INFECTIOUS CATARRH OF THE ALBINO RAT

I. Experimental Transmission in Relation to the Rôle of Actinobacillus muris

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Plate 21

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Since 1934 we have had under observation a native infection of the upper respiratory tract in the albino rat which results in a high rate of otitis media and rhinitis. The present paper is concerned with the experimental transmission of this infection and with certain changes which it has undergone with successive passages. In view of our earlier observations with Gowen (1) on middle ear disease in the rat, attention was paid also to the significance of *Bacillus actinoides* as an etiological factor.

In 1918 T. Smith (2) isolated a filamentous microorganism, further characterized by terminal bulbous sheaths, from the pneumonic lungs of calves and named it *B. actinoides*. In Bergey's (3) manual it is now listed as *Actinobacillus actinoides*. A morphologically similar bacterium was recovered by Jones (4) in 1922 and by Nelson (5) in 1930 from the lung and the middle ear, respectively, of albino rats. Our examinations included 50 adult rats (12 to 22 months old) with otitis media, an encapsulated bacterium being obtained in a pure or mixed state from 35 of 80 middle ear cultures (43 per cent). Although certain cultural and immunological differences were noted in comparison with the type species of T. Smith, the rat bacterium was unquestionably closely related and the name *B. actinoides* variety *muris* was suggested (6, 7). A new species *Actinobacillus muris* appears to be justified and we shall refer to it by that name. The organism, in pure culture, was established in the middle ear of normal rats by puncturing the external membrane and injecting directly into the tympanic cavity (8). In later unreported experiments it was also established in the middle ear following the nasal instillation of exudate from natural cases of otitis, but was not recoverable after the intranasal injection of *A. muris* in pure culture.

The relation of *A. muris* to the microorganism known as *Streptobacillus moniliformis* is uncertain. The latter was isolated in 1925 from a febrile disease in man by Levaditi, Nicolau, and Poincloux (9) and named by them. Strangeways (10) in 1933 reported that a similar bacterium was a common inhabitant of the nasopharynx of rats which did not themselves show any clinical signs of disease. Mackie, van Rooyen, and Gilroy (11), also in 1933, recovered another similar organism from an acute fatal epizootic in mice. The serological findings of van Rooyen (12), reported in 1936, indicated the identity of

the Levaditi, Strangeways, and Mackie bacteria. Van Rooyen also concluded that this microorganism had no affinities with any genera of the Actinomycetaceae and suggested that the name *Haverhillia multiformis* originally proposed by Parker and Hudson (13) for a bacterium recovered during an epidemic of so called Haverhill fever be substituted. At present, however, the original designation of Levaditi is generally used. Klieneberger and Steabben (14) in 1937 pointed out that *S. moniliformis* was morphologically similar to *B. actinoides* of T. Smith and to the rat bacterium here referred to as *A. muris*. The descriptions by Klieneberger and others and the growth requirements, particularly as reported by van Rooyen, indicate the probability of a close relation. It has been impossible to make a direct comparison as none of the original cultures of Smith, Jones, or Nelson are alive. Fig. 1 shows *A. muris* made from unstained pooled sediment which was collected about 9 years ago from serum agar cultures and suspended in saline without any preservative. The sheaths which comprise the bulk of the growth are as well defined now as in the original cultures. These globular bodies which we believed to be directly associated with *A. muris* are interpreted by Klieneberger (15) as components of the pleuropneumonia-like or L1 organism regarded by her as a symbiont of *S. moniliformis*. They are well illustrated in Figs. 1 and 4 of her paper of 1940 (16). Dienes (17) and Dawson and Hobby (18), however, regard the L1 organism of Klieneberger as a variant of *S. moniliformis* and not as an independent entity.

Source of the Infection

The history of the rat colony from which the infective material employed in the following experiments was originally obtained is of interest in connection with the distribution of *A. muris* under natural conditions, and hence is outlined in detail.

In January, 1934, at the request of Dr. G. L. Graham, we began a survey of a group of brown hooded rats maintained at The Rockefeller Institute in Princeton for work in helminthology. From time to time rats of different ages were removed and autopsied. These examinations indicated that sporadic otitis media was present in the colony, 9 of 64 animals autopsied between January and March showing an inflammation of one or both middle ears. *A. muris* was identified in cultures from 5 of the infected animals. Involvement of the nasal passages was not observed and most of the rats during this period appeared to be in good physical condition, there being no snuffling nor other external indication of infection.

