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BACTERIOLOGICAL AND CLINICAL STUDIES OF AN  
EPIDEMIC OF KOCH-WEEKS BACILLUS CON-  
JUNCTIVITIS ASSOCIATED WITH CELL  
INCLUSION CONJUNCTIVITIS.<sup>1</sup>

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PLATES 41 TO 43.

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While engaged in the study of conjunctivitis associated with epithelial cell inclusions, the occurrence of an epidemic<sup>2</sup> of conjunctivitis due to hemoglobinophilic bacilli enabled us to investigate the relationship between this organism and the epithelial cell inclusions.

Since the discovery of the inclusions by von Prowazek and Halberstaedter, who considered them to be the etiological agents of trachoma, an abundant literature has arisen. Opinions are still greatly divided as to the nature of the bodies, although the majority of investigators regard them as an independent organism, as claimed by von Prowazek and Halberstaedter. They do not, however, commit themselves to the statement as to whether the organism represents the causative agent of trachoma or not. We were inclined to the belief that it constitutes an independent organism which produces a true conjunctivitis of a more or less characteristic clinical course. Herzog<sup>3</sup> considered them to be mutation forms of the gonococcus, while Williams<sup>4</sup> views them as the cell inclusions of various organisms, such as the hemoglobinophilic bacillus and allied species.

<sup>1</sup> Read before the Ophthalmological Section of the New York Academy of Medicine, May 17, 1915.

<sup>2</sup> Service of Dr. Martin Cohen at the Randall's Island Hospital, New York.

<sup>3</sup> Herzog, H., *Arch. f. Ophth.*, 1910, lxxiv, 520.

<sup>4</sup> Williams, A. W., *Collected Studies from the Bureau of Laboratories, Department of Health, City of New York*, 1912-13, vii, 159-247; *Jour. Infect. Dis.*, 1914, xiv, 261.

Although by means of a special method an organism strikingly similar to the epithelial cell inclusions has been made to grow in pure culture,<sup>5</sup> their pathogenic properties, nevertheless, have not yet been established.

As a result of our previous studies a tentative conclusion had been reached that infection of the conjunctiva with these organisms produces an independent conjunctival disease which may properly be described as cell inclusion conjunctivitis. This organism may be present alone, or it may be associated with other pathogenic organisms.

*Outbreak and Course of the Present Epidemic of Koch-Weeks Bacillus Conjunctivitis.*

Prior to the outbreak of the Koch-Weeks bacillus epidemic,<sup>6</sup> one of us (Cohen) had under observation for about five months 10 cases of inclusion conjunctivitis and 4 cases at the outdoor department of the Post-Graduate Hospital. In these cases, smears (and in a few cases, cultures) were taken weekly and examined (Noguchi), but no Koch-Weeks bacilli were found. On October 3, 1914, one of the inclusion cases under observation at the Island developed an acute conjunctival inflammation. In the conjunctival smears numerous Koch-Weeks bacilli, as well as epithelial inclusions, could be demonstrated. The acute conjunctival inflammation spread to the remaining nine inclusion cases. The smears from all showed inclusions as well as numerous Koch-Weeks bacilli.

In an adjoining ward there were under treatment 15 cases of follicular conjunctivitis, 3 cases of interstitial keratitis, and 1 case of chronic dacryocystitis. 13 of these contracted the Koch-Weeks bacillus infection. During the entire course, which lasted from two weeks to seven months, no pathogenic organisms but the Koch-Weeks bacilli could be demonstrated in the smears and cultures.

There were 7 patients in the trachoma ward. 2 of these showed simultaneous presence in the conjunctiva of inclusion and of Koch-

<sup>5</sup> Noguchi, H., and Cohen, M., *Arch. of Ophth.*, 1914, xliii, 117; *Jour. Exper. Med.*, 1913, xviii, 572.

<sup>6</sup> In the present article the hemoglobinophilic bacilli found in these cases were designated as Koch-Weeks bacilli, in order to conform with the usage of this term in ophthalmology and bacteriology when speaking of this class of organisms.

Weeks bacilli. The latter organisms were present only in cultures, as there were possibly too few to be recognized in the smears. This condition was present for a short time during this epidemic. The infection with the Koch-Weeks bacillus apparently produced no change in the clinical picture of these trachoma cases. The 4 inclusion cases not treated on the Island showed at no time during the past year the Koch-Weeks bacillus, either in smears or cultures; not even during a relapse or reinfection, of which one of these cases had two, could they be demonstrated. Table I shows the bacteriological findings in the cases on which the present report is based.

TABLE I.

Organisms present.	No. of cases.
1. Epithelial cell inclusions alone .....	6
2. Epithelial cell inclusions at first, with subsequent Koch-Weeks infection	17
3. Koch-Weeks bacillus alone .....	13
4. Koch-Weeks bacillus at first, with subsequent appearance of inclusions	2
5. Pneumococcus with inclusions .....	1
Total .....	39

In acute catarrhal conjunctivitis it is at times difficult, or impossible, to determine the causative organism from the clinical manifestations alone. This is due to the great variability in virulence of the usual infecting organisms, and to the resisting qualities of the individual patient. Yet in many of the acute infections of the conjunctiva, the etiological diagnosis as made by the clinical appearance could be confirmed subsequently by the bacteriological diagnosis, especially in the subacute state. In mixed infections, such a diagnosis is much more difficult.

