Manifestations of ocular irritation, indicative of conjunctivitis, are not infrequently observed in experimentally infected mice. The interpretation of this reaction is handicapped by the lack of information concerning its occurrence in animals maintained under normal conditions. An extensive and persistent outbreak of conjunctivitis in a colony of white mice afforded ample material for study and led to the present inquiry into its pathogenesis and epidemiology.

The Distribution and Manifestations of Conjunctivitis in the Colony of Infected Mice

Manifestations of conjunctivitis were first observed during the winter of 1947 in experimentally infected mice of the Princeton strain (non-Swiss). Inspection of the breeding colony, which numbered approximately 750 mice, revealed similar signs of ocular involvement in a high percentage of the recently weaned young. The newly born mice are held in the breeding cages until the 3rd week of life when they are weaned and segregated in groups of 20 according to sex. Through about the 5th week after birth the number of mice of both sexes which showed evidence of conjunctivitis at a given time varied from 20 to 80 per cent. Periodic examination indicated that spread of the disease was progressive and that most of the young mice were ultimately affected. The acute signs of conjunctivitis began to abate as the mice matured and by the 10th to the 12th week of life many of them appeared normal. In some animals, however, the reaction was persistent and was recognizable after a period of months.

In most instances even the acute signs of conjunctivitis are far from striking and unless specifically looked for might readily be missed. The eyelids are somewhat swollen and may be coated with a sticky exudate which tends to glue them loosely together. If the lids are open there is an increased amount of fluid, which may be clear or turbid. The volume of fluid which can be removed on aspiration is definitely increased in comparison to that removable from a normal mouse. The hair adjacent to the eye may be wet and matted, resulting in an encircling ring. Occasionally there is a slight encrusting with blood. During the acute period of the disease both eyes are commonly involved.
Two complications may occur but are relatively uncommon, the estimated incidence not exceeding 1 per cent. A few mice in the presence of acute conjunctivitis show prominent, nodular, subcutaneous swellings adjacent to the eye. This lesion has been observed only in young mice and always restricted to one side. An occasional mouse may show involvement of the eye itself which is distended outward with fluid. It usually becomes opaque and vision is lost.

The inflammatory reaction is limited to the eye and its appendages. In the absence of other infections there are no additional indications of illness, and at autopsy the respiratory tract, middle ears, and visceral organs are uniformly normal.

The Bacteriological Examination of Exudate from the Eyes of Infected Mice

Young Princeton mice showing obvious signs of conjunctivitis were removed directly from the breeding colony and anesthetized with ether. The tip of a capillary pipette was inserted beneath the eyelids and any fluid adherent to the conjunctiva and the outer surface of the eye was removed by suction. Air-dried films were prepared from the exudate after mixing it with a drop of water on a glass slide. These films were stained by the Gram method and also with the polychrome Wayson stain (1). This stain, which affords a color contrast, proved to be particularly useful and was employed as routine.

The first slides studied showed minute spherical bodies which stained blue with the Wayson stain and were Gram-negative. They were arranged in varying-sized, extracellular clumps and were also regularly present in or on the large, conjunctival, epithelial cells. More than 100 weaned mice with conjunctivitis have now been examined and all have shown similar bodies in exudate from the eye.

There was a considerable variation in the number of the bodies from animal to animal but in general they were numerous and readily found. Within the epithelial cell they were most commonly arranged in small clumps or distributed as discretely spaced particles over much of its surface (Fig. 1). There were also observed, at times, much larger intracellular structures which were circular or irregularly shaped and stained blue with the Wayson stain (Fig. 2). Some of these structures were compact and undifferentiated while others were granular in appearance and showed small discrete particles breaking away from the periphery. There is little question that they represent colony-like aggregates of the minute bodies. Polymorphonuclear leucocytes were commonly present, though generally few in number, and in some the small particles were clearly visible. The films also showed miscellaneous bacteria among which Gram-negative bacilli predominated and in some exudates were very numerous. These bacteria were not specifically identified.

The morphologic appearance of the minute bodies, particularly their variable arrangement within epithelial cells, was suggestive of an agent of the trachoma-infectious blennorrhea group. The outcome of cultural studies soon indicated, however, that the resemblance was purely superficial.

