QAL MILES

NELSON (1948)

Reprinted from the Journal of Infectious Diseases
March-April, 1948, Vol. 82, pp. 169-176

THE NASAL TRANSMISSION OF PLEUROPNEUMONIA-LIKE ORGANISMS IN MICE AND RATS

JOHN B. NELSON

From the Department of Animal and Plant Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.

Recognition of the virus-like agent associated with endemic pneumonia of the albino rat has been largely dependent on its behavior in mice following nasal instillation of lung suspensions.[1,2] The appearance of pneumonia and otitis media in mice after direct transfer from the rat proved to be reasonably sound indication of the presence of the agent. The serial passage of lung suspensions in mice introduced, however, an opportunity for the development and carriage of intercurrent infection. During the course of one passage series, in which normal lung was being used as an appropriate control, a secondary disease likewise characterized by pneumonia and otitis media was indeed encountered. At autopsy, however, pleuropneumonia-like organisms were isolated from the lungs and middle ears of the infected mice. The general resemblance between the two diseases prompted further study of the associated bacteria. The resulting observations which pertain to the behavior of pleuropneumonia-like organisms on nasal instillation in mice and rats add an emphasis to their activity not brought out in the work of others and are presented as a supplementary appraisal of these bacteria.

Since the introduction of the term pleuropneumonia-like organisms by Klieneberger[3] in 1935, she and others have carried out extensive studies on the habitat, identification, and pathogenicity of these bacteria. This work was reviewed by Sabin[4] in 1941 and comparatively little information has been added since that time. Mention will be made here only of those studies that bear directly on the present work.

Klieneberger and Steabben[5] were the first workers to show that pleuropneumonia-like organisms are associated with pneumonia in rodents. In 1937 they isolated such bacteria from the lung lesions of albino rats. This work was extended by them[6] in 1940 but was confined to pulmonary examination. They again recovered the organisms from the lung, both in the presence and absence of lesions, but were unable to reproduce pneumonia in the rat by the experimental introduction of pure cultures.

Sabin[7] in 1939 and Sabin and Johnson[8] in 1940 reported the isolation of pleuropneumonia-like organisms from the conjunctiva and nasal mucosa and occasionally from the trachea and lung of normal carrier mice. Sullivan and Dienes[9] in 1940 observed pneumonia in mice anesthetized with ether, while making blind nasal passages of normal mouse lung at intervals of 4–6 days. Pleuropneumonia-like organisms were recoverable from the lungs but the pathogenicity of these cultures was apparently not determined.

Edward[10] in 1940 likewise observed pneumonia in mice following the nasal injection of a normal lung suspension. In two subsequent passages 10 of 11 mice showed lung lesions at autopsy on the 10th day. Three strains of pleuropneumonia-like organisms were isolated from the lungs of these mice. The second subcultures of the three strains produced pneumonia in three, two, and 4 of 5 mice, respectively, on nasal injection. A loss in pathogenicity was noted on continued cultivation: the 7th subculture of one strain produced

Received for publication October 9, 1947.

1. Nelson, J. B. 1946, J. Exp. Med. **84:** 7–14.
2. Nelson, J. B. 1946, J. Exp. Med. **84:** 15–23.
3. Klieneberger, E. 1935, J. Path. and Bact. **40:** 93–105.
4. Sabin, A. B. 1941, Bact. Rev. **5:** 1–67.
5. Klieneberger, E., and Steabben, D. B. 1937, J. Hyg. **37:** 143–152.
6. Klieneberger, E., and Steabben, D. B. 1940, J. Hyg. **40:** 223–227.
7. Sabin, A. B. 1939, Science **90:** 18–19.
8. Sabin, A. B., and Johnson, B. 1940, Proc. Soc. Exp. Biol. and Med. **44:** 569–571.
9. Sullivan, E. R., and Dienes, L. 1940, Proc. Soc. Exp. Biol. and Med. **41:** 620–622.
10. Edward, D. G. ff. 1940, J. Path. and Bact. **50:** 409–418.

pneumonia in two of 6 mice, but the 15th subcultures of the other two strains resulted in pneumonia in only one of 11 mice injected.

Horsfall and Hahn[11] in 1940 encountered pleuropneumonia-like organisms in the pneumonic lungs of mice during their work with the virus of mouse pneumonia. These organisms were isolated from mice both in the presence and the absence of the virus and also in the presence of influenza virus. They were not further studied.