In a few instances, even smears and cultures of the conjunctival scrapings fail to clear up the diagnosis, as no pathogenic organisms can be found. That the microscope alone is not sufficient to exclude the presence of an organism is shown by the fact that the scrapings from cases of trachoma which showed neither inclusions nor the Koch-Weeks bacilli (as proved similarly by cultures), when inoculated on the conjunctiva of the higher apes (baboons) produced symptoms similar to those which are seen in inclusion cases in man. They were, however, of a milder type and lasted only about ten days. The conjunctival scrapings from the inoculated animals re-

vealed the presence of epithelial inclusions, but no Koch-Weeks bacilli or allied organisms appeared in the smears or in the cultures.

We shall now discuss the clinical manifestations in the group of cases mentioned in Table I.

The clinical course of inclusion conjunctivitis has already been described by Cohen.<sup>7</sup> In the early stage, the clinical manifestations resemble those seen in the Koch-Weeks bacillus infection, and it is only by the examination of smears or cultures that the diagnosis can be positively made. Briefly, they are as follows: In the beginning there is moderate edema of the lids, with mucopurulent secretion. Somewhat later isolated reddish translucent follicles appear in the lower palpebral conjunctiva and upper folds, these two sites becoming involved simultaneously. At a later stage, the upper palpebral conjunctiva takes on a brick red color and assumes the characteristic granular appearance. These regular and progressive manifestations of the disease retrogress after about two months by absorption of the contents of the follicles and papules. These disappear first from the upper portions of the conjunctiva, when the conditions resemble those of cases of follicular conjunctivitis; and later, in about three months, they begin to disappear from the lower conjunctiva. At the end of four months, the conjunctiva is again in a normal condition.

This is the usual course, but at times there are deviations, due to external or unknown conditions, or to a previous affection of the conjunctiva; as, when grafted on a follicular conjunctivitis, the original follicles become larger and new ones make their appearance. In the stage of retrogression the papules become absorbed and the original follicles, owing to relaxation of surrounding pressure, again become prominent, the course being then one of follicular conjunctivitis. In 2 cases of inclusion conjunctivitis, there remains solely, at the end of nine months, a fine papillary condition of the upper tarsal conjunctiva; one of these two cases has had two relapses or infections.

It may be mentioned here in passing, that of the 75 cases of inclusion conjunctivitis studied by us during the past five years, 58 cases are still under observation of Dr. Cohen. 2 of these still show a diffuse and linear cicatrization of practically the entire palpebral conjunctiva, but no corneal or other involvement is present. 2 other cases of this group still show a fine papillary hypertrophy of both tarsal conjunctivæ, with no corneal or other complications. The remaining 54 cases have remained normal for the past four and a half years. 17 cases were observed for six months, and were normal when last seen.

4 patients with follicular conjunctivitis became infected with inclusions. The follicles previously present became enlarged and congested, and new papules and follicles appeared on the upper tarsal conjunctiva and the conjunctiva of the lower lid. These cases had the appearance of a severe type of inclusion conjunctivitis.

For several weeks after the beginning of the disease, the smears in all cases showed an abundance of epithelial cell inclusions. Toward the end of the disease, the number of inclusions gradually diminished. Yet after three months' duration, although no inclusions were demonstrable in the smears, the clinical aspect of the disease was usually still evident.

<sup>7</sup> Cohen, M., *Arch. of Ophth.*, 1913, xlii, 29.

On Oct. 23, 1914, one of the inclusion cases on the Island developed a slight conjunctival inflammation, which smears and cultures showed to be due to the Koch-Weeks bacillus. In the succeeding two weeks, as previously mentioned, additional cases of inclusion conjunctivitis contracted the Koch-Weeks bacillus infection. Conjunctival smears and cultures examined weekly for the past several months showed at first the simultaneous presence of inclusion and Koch-Weeks bacilli. In the succeeding two months, the findings varied, at times only one of the two organisms being present, at other times, both. Possibly this was due to relapse or reinfection. In the next two months, although no organism was present in smears and cultures, there was still evidence of inflammation. Ultimately the conjunctiva became normal in all but one case. In this case there is still at the end of a year evidence of an inclusion conjunctivitis in the retrogressive stage.

In an adjoining ward, where mild follicular conjunctivitis cases were being treated, 13 contracted the Koch-Weeks bacillus infection. Previous to the Koch-Weeks infection, all these cases showed over various areas of the conjunctiva follicles varying in number and size, and showing no inflammatory reaction. The first case in this ward developed on Nov. 1, 1914. Only 3 of the 16 cases escaped the Koch-Weeks infection. The Koch-Weeks bacillus was present in great numbers, but no other pathogenic organisms could be demonstrated throughout the entire course of the epidemic, which lasted six months.

The clinical manifestations in these follicular cases were as follows. There were slight edema of the lids and conjunctival congestion with mucopurulent secretion. Both eyes became successively involved. The acute infection lasted six to eight weeks. At the end of this period, the original follicles were still present.

In one of the typical follicular cases it was difficult to determine whether the acute infection was due to the Koch-Weeks bacillus or to the epithelial inclusions. The symptoms were those of a severe inclusion conjunctivitis. But notwithstanding repeated examinations extending over two months, no inclusions could be found. Nevertheless, judging from the clinical course and from the fact that cell inclusions are often absent in the subacute stage, this and similar cases may be considered due to epithelial inclusions.

2 cases of interstitial keratitis on the Island Eye Ward and 1 at the Dispensary had normal conjunctivæ, when they suddenly developed an acute catarrhal conjunctivitis. In the smears and cultures, which were taken weekly for four months, the Koch-Weeks bacillus was present, even at a time when the conjunctiva was free from inflammation. The inflammatory stage lasted from six to eight weeks. Inclusions could at no time be demonstrated in the smears.

The clinical appearance in these cases was moderate edema of the lids, and conjunctival congestion with mucopurulent secretion. In one case there was present a small scleroconjunctival hemorrhage with a small phlyctena. Conjunctival furrows or folds also appeared in those cases when the congestion diminished.