Conjunctival exudates were inoculated on a solid medium by expelling the contents of a capillary pipette into a drop of sterile saline solution which was then rubbed over its surface. The inoculated plates, which previously had been chilled in the refrigerator, were sealed with Scotch tape and incubated at 37°C for 5 to 7 days. The medium which was regularly used was
approximately 30 per cent horse serum-nutrient agar containing 2500 units of commercial penicillin (0.25 cc. of a stock 10,000 unit saline solution of potassium penicillin G).

The first exudates cultured showed small colonies barely visible without magnification. In subsequent examinations similar colonies were regularly obtained from exudates in which the minute bodies were observed microscopically. Practically all the inoculated plates showed a pure growth of these colonies, which were characteristic and uniform in appearance. In some instances a few large colonies, generally molds, were also present. A diffuse growth of minute, pleomorphic, Gram-negative bacteria was regularly obtained in horse serum-bouillon on transfer from the small colonies. These bacteria were undoubtedly identical with the minute bodies observed in films and were clearly representative of the pleuropneumonia-like group of organisms.

Biological Characteristics of the Associated Pleuropneumonia-Like Organisms

The presence of pleuropneumonia-like organisms in washings and cultures from the eyes of white mice has been reported by several workers.

In 1937 a communicable disease of white mice, termed infectious catarrh, was described by the writer (2). Its etiology was attributed to minute, pleomorphic, Gram-negative cells which were descriptively referred to as "coccobacilliform bodies." The term "pleuropneumonia-like organisms," originally introduced by Klieneberger (3) in 1935, has now become adopted, at least tentatively, as the name for bacteria of this particular morphologic type. Although the coccobacilliform bodies which were originally isolated from chickens (4), then from mice (2), and later from rats (5), showed certain cultural differences from the bacteria which are now recognized as pleuropneumonia-like organisms, the similarity between the two types of agents is so close that there is little reason for separating them.

Infectious catarrh was originally observed in 1935 in a group of Swiss mice which had been introduced from an outside source for temporary use (2). All experimental studies were made, however, in non-Swiss mice of the Princeton strain. Two forms of conjunctivitis were observed in the latter mice, the signs of the disease, as described, being identical with those in the present outbreak. One form was present in un.injected mice removed directly from the breeding colony. The second form, which differed from it only in the presence of polymorphonuclear leucocytes and coccobacilliform bodies in films from the eye, was observed in mice which had been injected intranasally with catarrhal exudates. The minute bodies were present in considerable numbers in the large epithelial cells and it was assumed that they were identical with those associated with infectious catarrh. A photomicrograph which was presented at that time is essentially duplicated by Fig. 1 of the present paper.

The coccobacilliform bodies described in 1937 from mice with infectious catarrh were cultivable only in the presence of substances extracted from 10-day chick embryo tissue. There was no evidence of growth in fluid, defibrinated horse blood at the base of slanted nutrient agar, the only medium containing blood which was then used. A second organism which was not pathogenic for mice was found to be irregularly present however in this medium, on inoculation with catarrhal exudates, and was readily maintained by transfer. This organism which resembled the coccobacilliform bodies in size and shape but formed large compact clumps on cultivation was referred to as the X bacillus (2).

Sabin (6), in 1939, noted that mice injected intraocularly with certain exudates (rheumatic
fever) and also with broth and normal synovial fluid yielded positive pleuropneumonia-like cultures from the eye with great regularity. Nutrient agar enriched with approximately 30 per cent horse serum was commonly used by this time for the cultivation of these bacteria. With the Rockefeller Institute (New York) Swiss stock at least 4 of each group of 6 mice (3 weeks old) gave positive cultures in ten different experiments. Further studies indicated that pleuropneumonia-like organisms are commonly found on the external surface of the eye under natural conditions. They are often present in large numbers and unassociated with other bacteria. Two other strains of mice, one Swiss and one non-Swiss, showed a similar condition but with a lower rate of carriage. Evidence was also obtained that the pleuropneumonia-like organisms were present on the mucosa of the nose and the accessory sinuses.