Andrewes and Welch[12] in 1946 reported the recovery of pleuropneumonia-like organisms from the pneumonic lung of one of several mice originally injected with unrelated material. Mice inoculated intranasally with serum-broth cultures of this strain, which proved to be motile, showed lesions in the lung when killed 6–23 days later.

MATERIALS AND METHODS

The mice used in the following experiments were from a colony maintained at The Rockefeller Institute in Princeton and designated the Princeton strain. This colony was started in 1922 with breeders obtained from a local dealer. It has undergone a number of epizootics involving the central nervous system and the digestive tract but has consistently escaped communicable involvement of the respiratory tract. Prior to 1945 attempts to isolate pleuropneumonia-like organisms from the eyes and the respiratory tract of mice removed directly from the colony consistently failed.

The albino rats were from the selected colony started by Nelson and Gowen[13] in 1931 and maintained uninterruptedly with no outside additions. Rats from this colony have shown no disease of the respiratory tract other than endemic pneumonia and have been consistently free from infection with pleuropneumonia-like organisms.

At autopsy the lungs were routinely removed and examined with the aid of a dissecting microscope. The middle ears and nasal passages were exposed and aspirated with a capillary pipette. Lung and exudate suspensions were prepared in a concentration of approximately 10% using normal saline. In dealing with normal animals the entire lung was used; with pneumonic animals the involved portions of the lung were generally selected. The lung tissue was finely minced with scissors and ground by hand in a glass tissue grinder. Nasal injections were made in mice weighing 15–18 g and in rats two to three months old, 5 animals being used with each experiment. The suspensions were dropped on the nares of the animals, previously anesthetized with ether, using a syringe and needle. The volume of the inoculum was usually 0.05 cc for mice and 0.1 cc for rats. The injected animals were maintained in isolation units for a period of at least 4 weeks.

Initial cultures from injected animals were made on solid medium using a loopful of suspension. The medium employed was veal infusion agar, p_H 8, reinforced with 30% horse serum. The inoculated plates were sealed with scotch tape to reduce evaporation and incubated at 37 C for 5–10 days. They were examined every other day at a magnification of 100× using a compound microscope. Transfers were made by cutting out a block of agar on the stage of a dissecting microscope and rubbing it over the surface of freshly prepared solid medium or dropping it in fluid medium. Two forms of the latter were used, a column of one cc of defibrinated horse blood at the base of a nutrient agar slant and 30% horse serum bouillon (volume 7 cc). Transfers were made at two to three day intervals from horse blood agar cultures and at four to five day intervals from horse serum bouillon. The horse blood was drawn at the Institute in Princeton, being renewed every 2nd week. Serum removed while the blood was fresh did not produce the type of lung reaction described by Thomas and Kolb[14] following the nasal injection of human blood serum in mice.

The microscopic examination of cultures and exudates was made with air-dried films which were Gram stained using carbol fuchsin diluted 1:3 with distilled water as the counterstain.

EXPERIMENTAL

Results of the examination of mice injected with lung suspensions.—In order to control the passage of lung suspensions from mice infected with the virus-like agent of the albino rat, a series was begun in January 1942 in which 5 mice were injected intranasally with a suspension of normal lung from mice removed directly from the stock colony. These passages were continued at intervals of approximately a month until early in 1944, 24 transfers being made.

11. Horsfall, F. L., and Hahn, R. G. 1940, J. Exp. Med. **71**: 391–408.
12. Andrewes, C. H., and Welch, F. V. 1946, J. Path and Bact. **58**: 578–580.
13. Nelson, J. B., and Gowen, J. W. 1931, J. Exp. Med. **54**: 629–636.
14. Thomas, L., and Kolb, E. M. 1944, Proc. Soc. Exp. Biol. and Med. **55**: 1–4.

The lungs and middle ears of the 120 mice which were included in this series were uniformly normal at autopsy.

Work was not resumed until January 1945 when a second series was begun and maintained as before. All of the injected mice of the first 13 passages were normal at autopsy. Evidence of intercurrent disease appeared in the 14th passage, a single mouse showing pulmonary lesions when killed. The subsequent course of events is summarized in table 1.

During the outbreak of pleuropneumonia chattering was observed and some of the infected mice failed to gain weight. Except during this period, chattering was not apparent, all of the mice appeared normal, and there was a marked gain in weight. There were no fatalities in either series of passages.