As regards the last group in the table, 2 cases at first showed the Koch-Weeks bacillus in the conjunctival smears. Later, inclusion cells made their appearance, associated with their clinical manifestations. In the first case there was present

a mild catarrhal conjunctivitis, papules being present on the lower tarsal conjunctiva alone. In this respect it differed from other inclusion cases where the papules are present also on the upper tarsal conjunctiva. It is possible that the lesions at times can only be detected microscopically. A second examination in this case revealed the epithelial inclusions. Perhaps the inclusions were missed in making the first examination, as it is likely that in some stages of the disease only a few epithelial cells contain inclusions.

The second patient originally had a follicular conjunctivitis. He then contracted an acute catarrhal conjunctivitis, the secretion containing for three weeks the Koch-Weeks bacillus alone. At the end of this time, when the conjunctivitis improved, he again developed an acute conjunctival inflammation, and the smears showed numerous inclusions and a few Koch-Weeks bacilli. This case then followed the usual course of an inclusion conjunctivitis, the conjunctiva ultimately returning to a normal condition.

In a ward assigned to minor eye affections, where there were a few mild Koch-Weeks cases, a routine examination of the conjunctivæ of all the cases was made, in order to determine the presence of the Koch-Weeks bacillus. 2 cases showed the Koch-Weeks bacilli, in spite of the fact that the conjunctivæ were entirely normal throughout the whole period. In another case in the same ward degenerated Koch-Weeks bacilli were found in the conjunctival smears. This case then developed an acute inflammation, showing all the symptoms of an inclusion conjunctivitis with the added findings of epithelial cell inclusions in the smear examination.

In our routine examinations made during the past five years Koch-Weeks bacilli have rarely been found. Recently we encountered eight cases of pneumococcal conjunctivitis. Clinically these cases resembled acute catarrhal conjunctivitis, due to the Koch-Weeks bacillus, and were so diagnosed before the smears and cultures showed the presence of numerous pneumococci without any Koch-Weeks bacillus. The conjunctival secretions in these cases were typically serous, being also associated with diffuse congestion. The duration was from one to two weeks. One of the patients, included already in this paper, had originally an inclusion conjunctivitis. Later, he contracted a Koch-Weeks bacillus infection. After four weeks, when apparently cured, another acute inflammation appeared, which was due to the pneumococcus. This infection was followed by the appearance of inclusion conjunctivitis, the smears showing both pneumococcus and inclusion cells. At present, the clinical symptoms are those of an inclusion conjunctivitis in a state of relapse or reinfection.

The treatment adopted in all these cases was irrigation of the conjunctival sac with a saturated solution of boric acid every two hours, and the application to the conjunctiva, once a day, of a silver nitrate solution, until the acute symptoms had subsided, when irrigation alone was continued. This method of treatment had practically but little effect on the course of the inclusion cases, whereas in the Koch-Weeks infections decided improvement was observable. The disappearance of the organisms from the smears and culture was, however, not at all influenced by the application of the silver nitrate.

*Bacteriological and Experimental Studies of the Present Epidemic.*<sup>8</sup>

Our technique for obtaining smears and cultures was as follows. The upper lid was everted and then the short end of a sterile slide was gently rubbed over the upper tarsal conjunctiva where there is least likelihood of contamination. By means of a platinum loop, a part of the scrapings removed was used for the purpose of cultivation and animal experiments. The remainder of the materials on the slide was transferred to another slide where a thin spread was made, and examined by means of the Giemsa or Gram stain.

Since our problem deals with the Koch-Weeks infection, the culture media employed were chiefly blood agar and tissue ascitic fluid. The cultural findings are summarized below.

In the present epidemic it was noticed that various strains of the so called Koch-Weeks bacilli or hemoglobinophilic organisms in cultures varied in size to such an extent that they may be divided into a thin (Figs. 4 to 7), a medium (Figs. 8 to 11, 20 to 23), and a coarse type (Figs. 16 to 19), according to their morphology. The coarse variety were bacillary or coccoid (Figs. 12 to 15) and resembled culturally and morphologically some strains of *Bacillus influenzae* (Figs. 24 and 25) derived from the respiratory organs; while the thinner variety was much narrower and often shorter. After several days' cultivation at 37° C. on blood agar, some of the organisms became somewhat granular and unevenly stainable; when found in large masses they often assumed the appearance of the granular forms of the so called trachoma bodies (Figs. 5 to 7, 9 to 11, 13 to 15, 17 to 19). But these granules did not break down so as to approach the minuteness of the elementary bodies (Figs. 32, 33, 36, 39, 43, 45) which are found in the uncomplicated cases of inclusion conjunctivitis or trachoma. The degenerated or involuted bacilli do not have the sharp contour of the elementary granules. Transplants made from these somewhat degenerated granular masses of the hemoglobinophilic organisms give a good growth of typical bacillary or semi-coccoid forms. The character of the colonies on the blood agar is also distinctive, in that they remain minute, sharply elevated with a pointed top, and appear more grayish than the colonies of the

<sup>8</sup>This part of the work was carried out at The Rockefeller Institute.

influenza type varieties, which show a tendency to spread and are of a more dewy aspect. It is quite possible that the minute type constitutes a group by itself, but further study is required to determine its relation to the other groups. At all events several strains of influenza bacillus<sup>9</sup> derived from cases of meningitis and pneumonia appear quite different from the conjunctivitis strains in their morphological features (Figs. 24 to 28). The frequency with which organisms of different type were found in the present epidemic is shown in Table II.

TABLE II.