Additional observations of Sabin and Johnson (7) in 1940 indicated that the carriage of pleuropneumonia-like organisms by mice was probably not a transitory phenomenon, 6 strains being isolated from the conjunctiva and nasal mucosa of 4 out of 10 mature mice (6 months or older). It was believed probable that the carrier state was the result of contact infection acquired after birth, a positive culture being obtained from the nose of 1 out of 20 5 day old mice from infected mothers.

The morphologic and cultural characteristics of the pleuropneumonia-like organisms associated with the present outbreak of conjunctivitis and termed the “conjunctival strain” have been studied in some detail and compared with those of a strain associated with respiratory disease in mice and designated the “catarrhal strain.” Comment will be made only on differences in behavior of the two strains. The methods used were a duplication of those reported by us (8) and will not be redescribed.

Serum-agar plates inoculated with eye exudates or with fluid cultures showed minute colonies which reached their maximum size on the 4th to the 5th day at 37°C. If the suspensions were undiluted most of the colonies were closely packed and ranged in size from 25 to 100μ, with only an occasional larger form. With dilute suspensions the colonies were well spaced and regularly larger, varying from 150 to 300μ in diameter. Examined by transmitted light at a magnification of X 100 the larger colonies were distinctive in appearance, showing a foamy internal structure composed of small vacuoles and granules. There was a tendency for these components to be arranged as irregular streams which radiated outward from the center. A darker central area was sometimes observed but was not characteristic. The smaller colonies, under 100μ in diameter, commonly showed vacuoles and granules but not striations. In referring to these colonies, which were quite unlike the uniformly granular ones of the catarrhal strain, the term “radial type” will be used (Fig. 3).

Growth of the catarrhal strain in defibrinated horse blood at the base of slanted nutrient agar was followed by a slight degree of hemolysis. Films prepared from the fluid, 48 hours after inoculation, showed a sparse distribution of discrete bacteria. Growth of the conjunctival strain in this medium was not followed by hemolysis and in films the bacteria were arranged as tightly packed clumps of tiny spherical particles. These aggregates varied considerably in size, measuring up to 50μ in diameter, and were identical in appearance with those of the previously described X bacillus (2).

Microscopic preparations were made from 48 hour old horse serum-bouillon cultures of both strains. The bacteria were sedimented, washed once, and stained with Giemsa for 30 minutes. With pleuropneumonia-like organisms from cultures this method gave better results than did the Wayson stain. The conjunctival strain consistently showed a greater number of ring and small spherical forms with a corresponding decrease in the number of filaments and bacillary elements. In some preparations the latter forms were apparently absent.
Additional Observations on the Distribution of the Pleuropneumonia-Like Organisms in Mice

Cultural studies were made on several groups of mice of the Princeton strain to determine the relation of age and experimental handling to localization of the pleuropneumonia-like organisms and the relation of the latter to the periorbital nodules which occasionally accompanied the ocular reaction.

Examination of Adult Female Mice.—Pleuropneumonia-like organisms of the radial type were isolated from the eyes and the nasal passages of 18 (72 per cent) out of 25 females breeders, 8 to 10 months old. The amount of growth obtained from the nasal cultures was meager, the number of colonies being commonly less than 100. The lungs, middle ears, and nasal passages of these mice were normal. The eyes of 4 showed reduced signs of conjunctivitis.

Examination of Unweaned Mice.—No growth was obtained from the unopen eyes of 12 unweaned mice, 5 to 7 days old. Pleuropneumonia-like organisms were present in films from the eyes of the 5 breeders with which the young were in contact but signs of conjunctivitis were observed in only 1. Positive cultures were obtained from the open and inflamed eyes of 7 and from the nasal passages of 2 out of 14 unweaned mice, 15 to 18 days old. No growth was obtained from 6 with normal eyes and from 1 with conjunctivitis. Pleuropneumonia-like organisms were present in films from the eyes of the 5 breeders from the same cage. Two of these mice showed conjunctivitis.

Examination of Mice Injected Intranasally with Bacteria-Free Suspensions.—Radial type pleuropneumonia-like colonies were obtained from 31 (73 per cent) out of 42 nasal cultures and from 1 (2 per cent) out of 47 lung cultures from Princeton mice injected intranasally 4 to 5 weeks earlier with bacteria-free suspensions of rat lungs. The number of colonies varied from 10 or less (in 23) to 100 or less (in 9). Aside from conjunctivitis all these mice were normal at autopsy.