TABLE 1.—*Findings in a mouse passage series begun with normal lung.*

Mouse No.	No. of passage with condition of lung and middle ear at autopsy															
	13		14		15		16		17		18		19		20	
	L	ME	L	ME	L	ME	L	ME	L	ME	L	ME	L	ME	L	ME
1	−	−	−	−	−	−	+*	+	−	+	−	+	−	+	−	−
2	−	−	−	−	−	−	+	+	+	−	−	+	−	+	−	−
3	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	−
4	−	−	−	−	−	−	−	−	+	−	−	+	−	+	−	−
5	−	−	+	−	−	−	+	+	+	+	−	+	−	+	−	−

L = lung; ME = middle ear.
* + indicates pneumonia or otitis media.

The experimental production of pleuropneumonia in mice

A. *By the injection of lung suspensions and middle ear exudate.*—The disease which appeared during the course of the normal lung passage was readily established in mice by the nasal instillation of pneumonic lung suspension and middle ear exudate. It was also readily maintained in mice by the nasal transfer of middle ear exudate, 9 passages being made at monthly intervals. Recovery of pleuropneumonia-like organisms was sufficiently constant, in the absence of any other pathogenic agent, to warrant the diagnosis of rodent pleuropneumonia and this term will be used from here on. At autopsy the scope of the

TABLE 2.—*Comparative rates of pneumonia, otitis, and rhinitis in mice injected intranasally with suspension of exudate from the middle ear.*

Source of inoculum	No. of mice examined	Rate, in %, of		
		Pneumonia	Otitis	Rhinitis
Rat pneumonia (virus-like agent)	100	96	94	31
Mouse pleuropneumonia	100	54	84	16

disease paralleled that of endemic pneumonia in mice, being limited to the lung, middle ears, and nasal passages. However, the comparative rates of involvement, which are presented in table 2, indicated a significant difference in the rate of pneumonia. The bacteriological findings were also quite different; to date pleuropneumonia-like organisms have never been isolated from mice infected with the virus-like agent.

Involvement of the middle ears was commonly bilateral and indicated by the presence of a copious purulent exudate. Pneumonia was generally manifested by the complete consolidation of one or more lobes. It was less regularly restricted to the right middle and azygous lobes than in the disease produced by the virus-like agent. Involvement of the nasal passages was indicated by a definite mucopurulent exudate. Exudate from both the middle ears and nasal passages showed numerous polymorphonuclear leucocytes on microscopic examination. Morphologic forms typical of the pleuropneumonia-like organisms were often demonstrable in Gram-stained films. The presence of Gram-negative granules and rods within leucocytes was a valuable aid in the differential diagnosis of pleuropneumonia and the disease produced by the virus-like agent. Similar microscopic elements were never observed in exudate films from mice infected with the latter disease.

The mortality in mice during the period of observations, which was occasionally extended to two months, was low. Ten deaths occurred in a total of 110 mice injected with lung or exudate suspensions. The data presented in table 2 were compiled from the survivors. Only 1 of the 100 mice which were examined failed to show any evidence of involvement at autopsy.

Chattering was the only sign of the disease which was at all regular. It was usually audible by the 3rd week after injection but was by no means constantly noted. Many of the injected mice showed no other indication of disease and gained weight normally during the period of observation.

B. *By the injection of pure cultures.*—Pleuropneumonia was also readily produced by nasal instillation of the specific organisms in pure culture. The manifestations of the resulting disease largely duplicated those produced by the injection of lung and exudate suspension. Pleuropneumonia-like organisms were again recoverable from the loci of involvement. The disease which resulted from nasal transfer of the organisms in pure culture was likewise maintained by passage.

TABLE 3.—*The transmission of pleuropneumonia by direct contact.*

Experiment No.	No. of mice injected	No. of mice with			No. of contact mice	No. of days in contact	No. of mice with		
		Pneumonia	Otitis	Rhinitis			Pneumonia	Otitis	Rhinitis
1	5	2	3	1	5	28	0	0	0
2	5	2	5	0	5	28	0	0	0
3	5	3	4	1	5	45	0	1	0
4	5	1	5	0	5	50	0	0	0
5	5	2*	4	0	5	60	0	0	0
6	5	1	3	1	5	64	0	3	0

* 1 mouse died during the period of contact.