Organisms present.	Types.		
	Coarse.	Medium.	Thin.
1. Epithelial inclusions first, with subsequent Koch-Weeks infection (17 cases) .....	I	7	9
2. Koch-Weeks bacillus alone (13 cases) .....	I	10	2
3. Koch-Weeks bacillus first, with subsequent appearance of inclusions (2 cases) .....	I (round type)	I	

As will be seen from Table II, the thin variety occurred very frequently in the cases in which the patients had been previously infected with the inclusions. In the simple Koch-Weeks cases the medium was predominating. It is significant that in the smears taken from the cases infected with the inclusions and Koch-Weeks bacilli it is not difficult to differentiate the initial bodies of the former from the bacilli, because the initial bodies are much larger and oval in shape, and take up a deeper blue stain. Fig. 3 shows a typical inclusion near the nucleus and some Koch-Weeks bacilli in the same cell. The hemoglobinophilic bacilli are seen to be taken up by the polynuclear leucocytes more frequently than by the epithelial cells (Fig. 1), while the inclusion bodies are chiefly epithelial and seldom leucocytic in nature (Figs. 29 to 45). So far as could be ascertained in stained specimens the bacilli remain well preserved within the cells and do not seem to disintegrate into granules (Fig. 2). On the other hand, the initial bodies are often ill defined and do not show any distinct bacillary forms. They are far more

<sup>9</sup> We are indebted to Dr. Martha Wollstein for these strains, for which we here wish to express our thanks.

pleomorphic than the hemoglobinophilic bacilli (Figs. 29, 34, 37, 40, 41, 43, 46, and 47).

The isolation of the hemoglobinophilic organisms, irrespective of the type, was easily accomplished by means of the blood agar; but in many instances the preliminary microscopical search in the smears failed to demonstrate the presence of a few organisms, until they could be found by the cultural procedure. It must be understood that in cases where the inclusions only were found, repeated efforts to isolate the hemoglobinophilic organisms were made, with, however, invariably negative results. In this connection it may be added that a number of cases clinically diagnosed as trachoma was also studied in order to see if any hemoglobinophilic organism could be isolated. The results were uniformly negative. A few cases were also found in the same wards, which may have been infected through contact with the inclusion cases and which, although carefully observed from the earliest stage of the disease, did not at any period show the presence of any hemoglobinophilic bacilli.

*Transmission of the Inclusion Bodies to Animals.*—In order to find out whether the inclusion virus ever exists in the form of a hemoglobinophilic bacillus, scrapings from the conjunctivæ of these cases were inoculated into the conjunctiva of a baboon. Prior to the inoculation the conjunctiva of the baboon was examined to ensure the absence of such a bacillus. Within seventy-two hours after the inoculation the conjunctiva showed moderate congestion, edema, and a few minute papules; a small quantity of a mucopurulent discharge was present at the inner canthus. The smears and culture were made from the conjunctival scrapings. The result showed that there were numerous cell inclusions, but no hemoglobinophilic organisms<sup>10</sup> (Figs. 46 to 51). The examinations were continued regularly for a period of two weeks and the results were unvarying. As was previously shown by various investigators, the von Prowazek bodies are transmissible to higher apes, but the hemoglobinophilic or Koch-Weeks bacillus is not.

*Attempts To Produce Koch-Weeks Bacillus Conjunctivitis in Animals.*—Several attempts were made to transfer the hemoglobino-

<sup>10</sup> Cultures made after 24 and 48 hours were also negative in regard to the hemoglobinophilic bacillus.

philic organisms to the conjunctivæ of rabbits (young and adult) and monkeys (baboon and several *Macacus rhesus*), by introducing several loopfuls of the twenty-four hour blood agar cultures of different strains into moderately abraded surfaces of conjunctivæ. The results were completely negative, except in the case of one rabbit, where the organism was still recoverable after twenty-four hours. It seems remarkable that such a large quantity of pure cultures of freshly isolated strains of these organisms from the cases where the inclusions were also present should fail to reproduce the conjunctivitis, in view of the fact that a comparatively small number of the inclusions as contained in the scrapings from a patient can readily reproduce the inclusion conjunctivitis.

From this a conclusion may be warranted that in a conjunctivitis where the inclusion and the hemoglobinophilic bacilli are simultaneously present, two pathogenic factors can be separated by means of transmission of the material into the conjunctiva of a suitable animal (baboon) in which the inclusion virus alone implants itself upon the new host, while the bacilli quickly disappear from the inoculated conjunctiva. For man both organisms are pathogenic, but for the baboon only the inclusion virus is capable of producing infection.

On the other hand, this may not exclude the possibility, as asserted by Williams and her associates, that a conjunctivitis due to the Koch-Weeks bacillus may also show some cell inclusions; since under certain experimental conditions a very suggestive phenomenon, to be related below, has been observed.

*Attempts To Produce the Koch-Weeks Bacillus Epithelial Inclusions in Animals.*—In order to determine experimentally whether or not the hemoglobinophilic bacilli when taken up by epithelial cells will undergo the morphological changes which lead to the formation of so called inclusion bodies, an intratesticular inoculation of the rabbit with pure cultures of the hemoglobinophilic organisms isolated from the cases already mentioned was resorted to. The local reaction which follows consists of edema of the scrotum and induration of the testicular parenchyma within twenty-four hours. The edema gradually disappears within the next few days, while the testicular induration remains more or less the same for about five days, after which it commences to recede. In some instances the

