva was hyperemic and infiltrated. During the first ten days there was moderate secretion.

3. Two sphinx baboons. These developed a type of follicular conjunctivitis more comparable to that seen in the human eye. The disease resembled mild cases of the follicular type of swimming-bath conjunctivitis. Follicles were most pronounced on the lower lid, but were well developed in the upper fornix. The upper tarsal conjunctiva was hyperemic and infiltrated but showed no follicle formation. Secretion was considerable during the first two weeks of the infection. The baboon appears to be the most logical experimental animal."(59)

4. Man. "Two human volunteers with blind eyes were

available for inoculation. Both were elderly individuals with normal conjunctivae.

Experiment # 1. Scrapings from the culdesac of a patient with active case of inclusion blennorrhea were divided into two parts. One was for bacteriological studies, and the other was for inoculation. The latter was applied to the conjunctiva of the right eye after gentle scarification. No changes were noted in the eye until the morning of the seventh day, when the lids were glued to-gether by secretion. Inflammation increased until the tenthday when it began to regress. The bulbar conjunctiva was only slightly hyperemic, the conjunctiva of the lower lid being involved predominately. The picture was one of a diffuse papillary conjunctivitis. No follicular development was noticed at any time during the disease. Symptoms gradually abated, with complete return to normal by the nineteenth day. There was no corneal involvement, and the left did not become infected. Inclusions were numerous from the first day through the disease. There were no pathogenic bacteria. C. xerosis was present, which failed to induce thedisease when inoculated into the auther (Thygeson) own eye"(59)

Experiment # 2. This was similar to Experiment # 1, except that the second eye became diseased on fourteenth day of the disease. It was a picture of a severe papillary conjunctivitis, healing of both eyes,with no scars, by the end of the fourth month. There were no follicles, scars or corneal complication. C. xerosis and Staph. albus were cultured. These failed to induce the disease when inoculated into the author's (Thygeson) own eye." (59)

SPECIAL STUDIES BY THYGESON AND OTHERS

? The first extensive study to be discussed under this heading will be a rather detailed account of the first series of cases on inclusion blennorrhea studied by Thygeson which was reported in 1934. (59)

In this study, seventy-seven cases of conjunctivitis occurring in infants born at the University of Iowa Hospital over a period of fifteen months were used.

Bacteriological studies in these cases showed the following:

Staph. aureus (hemolytic)	41 cases
D. pneumoniae	13 cases
H. influenzae	5 cases
D. pneumoniae	4 cases
B. coli	1 case
Lacrimal Conjunctivitis	
D. pneumoniae	1 case
D. pneumoniae and H. influenzae	1 case
Bacteriologically Negative	11 cases

It is interesting to note that no cases of gonococcic infaction occurred in the hospital series, a fact, illustrting the value of the Crede' method of prophylaxis.

The eleven cases with negative bacteriology constituted a distinct group, characterized by:

1. Onset five to nine days after birth
2. Resistance to silver nitrate
3. Long duration
4. Presence of basophilic heterogeneous inclusion bodies in the cytoplasm of certain epithelial cells.

All, but one,of the eleven cases were subjected to intense bacteriologic studies. The following plan was followed. "Cultures were taken every second day during the entire period of hospitalization. Blood agar was the medium of choice, but supplementary cultures were made on Noguchi, semi-solid leptospira medium, ascitic fluid blood agar, chocolate agar, Loeffler's blood-serum medium, and Noguchi's medium for the culture of <u>Treponema pallidum</u> . Both anaerobic and aerobic methods were utilized. Material for culture was obtained by scraping the affected conjunctiva with a platinum spatula or loop. Occasionally, the secretions alone with out epi-

thelial cells, by means of sterile cotton applicators, were used. Incubation was at 37 degrees Centigrade.

Air contamination was eliminated, in so far as possible,by subjecting uninoculated tube and plates to the same manipulations as for the inoculated ones. Lumbroso (43) did not control his studies, and thus, may account for his bizarre bacteriological findings.