Six different cultures of the pleuropneumonia-like organisms isolated from the mice injected with lung or exudate suspensions were tested in mice, 5 animals being used with each culture. The first transfer to a fluid medium from the initial serum agar plate was employed in each instance. Five of the cultures were 48-hour growths in horse blood agar and one was a 72-hour growth in horse serum broth. The mortality which followed the nasal injection of these cultures was somewhat greater than that which attended the injection of lung and exudate suspensions, 26 as compared with 10%. Otherwise the results of the two series were much the same. The disease produced by the first pure culture isolated was continued in mice for 6 consecutive passages.

Two pure cultures of the pleuropneumonia-like organisms were injected intranasally in mice after 20 transfers in horse blood agar. There was no significant change in their activity as the result of continued cultivation. Two of the 10 mice died. Four weeks after injection all of the 8 survivors showed otitis at autopsy and 4 of them pneumonia.

C. *By direct contact.*—As indicated in table 3 pleuropneumonia was not readily communicable by direct contact. The morbidity rate in the originally injected mice was nearly 100% whereas the corresponding rate in the mice which were exposed to them was 13%. The only manifestation encountered at autopsy in the contact mice was an inflammation of the middle ear from which the specific organisms were isolated. It may be of significance that the two groups of exposed mice which showed evidence of disease at autopsy were in contact with infected ones which included one mouse with nasal involvement.

In the 6 contact experiments, 5 mice were injected intranasally with active lung or exudate suspensions and 5 normal mice placed immediately in the same cage. At the end of the exposure period all of the mice were killed and autopsied. One of the injected mice in experiment 5 died before this period was ended. In addition to the middle ear cultures from the 4 contact mice which showed otitis, cultures were also made from the pooled lungs of the exposed mice in experiments 4, 5, and 6 and of the pooled nasal washings from the contact mice in experiment 2. Pleuropneumonia-like organisms were obtained from the middle ear cultures but not from the lung and nasal cultures. The specific organisms were demonstrable in middle ear exudate from the originally injected mice of all 6 experiments.

The infectivity of nasal suspensions

In the preceding work on rodent pleuropneumonia all of the nasal injections were made with suspensions of pneumonic lungs or of exudate from the middle ears. The specific organisms were commonly demonstrable in cultures from both loci and the activity of the corresponding suspensions was comparable. Involvement of the nasal passages, indicated by the presence of a mucopurulent exudate, was much less commonly encountered. Two additional transmission experiments were made to determine the infectivity of suspensions from the nasal passages in the presence and in the absence of a local inflammatory reaction.

TABLE 4.—*The comparative infectivity of nasal exudate and nasal washings.*

Experiment No.	Mouse No.	Examination of mice from which the nasal suspensions were made			Nature of the suspension	Mouse No.	Examination of mice infected with the nasal suspensions		
		Pneumonia	Otitis	Rhinitis			Pneumonia	Otitis	Rhinitis
1	1	+	+	−	Nasal exudate	1	+	+	+
	2	+	+	+		2	+	+	+
	3	+	+	+		3	+	+	+
	4	+	+	+		4	+	+	+
	5	+	+	+		5	+	+	+
2	1	+	+	−	Nasal washings	1	−	+	−
	2	+	−	−		2	−	+	−
	3	+	+	−		3	+	+	−
	4	−	+	−		4	−	−	−
	5	−	+	−		5	−	+	−

The results of these experiments, which are summarized in table 4, indicated that both nasal washings and nasal exudate were infective on nasal instillation in mice and that pleuropneumonia-like organisms could be recovered from the upper air passages in the absence of any apparent local reaction. From the findings of an experiment which is not reported in detail it may be noted, in this connection, that the specific organisms were not demonstrable in the nasal passages before the 2nd week after injection although they were present in the lung at the end of the 1st week.

The nasal exudate used in experiment 1 was obtained from a group of 5 mice killed 4 weeks

after the nasal injection of a pneumonic lung suspension. At autopsy, 4 of the mice showed a moderate amount of mucopurulent exudate in the nasal passages. Pleuropneumonia-like organisms were demonstrable in Gram-stained films from 2 of the animals and were recovered from the pooled exudates cultured on serum agar plates. Numerous leucocytes were present in films from each of the 4 mice. On subsequent injection pleuropneumonia was produced in each of 5 mice.

The nasal washings used in experiment 2 were obtained from 5 mice injected with middle ear exudate and killed 4 weeks later. The nasal passages were normal at autopsy and saline washings showed neither leucocytes nor the specific organisms. Pleuropneumonia-like colonies were demonstrable, however, on serum agar plates from 4 of the suspensions. They were few in number, varying from 5 to 10 per plate. The pooled nasal washings produced pleuropneumonia in 4 of 5 mice on nasal injection.