rabbit succumbed to septicemia and probably to intoxication as a result of the introduction of the cultures. The organs were removed at intervals of twenty-four hours, three days, and six days, and then fixed in sublimate alcohol, and stained by Giemsa's acetone method,<sup>11</sup> and, if overstained, treated with a 10 per cent solution of *Glycerin-äthermischung* (Grübler) for a few minutes, as advocated by one of us.<sup>12</sup> The results show that the injection of the bacilli is followed by an intense leucocytosis, in which the polynuclears invade the tubules in groups. In the twenty-four hour specimens the organisms are still well distributed along the interstitial spaces; in the three day specimens one notices numerous masses of agglutinated bacilli here and there within the tubular lumina or along the connective tissue. These masses take on a purplish hue and appear granular in structure and indefinite in outline. They are on the point of disintegration. The granules within and about these bacterial masses are not so minute as to be mistaken for the elementary bodies of the inclusion. In the six day specimens some clumped bacteria were found within the polynuclear leucocytes, but a diligent search failed to show any typical epithelial cell inclusions. In these six day preparations the number of the bacterial clumps is smaller than at an earlier period. There were no granules small enough to be regarded as the typical elementary granules. While a careful comparison of the inclusions and the bacterial clumps just referred to will reveal the difference between them, this is not always easy to accomplish (Figs. 53 to 55). Fig. 52 shows a mass of granules from a case of mixed infection of Koch-Weeks bacilli and the von Prowazek inclusions, and it appears difficult to determine whether they represent the degenerated bacilli or the inclusion granules. The deep stain of the mass and the absence of any free bacilli around it seem to indicate that it belongs to the latter kind. It is also possible that some of the clumped granules found in the conjunctival smears from cases of Koch-Weeks infection might have been interpreted as the cell inclusions and classified with the von Prowazek inclusions.

*Attempts To Transmit the Inclusion Bodies to a Parenchymatous Organ in Animals.*—Efforts were also made to transmit the von

<sup>11</sup> Giemsa, G., *Deutsch. med. Wchnschr.*, 1909, xxxv, 1752.

<sup>12</sup> Noguchi and Cohen, *Proc. N. Y. Path. Soc.*, 1910, x, 20.

Prowazek bodies from uncomplicated inclusion cases to the testicles of rabbits, since this organ offers an excellent medium of growth to various highly parasitic organisms which otherwise cannot be easily cultivated.<sup>13</sup> The scrapings of conjunctivæ from 4 different patients were inoculated into the testicles of 8 rabbits; but in spite of the large number of the inclusions contained in the conjunctival scrapings used for this purpose, no success was obtained along this line. The testicles showed within twenty-four hours some induration and edema, but after a few days resumed their normal condition. Tissues removed after twenty-four hours, three days, and six days failed to show any cell inclusions when examined in smears and sections. No hemoglobinophilic organism was found in cultures made from the tissues. This negative finding also tends to strengthen the view that the cell inclusions found in these cases were not the Koch-Weeks bacilli, for if they had been it would have resulted in the production of Koch-Weeks orchitis.

## CONCLUSIONS.

1. There are cases in which epithelial cell inclusions may alone be present in the conjunctival smears. In such cases no other pathogenic organisms, such as the Koch-Weeks bacillus or the pneumococcus, can be demonstrated in smears or cultures.
2. The conjunctiva can become simultaneously infected with the inclusion bodies and Koch-Weeks bacilli or other organisms.
3. In cases of acute or subacute conjunctival inflammations due to mixed infections the clinical features of each infection may be present. The course of the inflammation is, however, more prolonged.
4. Within recent years, the Koch-Weeks bacillus has only seldom been found in our routine examinations.
5. The epidemic studied was of a severe type.
6. Clinically it is practically impossible to distinguish pneumococcal conjunctivitis from the Koch-Weeks conjunctivitis. Bacteriological examination of smears and cultures is the only means by which the etiological diagnosis can be definitely established.
7. Conjunctivæ of certain species of monkeys are susceptible to

<sup>13</sup> Noguchi, H., *Jour. Exper. Med.*, 1915, xxi, 539.

the von Prowazek inclusion bodies, but not to the hemoglobinophilic bacilli isolated from cases of epidemic conjunctivitis.

8. The injection of conjunctival scrapings containing the von Prowazek cell inclusions into the testicles of rabbits produces no cell inclusions in the latter, while the injection of a pure culture of the hemoglobinophilic bacilli causes an acute inflammation accompanied by numerous clumps of the organisms, simulating the von Prowazek bodies at certain stages of their evolution.

9. There exists an apparent morphological similarity between the degenerated forms of this variety of the hemoglobinophilic bacilli and the cell inclusions, both in cultures and in experimental orchitis in the rabbit. But, as a rule, the elementary bodies of the latter are much smaller and more sharply defined than the smallest granules of the former, while the initial bodies are bigger, more intensely stainable, and less definite in their contour than the hemoglobinophilic bacilli found in the infected conjunctivæ.

#### EXPLANATION OF PLATES.

All the photographs were made from film preparations, except where otherwise stated. They were stained with Giemsa. The enlargement is uniformly 1,000 diameters.

#### PLATE 41.

FIG. 1. This shows a mass of the Koch-Weeks bacilli in a film preparation from an uncomplicated case of Koch-Weeks conjunctivitis. The bacilli belong to the thin type and some scattered examples appear coccoid. By focusing, the mass is seen to be composed of numerous well defined bacilli which cannot be confused with the inclusion granules.

FIG. 2. An epithelial cell from a case of uncomplicated Koch-Weeks conjunctivitis. Around the periphery of the cell and near the nucleus along the lower border numerous bacilli are seen to be attached to, or contained within, the cell body. There is, however, no difficulty in recognizing the bacilli as such in this instance.

FIG. 3. A film preparation from a case of mixed infection of the Koch-Weeks and the inclusion organisms. In the field the epithelial cell is seen to contain a densely stained mass of the initial bodies near the upper right border of the nucleus and numerous Koch-Weeks bacilli to the left, especially where there is a leucocyte.