Examination of Mice Injected Intranasally with the Conjunctival Strain.—No growth was obtained from the lungs of 25 Princeton mice which had been injected intranasally 4 weeks earlier with eye exudate or cultures of the particular strain of pleuropneumonia-like organisms. None of these mice showed any involvement of the middle ears, lungs, or nasal passages. Fifteen (73 per cent) of the nasal cultures were positive but there was no apparent increase in the number of radial type colonies which varied from 10 to several hundred.

Examination of Mice Injected Intranasally with the Catarrhal Strain.—Mice injected intranasally with the catarrhal strain of pleuropneumonia-like organisms showed no apparent change in the behavior of these bacteria as a result of the superimposed eye infection. The nasal implantation was followed by a high rate of otitis media and lower rates of pneumonia and rhinitis. Fifty out of 54 middle ear cultures yielded a pure growth of the catarrhal strain, while 4 (7 per cent) showed a much scantier growth of the conjunctival strain.

Examination of Mice with Periorbital Nodules.—Twelve recently weaned mice in which the conjunctivitis was accompanied by unilateral nodule formation were removed from the breeding colony for examination. The position of the nodule in relation to the eye varied but more commonly was located along a line between the eye and the ear. Postmortem inspection indicated that these nodules, which ranged in size from 5 to 8 mm. with an elevation of 3 to 4 mm., were in effect subcutaneous abscesses containing a voluminous, thick, purulent material.

In the presence of penicillin a heavy pure growth of radial type pleuropneumonia-like colonies was obtained from the eyes of the 12 mice and a much scantier growth from 5 of the abscesses. In the absence of penicillin there was a dense growth of miscellaneous bacteria from both loci. Gram-negative bacilli, of which 3 cultural types were observed, predominated. Their distribution in each pair of cultures was usually the same but varied from mouse to mouse.
Transmission of the Pleuropneumonia-Like Organisms to Swiss Mice

The morbidity of conjunctivitis in mice of the Princeton strain has persisted at a high level over a period of 21 months. The manifestations of this condition were so commonly encountered in young mice and ocular carriage of the associated pleuropneumonia-like organisms was so high in older animals that it was impossible to use them in an experimental study of the disease. Attention was then focused on a colony of Swiss mice which has been maintained by this department for some years. It was found that these mice were neither subject to conjunctivitis, under the conditions which prevailed in the breeding colony, nor to carriage of the pleuropneumonia-like organisms.

The colony of normal Swiss mice is kept in a different building from that which houses the Princeton strain subject to conjunctivitis, but both are cared for by the same attendant. The size of the Swiss colony varies but it is generally operated with approximately 1000 breeders. The method of husbandry is the same with both colonies.

The Swiss mice were frequently inspected over a period of a year, particular attention being paid to the weaned young. Manifestations of the communicable conjunctivitis were never observed. By far the majority of the mice showed bright and wide-open eyes with no indication of local irritation. Occasionally a mouse with a distended and opaque eye, accompanied by increased lacrimation, was encountered but this condition was not transmissible and pleuropneumonia-like organisms were not associated with it.

Twenty-five Swiss mice, 4 to 5 weeks old, were removed at random from the cages of weaned young for cultural examination. In the presence of penicillin serum-agar plates inoculated with washings from the normal eyes of these mice were bacteriologically sterile.

Transmission by Direct Contact

The experimental transmission of conjunctivitis to Swiss mice was first attempted by direct exposure for varying periods to naturally infected Princeton mice.

In five experiments contact was established by placing 5 normal Swiss mice, 12 to 15 gm. in weight, in the same cage with an equal number of Princeton mice with acute conjunctivitis, and in one experiment with a single diseased animal. The originally infected mice were removed after an interval of 3 to 4 weeks and autopsied. Each group of the exposed Swiss mice was held under observation for an additional period of 1 to 24 days and then killed. At this time stained films or cultures were made from the eyes.