Cultural and morphological characteristics of the pleuropneumonia-like organisms from mice

The microorganisms isolated originally from the mice of the normal lung series and later from those experimentally infected were readily cultivable on artificial mediums and conformed to the accepted growth pattern of the pleuropneumonia-like organisms. Microscopically they showed the wide variety of morphological elements which characterize these bacteria.

On serum agar plates the specific organisms produced small spherical colonies, ranging from 10 to 350μ in diameter. There was no growth downward into the medium. At a magnification of 100× the colonies were distinctly granular, the granules becoming coarser and darker as the colony aged.

Serum agar plates inoculated with lung and exudate suspensions generally showed a normal development of pleuropneumonia-like colonies in the presence of rapidly growing secondary bacteria. In a few instances satellitism was observed with a marked increase in the size of the specific colonies. In the presence of mold colonies and occasionally with bacterial colonies a degenerative or inhibitive effect was observed. Plates inoculated with middle ear exudate showed surprisingly few secondary colonies and not infrequently yielded a pure growth of the pleuropneumonia-like organisms.

In 30% horse serum broth a diffuse turbidity was apparent by the 2nd day and increased slightly through the 4th day, on incubation at 37 C. One culture was successfully transferred 60 times in this medium at intervals of three to five days.

In fluid defibrinated horse blood at the base of slanted agar the only visible manifestation of growth was a slight flash of hemolysis which stained the supernatant and seeped backwards into the agar. This change occurred on the 2nd or 3rd day of incubation and has been an almost invariable characteristic of this particular strain. The degree of hemolysis was somewhat greater with aged than with fresh blood. It was not observed in uninoculated tubes incubated for the same length of time. The organisms were readily maintained in this medium, remaining viable for at least 100 transfers at two to three day intervals. Serum agar plates inoculated from blood agar cultures showed innumerable specific colonies.

The best microscopic preparations were obtained from horse serum bouillon cultures. The organisms were sedimented after a growth period of three to four days and resuspended in a small amount of saline solution. Films made from such suspensions were stained with dilute Giemsa overnight and showed the usual pleomorphic elements. Characteristic rods and granules were also demonstrable in films made from the fluid portion of horse blood agar cultures and Gram stained. This method was not always successful but was nevertheless a useful aid in following the development of cultures.

The experimental production of pleuropneumonia in albino rats

A limited number of observations were made on the behavior of the pleuropneumonia-like organisms in the albino rat. They were successfully established by the nasal instillation of a mixed lung and exudate suspension from infected mice and were subsequently maintained for 6 passages. Twenty-nine of the 30 rats injected showed evidence of pleuropneumonia at autopsy. The localization of the specific organisms was similar to that in mice, being restricted to the respiratory tract and the middle ears, but the incidence of involvement was different. The rates of pneumonia, otitis media, and rhinitis in

the 30 rats of the passage series were 20, 70, and 80%, respectively. Pleuropneumonia-like organisms were commonly recovered from the middle ears and nasal passages. They were not isolated from the pooled lungs of 5 rats in the absence of pneumonia but were obtained from the lungs of three animals in which pneumonic foci were present.

Pleuropneumonia was also produced in rats by direct contact. The two experiments which were carried out suggested that the disease was somewhat more communicable in rats than it was in mice. Nine of the 10 animals that were used in these experiments showed evidence of pleuropneumonia at autopsy. The manifestations of the disease were commonly limited to the middle ears and nasal passages from which the specific organisms were re-isolated on culture. Two of the rats showed pneumonic foci in the lungs.

In a single experiment the specific disease was likewise produced in rats by a pure culture of the pleuropneumonia-like organisms, originally isolated from an infected rat of the passage series. Four weeks after nasal instillation of the culture 4 of the 5 rats showed characteristic manifestations at autopsy with recovery of the organisms.

The rat passages were made at monthly intervals by the nasal injection of middle ear exudate. At autopsy the majority of the injected rats showed a moderate to copious amount of mucopurulent exudate in the nasal passages. Many leucocytes were present in Gram-stained films and intracellular groups of pleuropneumonia-like organisms were commonly observed.

The contact experiments were made with passages 3 and 4 by placing 5 normal rats in the same cage with 5 injected ones, contact being established immediately after nasal instillation. The period of contact was ended after 4 weeks by the removal of the originally injected animals. The exposed rats were held under observation for 2 additional weeks and then killed.