FIG. 4. This represents a twenty-four hour old pure culture of a thin type of the Koch-Weeks bacilli on blood agar.

FIGS. 5, 6, and 7. The appearance of the same organism as in Fig. 4 after 3 days, 5 days, and 8 days, respectively, on the same medium at 37° C.

FIGS. 8, 9, 10, and 11. These represent the appearance of a medium type strain of the Koch-Weeks bacilli after 24 hours, 3 days, 5 days, and 8 days, respectively, on blood agar at 37° C.

FIGS. 12, 13, 14, and 15. These show the appearance of a strain of round or coccoid type of the Koch-Weeks bacilli in a 24 hour, 3 day, 5 day, and 8 day growth on blood agar, respectively.

FIGS. 16, 17, 18, and 19. These show the appearance of a coarse strain of the Koch-Weeks bacilli in a 24 hour, 3 day, 5 day, and 8 day growth on blood agar, respectively. This strain resembles *B. influenza* more than the others.

FIGS. 20, 21, 22, and 23. These show 4 different strains of the Koch-Weeks bacilli isolated from the mixed infection cases in the present epidemic. They belong to the medium type.

FIGS. 24, 25, and 26. These show 24 hour growths of 3 different strains of *B. influenza* isolated from cases of pneumonia.

FIGS. 27 and 28. These show 24 hour growths of 2 different strains of *B. influenza* isolated from cases of meningitis. Both strains were kept on artificial media for several years and tend to form threads more than they originally did (Wollstein).

## PLATE 42.

FIGS. 29, 30, 31, 32, and 33. These show the inclusions at various stages of evolution in the film preparations made from a case (Tho.) of inclusion conjunctivitis. This patient had been suffering from the inclusion conjunctivitis when he was superinfected with the Koch-Weeks bacilli, which, in due course, disappeared from his conjunctiva leaving the original condition little affected. These inclusions shown here were found in the conjunctiva long after the Koch-Weeks bacilli had disappeared in smears or in cultures. The minute elementary bodies in Figs. 32 and 33 bear no resemblance to the degenerated forms of the Koch-Weeks bacilli.

FIGS. 34, 35, and 36. These represent the inclusion bodies in an uncomplicated case (Sh.), that is, without any association with the Koch-Weeks bacilli. The character of the initial bodies in Fig. 34 would definitely dispose of any suggestion as to their being the Koch-Weeks bacilli. Fig. 36 shows a still unburst intracellular aggregation of the elementary bodies.

FIGS. 37, 38, and 39. Inclusions from another case (Sat.) of pure inclusion conjunctivitis.

FIGS. 40, 41, and 42. Inclusions from a case (Bol.) of trachoma. The initial bodies as shown in Fig. 40 are exceptionally coarse.

FIGS. 43, 44, and 45. Inclusions from an uncomplicated case (For.) of inclusion conjunctivitis. In Fig. 43 a thick mass of the initial bodies is seen to embrace the left side of the nucleus of a cell on the left, while on the right numerous elementary granules are scattered around the cell below. Figs. 44 and 45 show the steps of evolution of the inclusion bodies.

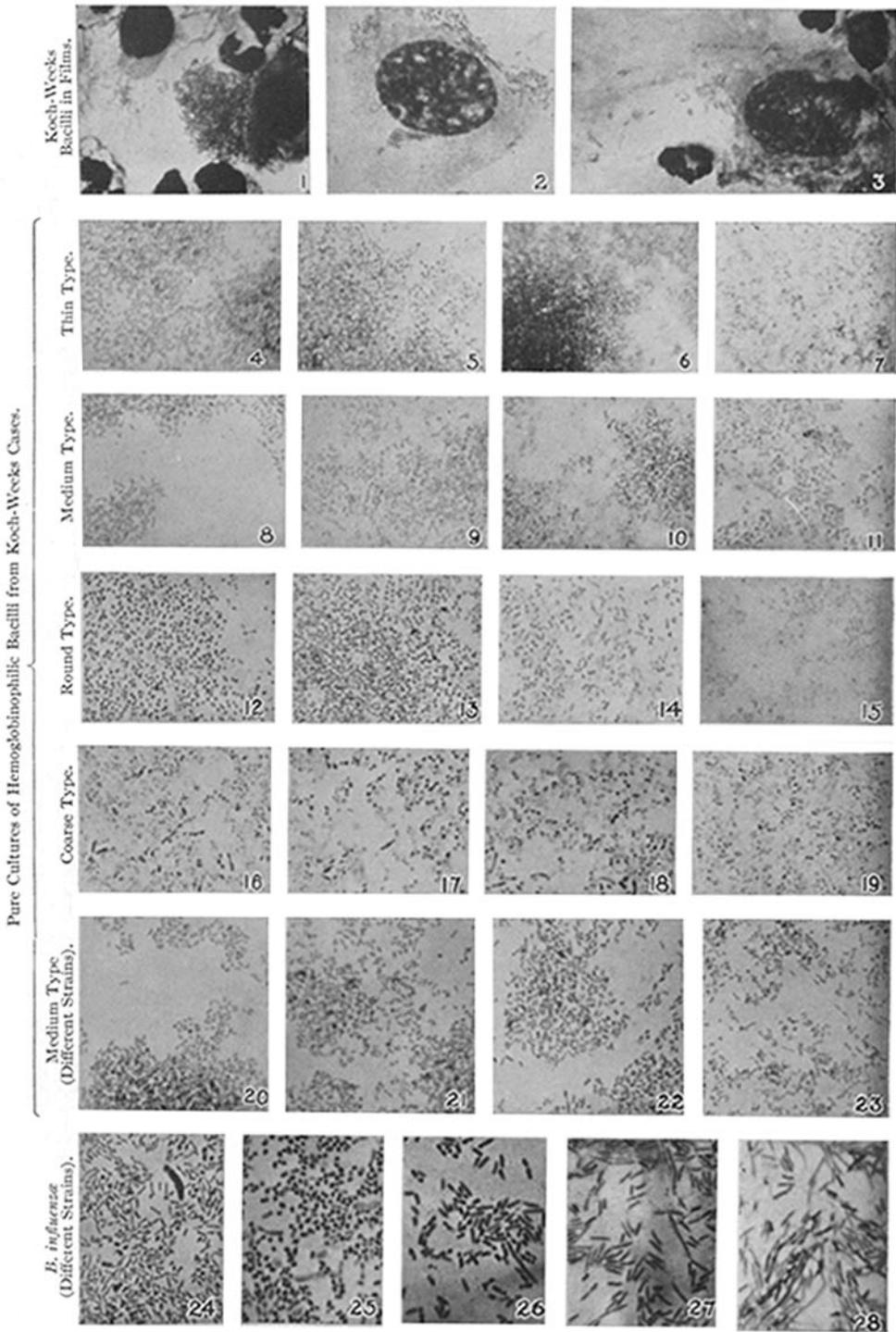
## PLATE 43.