The results of the six experiments are summarized, in part, in Table I. Conjunctival films from all the 26 infected Princeton mice showed pleuropneumonia-like organisms together with numerous miscellaneous bacteria among which Gram-negative bacilli predominated. Marked signs of conjunctivitis were observed in 22. Pleuropneumonia-like organisms were also present in the eye washings from 29 of the 30 exposed Swiss mice (96 per cent). An ocular reaction, limited to increased lacrimation, was observed in only 4 (13 per cent). The eye films from these mice showed comparatively few secondary bacteria. During the intervening period between exposure and autopsy
a few additional Swiss mice showed a transient watering of the eye but at no
time was there any evidence of an acute inflammatory reaction. At autopsy,
however, 15 of these mice did show an increased volume of conjunctival fluid
and this contained a few polymorphonuclear leukocytes. Seven of the 10 ex-
posed mice of groups 4 and 5 yielded a scanty growth of pleuropneumonia-
like colonies of the conjunctival type on culture from the nasal passages.

Stained films from the eyes of the 15 Swiss mice of groups 1, 2, and 3, made
before contact was established, had shown no pleuropneumonia-like organisms.
Films were again made on the 7th day of contact and at this time the organisms
under consideration were present in 9. Two films from group 2 and 4 from

<table>
<thead>
<tr>
<th>Group No.*</th>
<th>Time between exposure and autopsy</th>
<th>No. of mice with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Conjunctivitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>1</td>
</tr>
</tbody>
</table>

* 5 mice were exposed in each group.

group 3 were negative. The presence of these bacteria was not accompanied by
detectable signs of conjunctivitis.

A second transmission experiment involving contact between previously
exposed and normal Swiss mice was begun with the 6th group of Table I and
maintained for 10 passages.

Five normal Swiss mice were placed in the same cage with the 6th group of infected Swiss
mice and left with them for 40 days. This procedure was repeated 10 times in all as indicated
in Table II. At the end of each passage the previously exposed mice were autopsied and the
conjunctival washings examined microscopically or culturally. In some instances pleu-
ropneumonia-like organisms were also looked for during the period of contact.

The results of this experiment are summarized in Table II. In all, 44 of the
50 Swiss mice (88 per cent) exposed to infection during the 10 passages showed
pleuropneumonia-like organisms in the conjunctival washings. In most of these
fluids they were numerous whereas miscellaneous bacteria were few in num-
ber and were generally limited to Gram-positive micrococci. The volume of
the fluid was increased in 20 of the mice and polymorphonuclear leukocytes
PLEUROPNEUMONIA-LIKE ORGANISMS AND CONJUNCTIVITIS

were observed in 14. However, only 3 of the 50 mice (6 per cent) showed a definite conjunctivitis. A slight watering of the eye was sometimes noted during the period of exposure but was generally of transient duration. At autopsy the lungs, middle ears, and nasal passages of these mice were uniformly normal.

An additional contact experiment was carried out in Swiss mice to determine whether the disease and its associated bacteria were communicated by maternal transfer.

A normal, female, Swiss mouse with 6 nursing young, 7 days old, was placed in the same cage with 4 infected Princeton mice. Microscopic examination was made of conjunctival washings from both groups of mice and from 3 selected generations of Swiss mice which were subsequently reared from the young first exposed.

On the 15th day after contact was established pleuropneumonia-like organisms were demonstrable in eye films from the 4 Princeton mice, from the Swiss female, and from 5 of her 6 young. Pleuropneumonia-like organisms were also present in conjunctival washings from 2 of 5 mice in the first subsequent generation, from 3 of 6 in the second, and from 5 of 7 in the third. Approximately half of the eye films from the 25 exposed Swiss mice in this series showed a few polymorphonuclear leucocytes but only 3 of the mice (12 per cent) showed definite signs of conjunctivitis.