In March, 1934, the brown hooded colony was augmented by the purchase of 100 supposedly normal albino rats from an animal dealer. A week after its arrival a few rats within this group began to show obvious symptoms of disease, characterized by loss of weight, ruffing of the hair, snuffling, and hemorrhagic crusting around the nares and eyes. Within a week the infection had spread through the entire group and ultimately all of the rats either died or were killed. Between March and May, 50 of these rats were autopsied, cultures being made from the middle ears and nasal passages if exudate was present. 39 of them, 78 per cent, showed an inflammation of one or both middle ears, and 45, 90 per cent, an inflammation of the nasal passages. A pure or mixed growth of *A. muris* was obtained in cultures from 23, 58 per cent, of the rats with otitis media, and from 25, 55 per cent, of those with rhinitis.

Immediately after the arrival of the new rats and prior to the onset of symptoms, a number of them were placed in the same room with the brown hooded colony. Several weeks later symptoms characteristic of the infection began to appear in these rats. Spread of the infection was checked by appropriate quarantine measures and later eliminated by selective breeding, but sporadic cases continued to develop for a period of 12 months. 28 diseased rats, 10 of which were "twisters," were autopsied between April, 1934, and March, 1935. 26 of them showed unilateral or bilateral otitis and rhinitis, complicated by pneumonia in 12. *A. muris* was identified in 20 of the cultures from the middle ear and in 16 from the nasal passages. An actively motile Gram-negative bacillus, identified as *Brucella bronchiseptica* by fermentation tests, was isolated from the lung of one rat and from the upper air passages of a second. Similarly motile bacteria were also seen in serum broth cultures from the nasal passages of other rats but they were not further identified.

TABLE I

The Incidence of Pneumonia and Otitis Media by Age Groups in 200 Uninjected Selected Rats

Age	Number of rats	Number of pneumonia cases	Incidence of pneumonia	Number of otitis cases	Incidence of otitis
mos.			*per cent*		*per cent*
2–4	34	4	11	2	5
5–7	34	7	20	1	2
8–10	32	15	46	2	6
12 and over	100	58	58	3	3

The Nasal Instillation of Actinobacillus muris in Pure and Mixed Culture

Although earlier attempts to establish *A. muris* in the middle ear of the rat by the nasal instillation of pure cultures had been unsuccessful, additional experiments were carried out in 1935 with the recently isolated strains of that bacterium.

The rats used in these and the following experiments were from a special colony developed by Nelson and Gowen (19) in 1931 by selective breeding with the view of eliminating middle ear disease. Through May, 1931, 120 rats from this colony, varying in age from 2 to 16 months, were autopsied with no attendant otitis. Maintenance of this group has been continued to date, with no additions from outside sources, the condition of the rats being followed by occasional autopsies. Since 1931 a few cases of otitis have been observed, the incidence for the entire series of autopsies, which now includes 320 rats, being 2.5 per cent. Pneumonia continued to be prevalent, the morbidity rate for the entire autopsy series including animals of all ages being 31 per cent. The incidence of pneumonia and otitis by age groups in the 200 selected rats autopsied since May, 1931, is summarized in Table I. The otitis rate unlike the pneumonia rate showed no age correlation. A variety of miscellaneous bacteria were recovered in cultures from the middle ear but *A. muris* was not identified in any of them. The sporadic cases of otitis

in these rats are believed to have resulted from the chance development of non-specific bacteria in the middle ear cavity.

18 rats, 4 of which were 1 year old and the remainder 3 months old, were injected intranasally, after being etherized, with fluid or sediment from the base of 48 to 72 hours old horse serum agar cultures of *A. muris*. Three strains of the organism, subcultured 2, 7, and 4 times, respectively, were used. The rats were held under observation for 2 to 3 weeks, during which time they remained normal, and were then autopsied. One rat, a 3 months old animal injected with the third strain, showed a unilateral otitis. The specific bacterium was not isolated from the middle ear exudate but was identified in a culture from the nasal passages. The middle ears and nasal tracts of the other rats were normal and the cultures from them negative with respect to *A. muris*.

It was known that *Br. bronchiseptica* in appropriate dilution produced a transient inflammation of the upper air passages on nasal instillation in pure culture. Accordingly, an experiment was carried out to determine whether its simultaneous injection with *A. muris* could affect that organism in regard to its establishment and survival in the nasal passages of the rat. 20 etherized rats, 3 to 4 months old, were injected intranasally with a mixture of 48 hours old serum broth cultures of *A. muris* diluted 1:2 and *Br. bronchiseptica* diluted 1:250. 4 of the rats died on the 7th day with pneumonia and rhinitis but without otitis. The nasal instillation of *Br. bronchiseptica*, particularly in low dilution, is often followed by death with attendant septicemia and pneumonia. 14 of the rats remained normal in appearance and were killed after 4 to 6 weeks. 2 of them showed a unilateral involvement of the middle ear from which *A. muris* was isolated in culture. An inflammatory exudate was also present in the nasal passages of these rats but the specific bacterium was not identified in the mixed growth obtained on cultivation. The middle ears and nasal passages of the other rats were normal in the gross; stained films from the latter locus, however, showed varying numbers of polynuclear leucocytes.