In the pure culture experiment a 72-hour growth in horse serum bouillon was used. The culture was originally isolated from the middle ear exudate of a rat in the second passage group and had been transferred 3 times.

DISCUSSION

The situation encountered during the serial passage of normal lung suspensions in mice was similar to that originally reported by Sullivan and Dienes[9] and later by Edward.[10] In our experiments the transfer interval was longer but the outcome was the same. The passage of macroscopically normal lungs from mice showing no signs of disease ultimately resulted in the development of pneumonia and the recovery of pleuropneumonia-like organisms from the lung. It is probable that the appearance of these bacteria in our passage series was due to the chance inclusion of a healthy carrier in one of the groups of injected mice. Sabin[7] has noted that pleuropneumonia-like organisms may occasionally be carried in the lung of normal mice. The absence of pneumonia and otitis media in the 185 mice included in the first normal lung series and the second one up to the 14th passage intimates that the rate of carriage under natural conditions is extremely low in the Princeton strain of mice.

It seems surprising that no one has previously paid any attention to the middle ears in rodent pleuropneumonia. In the few papers that have dealt with the activity of the specific organisms in mice and rats the postmortem findings have been almost exclusively limited to the lung. Cultural examination of the nasal passages was made by Sabin[7] but the middle ears have been consistently ignored. Our autopsy results indicate that an inflammatory involvement of the middle ears is the commonest manifestation in mice under experimental conditions. A significant number of the injected animals showed no reaction in the lung at autopsy but did show otitis

media or occasionally rhinitis.

The importance of an examination of the middle ears and nasal passages is well brought out by the results of the transmission experiments in rats. Experimental establishment of the pleuropneumonia-like organisms in the rat had previously been in doubt. Klieneberger and Steabben[5,6] repeatedly recovered the organisms from rats in their natural habitat but were unable to produce any evidence that they could be established in the respiratory tract following injection by various routes. Their observations were limited, however, to the activity of the pleuropneumonia-like organisms in the lung.

If our observations had been confined to a pulmonary examination a comparable result would have been obtained. Examination of the middle ears and nasal passages clearly indicated, however, that the specific organisms could be established in rats of pleuropneumonia-free ancestry with the production of definite manifestations of disease. In comparison with the results obtained in mice the high rate of rhinitis observed in rats is of interest. The virus-like agent associated with endemic pneumonia is commonly carried in the nasal passages and the lungs of young rats, from the selected colony, in the absence of a pathologic reaction.[15] The rhinitis which often attended nasal instillation of the pleuropneumonia-like organisms may have resulted from the combined action of the two agents. The synergistic activity of these agents might also account for the pneumonia described by Klieneberger and Steabben.[5,6]

The behavior of the pleuropneumonia-like organisms under natural and experimental conditions in their animal hosts as well as under artificial cultural conditions is sufficiently different from that of the virus-like agent to warrant definition of the two associated diseases as unrelated entities. The position of infectious catarrh, which we described earlier as a native disease of mice and rats,[16,17] in relation to rodent pleuropneumonia is much less certain. In spite of obvious similarities between the coccobacilliform bodies of infectious catarrh and the pleuropneumonia-like organisms, there are significant cultural and biological differences which demand further study before a conclusion can be made.[18]

15. Nelson, J. B. 1948, J. Exper. Med. **87**: 11–19.

SUMMARY

A secondary infection with pleuropneumonia-like organisms was encountered during the nasal passage of normal lung suspensions in mice. The specific organisms were established in mice by the nasal instillation of lung and exudate suspensions, by pure cultures, and occasionally by direct contact.

The resulting disease resembled that produced by the virus-like agent of the rat in being restricted to the respiratory tract and the middle ears but was readily differentiated from it by cultural examination. The rate of pneumonia was significantly lower in the mice infected with pleuropneumonia, 54 as compared with 96%.

Pleuropneumonia-like organisms from the mice were also established in selected albino rats by nasal injection and by contact, their localization being chiefly limited to the middle ears and nasal passages.

The importance of a postmortem examination of the middle ears and nasal passages in the diagnosis of rodent pleuropneumonia is emphasized.

16. Nelson, J. B. 1937, J. Exp. Med. **65**: 833–841.
17. Nelson, J. B. 1940, J. Exp Med. **72**: 645–654.
18. Edwards, D. G. ff. 1947, J. Path. & Bact. **59**: 209–221.