Transmission by Nasal and Conjunctival Instillation

The preceding experiments indicated that the exposure of normal Swiss mice to infected Princeton mice resulted in a high ocular carrier rate of pleuro-

TABLE II

Results of the Exposure of Normal Swiss Mice to Swiss Mice Infected with Pleuropneumonia-like Organisms and the Maintenance of Infection by Passage

<table>
<thead>
<tr>
<th>Passage No.*</th>
<th>Time in contact</th>
<th>No. of mice with Conjunctivitis</th>
<th>Pleuropneumonia-like organisms</th>
<th>Leucocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>56</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>46</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

* 5 mice were exposed in each passage.
pneumonia-like organisms but a low rate of conjunctivitis. Two series of experiments were conducted to determine the outcome of nasal and conjunctival instillation in relation to the pathogenesis of the disease and the establishment of the associated bacteria.

Three groups of 5 normal Swiss mice 12 to 15 gm. in weight were injected intranasally, using ether anesthesia, with approximately 0.05 cc. of a saline suspension of pooled conjunctival washings from 5 Princeton mice with conjunctivitis. Three additional groups were similarly injected with 48 hour old, 30 per cent horse serum-bouillon cultures of 3 radial type pleuropneumonia-like organisms recently isolated from the eyes of infected Princeton mice. The injected mice were inspected frequently for a period of 3 to 4 weeks. At autopsy Wayson-stained films of conjunctival washings from each mouse were examined microscopically. Thirty per cent horse serum-agar plates containing penicillin were inoculated with individual nasal and conjunctival washings and with a suspension of the pooled lungs from each of the 6 groups of 5 animals. The inoculated plates were incubated at 37°C. for 7 days and examined microscopically at a magnification of X 100.

The results of the 30 intranasal tests were completely negative. None of the injected mice showed any indication of conjunctivitis during the period of observation. At autopsy the eyes were uniformly normal and there was no involvement of the lungs, middle ears, or nasal passages. The conjunctival films showed no pleuropneumonia-like organisms and all cultures were sterile in respect to these bacteria.

 Conjunctival instillations were made bilaterally in normal Swiss mice, using ocular washings from infected Princeton mice and recently isolated cultures of the pleuropneumonia-like organisms. Prior to injection the mice were anesthetized with ether and a small amount of the suspension was introduced under the eyelids with a fine capillary pipette. With each type of inoculum this was done only once in 10 mice but 5 times at intervals of 2 to 3 days in 6 mice. The injected mice were frequently inspected for a period of 2 to 4 weeks and then killed. At autopsy pleuropneumonia-like organisms were looked for microscopically in conjunctival films and culturally on serum-agar plates inoculated with individual ocular and nasal washings and with pooled lung suspensions from each group of mice.

Negative findings were again obtained from the Swiss mice which had received a single conjunctival instillation of ocular washings and a single and multiple instillation of pleuropneumonia-like organisms in culture. The 26 mice used in these 3 tests showed no signs of conjunctivitis during the period of observation or at autopsy. The lungs, middle ears, and nasal passages were normal. Pleuropneumonia-like organisms were not demonstrable in films or in cultures from the eyes, nasal passages, or lungs.

The 6 Swiss mice which had received multiple instillations of ocular washings showed no persistent signs of conjunctivitis either during the period of treatment or subsequent to it. A transient watering of the eyes was noted in 2 of the mice. Numerous pleuropneumonia-like organisms were observed, however, in stained conjunctival films made 7 to 14 days after the last instillation. Leucocytes were present in 4 of the films examined on the 7th day and in 3 on the 14th day. Cultures of ocular washings which were made from

Downloaded from on June 24, 2017

Published March 1, 1950
each of the 6 mice on the 14th day yielded a heavy growth of pleuropneumonia-like organisms. Cultures from the nasal washings were likewise positive but the number of colonies was much reduced.

DISCUSSION

The biological characteristics of the pleuropneumonia-like organisms isolated from the eyes of mice with conjunctivitis were sufficiently uniform and distinctive to warrant classifying them as a special strain. The most significant difference between these organisms and the ones associated with infectious catarrh was that of their localization in the host. The conjunctival strain was essentially restricted to the appendages of the eye and the nasal passages, whereas the catarrhal strain tended to multiply throughout the respiratory tract and particularly in the middle ears. The sparse growth which was commonly obtained in cultures from the nasal passages of mice with conjunctivitis suggested the survival of organisms carried there from the eye rather than actual multiplication.