The incidence of otitis media in the 38 rats which were injected intranasally with *A. muris* in pure or mixed culture was approximately 8 per cent. The rate was somewhat higher than that expected in rats from the selected colony but was far too low to enable one to attribute any etiological significance to that microorganism.

Transmission of the Infection by Nasal Instillation

Beginning in March, 1935, the infection naturally acquired by the brown hooded rats was established by nasal instillation in normal animals from the selected colony and has been maintained therein to the present time (March, 1940), the passages now numbering 37. During the first year a change occurred in the bacterial flora of the nasal passages and middle ears of the experimental animals and also in the manifestations of the infection. Thereafter a disease syndrome emerged which has continued to date with only minor fluctuations.

The initial passage was made in 3 young rats which were injected intranasally, after being etherized, with small unmeasured amounts of a saline suspension of pooled exudate

from the middle ears and nasal passages of 3 naturally diseased brown hooded animals. 10 to 12 drops of the suspension were applied to the nares, using a capillary pipette. This method was employed in all of the subsequent passages, although in most of them only middle ear exudate was used. 2 to 5 rats, 2 to 3 months old, were used with each passage and were kept together in an isolation unit, the period of observation being generally 4 weeks or more. At autopsy particular attention was paid to the condition of the nasal passages, the middle ears, and the lungs. In the culturing of exudates slanted nutrient agar with 1 cc. of horse serum diluted 1:2 with bouillon was used.

In the first 6 passages which were made between Mar. 1 and Apr. 15, 1935, 14 rats were injected. 6 of them died on the 2nd to the 13th day after the administration of exudate, and 3 others which were killed on the 2nd to the 4th day would probably have succumbed. Prior to death these rats showed snuffling, with hemorrhagic crusting around the nares and eyes, and were thin, with ruffed hair. At autopsy the lungs were pneumonic and the nasal passages contained a hemorrhagic or mucopurulent exudate, but the middle ears were normal. *A. muris* was identified in cultures from the nasal passages of 6, and *Br. bronchiseptica* in cultures from the nasal passages, lung, or heart's blood of 7 of the acute cases. 5 of the rats survived for a period of 4 weeks and were then killed. At autopsy all of them showed rhinitis, and 4 of them a unilateral or bilateral otitis media and pneumonia. *A. muris* was isolated from the middle ear exudate of 4, and *Br. bronchiseptica* from the lung or nasal passages of all.

After the 6th passage fatalities were rarely observed following the injection of exudate, only 3 deaths being recorded among 136 rats used in the 30 subsequent transmission experiments. Aside from intermittent snuffling and occasional twisting most of the infected rats showed no symptoms. They gained weight normally after injection and retained their original sleek appearance. The snuffling was of variable onset and duration but was not observed before the 10th day and tended to diminish after the 4th week. It varied in intensity from passage to passage and was most noticeable when the rats were moving actively in their cage. The snuffling was never accompanied by a nasal discharge and was not always observed even in the presence of a frank rhinitis.

In the absence of acute symptoms pneumonia continued to be prevalent through the 10th passage, made in October, 1935, and *Br. bronchiseptica* was commonly isolated from the lung and nasal passages. After the 10th passage pneumonia was observed infrequently at autopsy and save for several passages, which will be referred to later, *Br. bronchiseptica* was rarely encountered. *A. muris* continued as a sporadic member of the bacterial flora of the middle ears or nasal tract through the 12th passage, 2 cultures being isolated from 4 rats autopsied in January, 1936. It was not isolated with the next passage, the 13th, and to date has not reappeared in the infected rats.

In the early passages of this infection, obtained originally from the brown hooded rats, the injection of exudate was followed by acute symptoms, a high rate of pneumonia (64 per cent), and not uncommonly by death. Throughout this acute stage two bacteria, *A. muris* and *Br. bronchiseptica*, were conspicuous by the frequency of their isolation. After the 12th passage acute symptoms were rarely observed, fatalities were infrequent, and the pneumonia rate dropped to 18 per cent. Coincident with these changes in the behavior of the infection in the host was a change

in the bacterial flora: *A. muris* was no longer isolated from the middle ears and nasal passages and *Br. bronchiseptica* was only occasionally encountered in the respiratory tract. The injected rats continued, however, to show a high rate of otitis media and rhinitis, accompanied by snuffling. For the entire series of 37 passages which included 156 rats the pneumonia rate was 28 per cent, the otitis rate 63 per cent, and the rhinitis rate 87 per cent.