The results of these bacteriological findings are indicated in the following table: (59)

Case No.	Name	No. of Exams.	Result of findings
1	G	0	No bacteria seen in smears
2	R	5	C. xerosis, Staph. albus
3	B	30	C. xerosis, St. Albus, D. pneoum.
4.	K	4	C. xerosis
5.	F	28	C. xerosis, S. albus, C. hoffmani
6	A	5	C. xerosis
7	S	26	C. xerosis, S. albus, D. pneum.
8.	H	12	C. xerosis, St. albus
9	W	3	Staph. albus
10	M	8	Staph. albus, C. xerosis
11	H	2	C. xerosis

It will be seen that in no case was the disease complicated by syperimposed bacterial infection.

While no mixed infection occurred, the curious finding of a bacterial infection in one eye and an inclusion infection in the other was noted in two instances:

Case # 7. Bilateral conjunctivitis was noted

on the ninth day. Smears of the right eye revealed gram-positive cocci in clumps. Smears from the left eye showed no bacteria, but a moderate number of inclusion. Cultures from the right eye grew Strept. aureus (hemolytic), while cultures from the left remained sterile. Treatment with ½% silver nitrate ointment, t.i.d. was begun in the two eyes. The left eye was unaffected by it, but the right eye recovered rapidly and in five days had returned to normal. Seven days later, the right eye became acutely involved. Cultures were negative, but epithelial scrapings revealed inclusions. Thus, transfer infection from the left eye to the right eye had occurred.

Case # 10. This was similar to case # 7, but the second eye did not become involved by inclusions.

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A second series of cases were also studied and reported by Thygeson and Mengert in 1936 (63). The same technic and plan of study was used in this series as that reported by Thygeson in 1934 (59). A summary of the results, thus obtained, follows.

Eight additional cases of inclusion blennorrhea, differing in no essential from the eleven previous-

ly reported, are described.

The bacteriologic findings in these eight cases of inclusion blennorrhea is as follows: (63)

Case	Name	Examinations	Results
12	V	3	C. xerosis, Staph. albus
13	S	3	No growth
14	K	5	C. xerosis, Staph. albus, Staph. aureus.
15	H	2	Staph. aureus
16	H	1	Staph. albus
17	L	2	No growth
18	S	3	Staph. albus, aureus, and C. xerosis
19	G	3	C. hoffmani in 1 examination

Five of these cases were delivered in the hospital, while the other three were delivered elsewhere and were brought in for treatment of suspected gonorrheal ophthalmia.

Five cases had no secondary infection. In the three cases with Staph. aureus, the organisms disappeared within a few days under treatment with silver nitrate ointment ½%.

The clinical picture in these eight cases of inclusion blenorrhea presented no peculiarities.

In cases, 12, 13, 16, 17, and 19, the disease was severe, similating gonorrheal ophthalmia; it was bilateral from the onset, the discharge was profuse, purulent, and transitory pseudomembranes were noted during the first week of the

illness. The acute stage is superceded in ten to fourteen days by a chronic stage which did not differ from the chronic stage of milder forms. In cases # 14 and 15, the condition was mild and not readily distinguished from catarrhal conjunctivitis caused by D. pneumoniae and Strept. Aureus. Severe early infiltration of the conjunctiva of the lower lid in inclusion blennorrhea might perhaps have constituted a differential sign but similar infiltration has been observed occasionally in cases of conjunctivitis secondary to dacryocystitis in the new-born.

Thygeson, further, states, "It is obvious that inclusion bleenorrhea can not be diagnosed accurately on the basis of clinical findings alone. Axenfeld (2) has emphasized correctly the importance of a search for inclusion bodies in every case of conjunctivitis of the new-born. No corneal changes w were noted.

In cases # 15, 16 and 18 the infection was monocular at first, but it eventually became bilateral in all instances. In no instance did the second eye become involved in less than six days. Monocular involvement has generally been mild" (63)

The incubation period in inclusion blennorrhea, based on the preceding two series of studies, is presented in the following table:

Day of onset	Number of cases
4th	1
5th	6
6th, 7th & 8th	3 each day
9th	2
10th	3
11th	0
	21 cases

For comparison of the incubation periods of conjunctival infections in the new-born, the following table is included. (55)

Infective Organism	Incubation Period
Gonococcus	2-5 days
Inclusion Blennorrhea	5-10 days
Pneumococcus	36 hours
Influenza Bacillus	36 hours
Koch-Weeks Bacillus	36-48 hours
Staphylococcus	48 hours
Streptococcus hemolyticus	48 hours (?)
Diphtheria	?
Morax-Axenfeld Bacillus	48 hours
Colon Bacillus	2-3 days.