It is probable that the ocular inflammation which was observed in 1935 (2) in mice with infectious catarrh was identical with the presently described conjunctivitis and that the so called X bacillus which was encountered in horse blood agar cultures was actually a pleuropneumonia-like organism of the conjunctival strain. The relation of this strain to the pleuropneumonia-like organisms isolated by Sabin (6) from the external surface of the eyes of supposedly normal mice was less obvious. The morphologic and colonial characteristics of the respective organisms were somewhat different and the presence of those which he described was apparently not accompanied by conjunctivitis.

Observations on the mode of transmission indicate that conjunctivitis and the associated pleuropneumonia-like organisms are both acquired by direct ocular contact with fluids discharged from the eyes and nasal passages of infected mice. It is evident that conjunctival implantation of the organisms regularly precedes their nasal establishment. Ocular carriage of these bacteria occurs in a high percentage of adult animals though often in the absence of detectable signs of conjunctivitis. Unweaned mice are exposed to the disease by direct contact with adults and, as noted earlier by Sabin and Johnson (7), some of them become infected during the ensuing period of nursing. It is probable, however, that ocular development of the pleuropneumonia-like organisms does not occur until after the eyes are open. An additional opportunity for spread of the infection comes at the time the weaned mice are brought together from different breeding cages. The combined periods of exposure, before and after weaning, ultimately result in a morbidity rate which must be close to 100 per cent.

The outcome of the transmission experiments in Swiss mice brings further evidence that the pleuropneumonia-like organisms associated with conjunctiv-
Conjunctivitis have special affinity for the conjunctival mucosa. The low rate of ocular reaction detectable microscopically in mice infected with these organisms was unexpected and afforded only partial support for their conjectured etiological relation to the disease. It is of interest in this connection that the high recovery rate of pleuropneumonia-like organisms reported by Sabin (6) from the external surface of the eyes of Swiss mice was apparently not accompanied by manifestations of conjunctivitis.

In view of the ease with which pleuropneumonia-like organisms were recovered from the conjunctiva of naturally and experimentally exposed mice it is of interest that the organisms were so difficult to establish by artificial instillation. It is probable that many were rapidly eliminated from the conjunctiva by mechanical or biological means and that persistence may occur only after repeated implantation. The pleuropneumonia-like organisms which survived artificial introduction were implanted by the multiple conjunctival instillation of undiluted ocular washings. These results suggest that conjunctival establishment of the organisms, under natural conditions, is brought about by repeated contact aided by the presence of some protective substance, as mucin, in the conveying particles or droplets.

An attempt has been made to rear a group of infection-free mice of the Princeton strain. Several generations of specially selected mice have now been under observation for a period of 6 months and have shown no indication of ocular or nasal carriage of pleuropneumonia-like organisms. It is hoped that they can ultimately be used to study more conclusively the relation of these bacteria to conjunctivitis.

**SUMMARY**

An outbreak of conjunctivitis, unaccompanied by involvement of the respiratory tract, is reported in a colony of white mice.

A special strain of pleuropneumonia-like organisms was regularly isolated from the eyes and nasal passages of affected mice but not from the lungs or middle ears.

Ocular carriage of these organisms in the absence of an inflammatory reaction occurred in at least 50 per cent of the adult mice.

Transmission to the young was presumably initiated by parental contact, the organisms being recoverable after the eyes were open, and was continued after weaning by direct contact between cage mates.

These organisms were repeatedly established on the conjunctiva of normal Swiss mice by direct contact with infected animals and subsequently maintained there for ten successive passages.

Multiplication of the pleuropneumonia-like organisms, which was largely limited to the eye and its appendages, was accompanied by a low rate of conjunctivitis.
The multiple conjunctival instillation of ocular washings from infected mice was the only additional method of implantation of the organisms which was successful.

BIBLIOGRAPHY

EXPLANATION OF PLATE 15
Photographs by Mr. J. A. Carlile.
Fig. 1. Discretely arranged pleuropneumonia-like organisms in large epithelial cell from the conjunctiva of an infected mouse. Wayson stain. × 1560.
Fig. 2. Colony-like groupings of the organisms in a similar cell. Wayson stain. × 1560.
Fig. 3. Colonies of the conjunctival type of pleuropneumonia-like organisms. 4 day old serum-agar plate. × 100.