FIGS. 46, 47, 48, 49, 50, and 51. Different stages of the evolution of the inclusion bodies and the conjunctiva of a baboon experimentally infected with the scrapings of the affected conjunctivæ of man. The structure of the initial

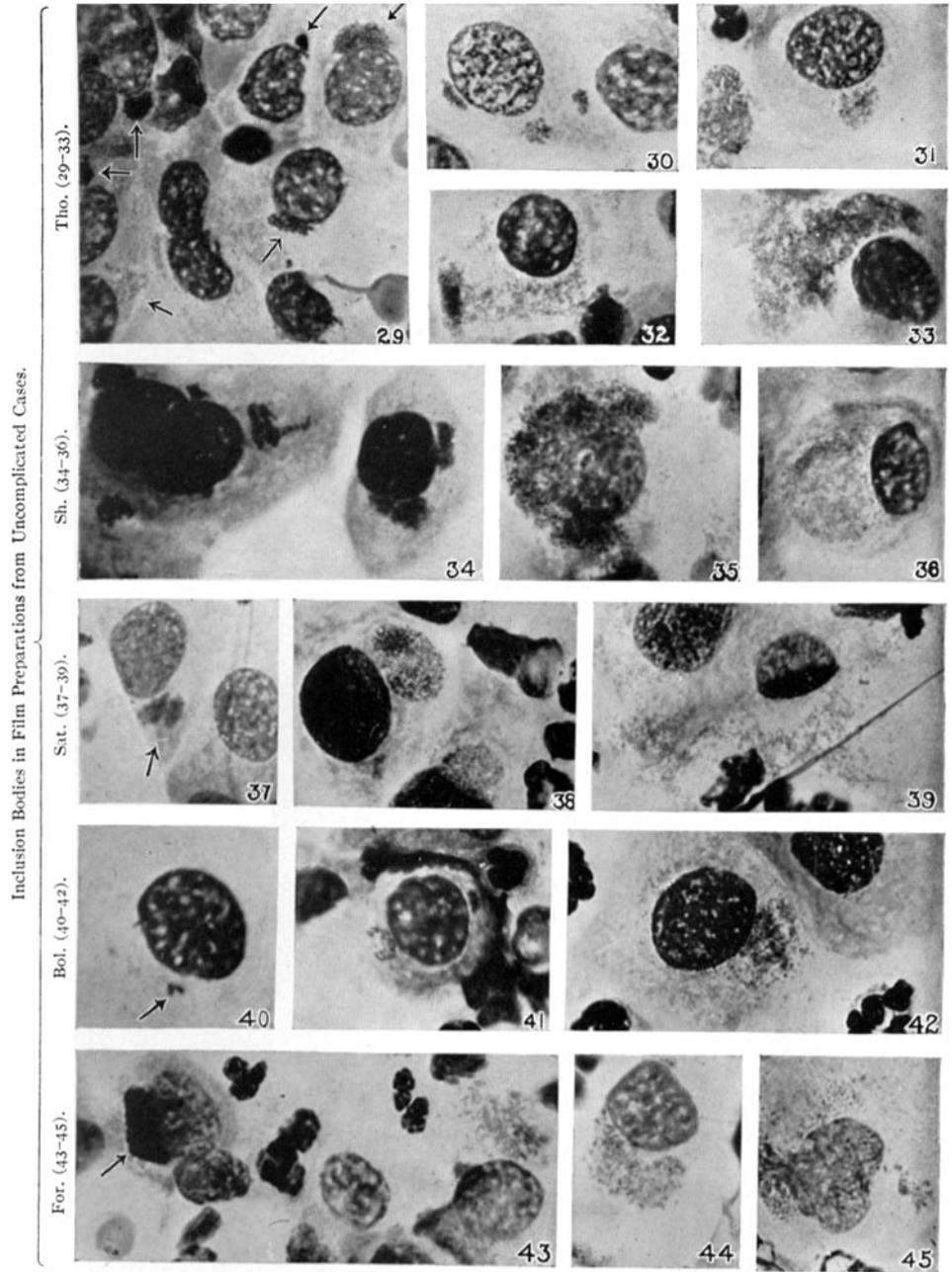
bodies in Figs. 46, 47, and 48 is quite peculiar and reveals no definite bacillary outlines. They are deeply stained, almost amorphous, and show indistinct coarse granules within the mass. The elementary bodies are even more minute than those in the human cases, as will be seen in the cell occupying the lower half of Fig. 48. In the same figure one sees an irregularly shaped mass of the initial bodies at the upper corner. In Figs. 49, 50, and 51 are shown the enormously distended cells in which the inclusion granules are rapidly multiplying.