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A more recent study of a series of conjunctivitis cases in the new-born was reported by Howard in 1938 (28) confirming the work and conclusions of Thygeson , just previously discussed. A brief summary of Howard's findings will follow.

"During the six months, January 1, to July 1, 1937, thirty cases of purulent ophthalmia in the newborn were observed from the obstetric and pediatric services of the Strong Memorial and Rochester Municipal Hospitals. Culture and smears were made in an attempt to confirm the bacteriologic findings of Thygeson.

"All inoculations were made on Bradford's medium, which was found to be an exeellent culture material for all conjunctival organisms, pathogenic and non-pathogenic, including the gonococcus. In all doubtful cases, special gonococcus cultures were made on chocolate agar plates and incubated in sealed glass jars containing 8-10 % carbon dioxide.

"Of the thirty cases of infants examined, a bacteriologic diagnosis was possible in twenty-two. In none of these were inclusions found in the Giemsa-stained smears. Intracellular groups of organisms were frequently seen in bacterial cases but they in no way resembled true inclusions as found in the non-bacterial cases.

"In eight cases, cultures were either sterile or grew only the non-pathogenic diphtheroids and non-hemolytic staphylococci. Seven of these eight cases showed typical cytoplasmic inclusions in successive

preparations of conjunctival secretions stained with Giemsa. In the eighth case, no etiologic agent was determined. This may represent a failure to isolate the causal organism, or the inclusion may have been missed. No case of gonorrheal ophthalmia was seen.

"These findings tend to substantiate Thygeson's statements that true cytoplasmic inclusion do not occur in conjunctivitis of known bacterial origin and that pathogenic bacteria are not found in inclusion blennorrhea". (28

In regard to mixed inclusion infections, "It is interesting to note that there was no evidence of mixed inclusion infection in four infants and six adults admitted to the hospital with gonorrheal ophthalmia" (63)

Julianelle, Harrison and Lange,late in 1938, after completion of their thrid series of studies dealing with the experimentaletiological aspects of inclusion blennorrhea advanced the following conclusions: (32)

"1. The bacteria cultivable by a variety of methods from inclusion blennorrhea are representative of the flora associated with the normal conjunctiva.

2. The bacteria so derived are non-pathogenic and they are incapable of inducing experimental inclusion blennorrhea in the monkey.

3. The infectious agent of inclusion blennorrhea passes through a Berkefeld V filter and collodion membranes of 0.6 micron A.P.D.

4. Such filtrate are bacteriologically sterile and induce experimental infection.

5. Attempts to cultivate the infectious agent in tissue cultures were not successful.

6. Such tissue culture were not infectious for monkeys and they did not contain inclusion bodies.

7. The virus is definitely related to the inclusion bodies. "(32)

CONCLUSIONS

As a result of the foregoing studies, the author wishes to present the following concluding statements:

1. Inclusion blennorrhea is definite and distinct clinical entity characterized by its onset five to ten days after birth, by its long duration and by the presence of cytoplasmic inclusion bodies in certain of the conjunctival epithelial cells.

2. The bacteria present in the disease are apparently not concerned etiologically.

3. The disease is transferable to the adult human conjunctiva, and produces a papillary or a follicular conjunctivitis identical with swimming-pool conjunctivitis in which the Halberstaedter-Prowazek type of inclusion bodies are found.

4. The virus nature of the agent of the disease is confirmed by its failure to grow on artificial media and by its passage through filters which retain conjunctival bacteria.

5. The identity of the minute elementary bodies found in the disease with the virus is indicated by

a. their constant presence in the disease,

b. their absence in conjunctivitis of bacterial origin,

c. their multiplication within the new hosts,

d. the infectivity of the filtrate containing elementary bodies,

e. the non-infectivity of filtrates not containing the elementary bodies.

6. The inclusion bodies are intracellular virus colonies in the various stages of development

7. The birth-canal origin of the disease is confirmed by the demonstration of the virus in the cervical epithelium of mothers of affected infants.

8. The venereal nature of the disease was confirmed in two cases of non-gonorrheal urethritis.

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