FIG. 52. This shows a dense mass of deeply stained granules in a film preparation from a case of mixed infection. This mass appears to be more of the von Prowazek type of body than of the degenerated Koch-Weeks bacilli.

FIGS. 53, 54, and 55. Sections of the rabbits' testicles inoculated with pure cultures of Koch-Weeks bacilli isolated from the mixed infection cases during the present epidemic. Fig. 53 shows that the organisms assume a coccobacillary form when examined within twenty-four hours after they were injected into the testicle. In photography they appear somewhat like the initial bodies in the inclusion cases, yet in actual examination of the preparations they are not difficult to distinguish as the Koch-Weeks bacilli. They are freely capable of cultivation when a minute portion of the testicle is transferred to the blood agar, while this is not the case with the initial bodies of the inclusion organism. In Figs. 54 and 55 the sections removed after 3 and 6 days, respectively, are shown in which large masses of agglutinated and somewhat ill defined bacilli are found in clear spaces within the lumen of the testicular tubules. They, too, present an appearance not unlike that of the inclusion organism found in experimental conjunctivitis in monkeys, as well as in an epidemic in man. Here, again, a culture on blood agar is quickly differentiated from the other kind, as the Koch-Weeks bacilli at this stage still thrive very well in culture, while the pure inclusion material does not give any growth with the hemoglobinophilic bacilli.

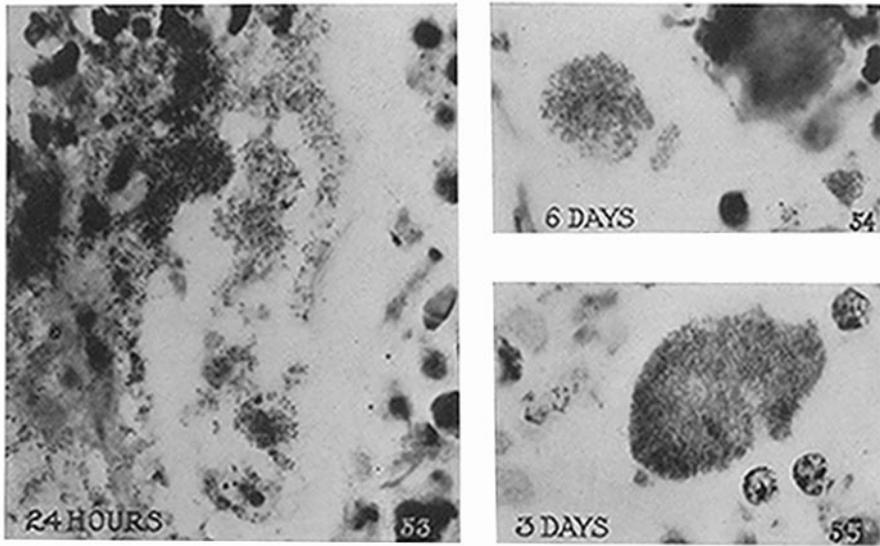
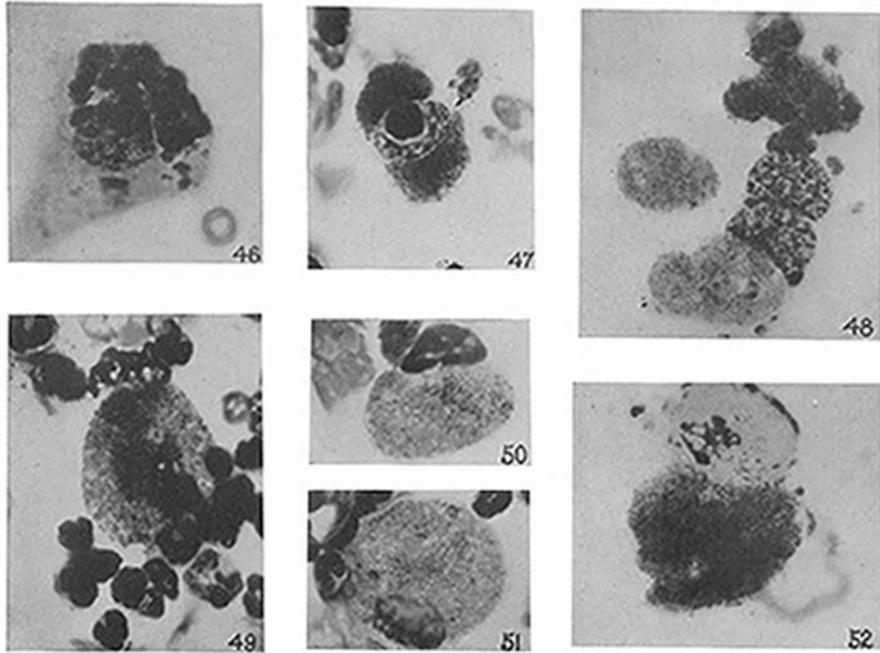


(Noguchi and Cohen: Epidemic of Koch-Weeks Bacillus Conjunctivitis.)



(Noguchi and Cohen: Epidemic of Koch-Weeks Bacillus Conjunctivitis.)

Experimental Inclusion Conjunctivitis in Baboon (46-51 .



Experimental Koch-Weeks Orchitis in Rabbit (53-55).

(Noguchi and Cohen: Epidemic of Koch-Weeks Bacillus Conjunctivitis.)