Of the 99 rats in which otitis was observed at autopsy, 44, distributed through 25 passages, showed a bilateral involvement. In 26 of the otitis cases, distributed through 16 passages, the middle ear exudate was under sufficient tension to cause an outward bulge in the tympanic membrane. Only 3 rats with twisting movements were encountered in the entire series of passages and these were also the only rats which showed an inflammation of the inner as well as the middle ear. One of them was from the 14th subpassage, killed on the 284th day after injection, and 2 from the 17th passage, killed on the 83rd day. The low rate of twisting symptoms may be attributed in part to the relatively short period that the rats were held after injection. The element of time may also have been a factor in the low pneumonia rate which was obtained after the decline of the acute *Br. bronchiseptica* infection. It may be added that the pneumonic manifestations which occurred during the early passages can be reproduced in normal rats by the nasal instillation of pure cultures of *Br. bronchiseptica* in low dilution.

In passage 26 a secondary infection with *Br. bronchiseptica* occurred and resulted in acute symptoms. One of the 5 rats injected at this time died on the 13th day with an advanced pneumonia. The other 4 showed frequent snuffling with hemorrhagic crusting around the nares and a considerable loss of weight. At autopsy on the 20th day the lungs of 3 were pneumonic. *Br. bronchiseptica* is apparently endemic in the isolated colony, being occasionally isolated from the lung or nasal tract of normal rats, and was presumably introduced with the rats used in this particular passage. Aside from occasional transient snuffling, usually confined to the inmates of a single cage, there are no visible signs of its presence in the colony.

Rhinitis in the infected rats was diagnosed by the presence of a mucopurulent or catarrhal exudate in the nasal passages. A rough quantitative estimation was made by aspirating with a capillary pipette. The amount of exudate was variable but was often sufficiently copious to be drawn up well into the stem of the pipette. In some rats a thicker material containing numerous epithelial cells but also rich in leucocytes was obtained. Even in the presence of a large volume of exudate there was no visible nasal discharge. In intercurrent *Br. bronchiseptica* infections the exudate was sometimes mixed with blood and there was hemorrhagic encrusting around the nares.

The Onset and Duration of the Infection

Precise data on the onset and duration of the catarrhal syndrome were not obtained. The incubation period, following nasal instillation, is probably not less than 10 days. This was the shortest interval between injection and autopsy with observed inflammation and snuffling. Most of the

passage rats were killed approximately 4 weeks after injection. The high incidence of otitis and rhinitis in 6 groups of rats which were held for longer periods, ranging from 12 to 40 weeks, was indicative, however, of a

TABLE II

Duration of the Catarrhal Syndrome in Experimentally Infected Rats

Passage No.	Number of rats	Length of time to autopsy	Number of rats with			
			Otitis	Rhinitis	Pneumonia	Symptoms
		days				
12B*	4	112	3	4	0	3 snuffle
13B*	4	117	4	4	3	3 "
14B*	3	284	3	2	2	0 "
						1 twists
15	4	91	4	4	0	4 snuffle
17	4	83	4	4	0	4 "
20	5	113	3	4	0	3 "
Totals.....	24		21 (87%)	22 (91%)	5 (20%)	17 snuffle (70%)

* Not included in the main series of passages.

TABLE III

Transmission of the Catarrhal Syndrome by Direct Contact

Passage No.	Number of rats	Length of time to autopsy	Number of rats with			
			Otitis	Rhinitis	Pneumonia	Symptoms
		days				
11	2	36	2	2	0	2 snuffle
12C*	4	41	3	4	1	4 "
24	5	58	3	4	0	4 "
25B*	5	36	4	5	0	3 "
36B*	5	57	4	4	0	3 "
Totals.....	21		16 (76%)	19 (90%)	1 (5%)	16 snuffle (76%)

* Not included in the main series of passages.

persistent state of inflammation. The actual rates of otitis and rhinitis in these rats are presented in Table II.

The rats of passage 12B, which were held under observation for 112 days, were the only ones in this series from which *A. muris* was isolated. 2 of the animals showed a unilateral and 1 a bilateral otitis, *A. muris* being identified in all 4 exudate cultures. It was also recovered from the nasal passages of 2 rats with rhinitis.

Transmission of the Infection by Direct Contact

In addition to transmission by the nasal instillation of exudate the infection was also maintained by direct contact, susceptible rats being placed in the same cage with one or more infected animals. The incidence of otitis and rhinitis in 21 rats used in 5 different contact experiments, 2 of which were included in the main passage series, was 76 and 90 per cent, respectively. The data obtained from these experiments are presented in Table III.

Contact passage 11 included 2 rats both of which showed a bilateral otitis at autopsy. *A. muris* was recovered from all 4 middle ear cultures and from 1 nasal tract culture. It was also isolated from both loci of 1 rat in passage 12c, otitis being observed in 3 of the 4 rats used in this experiment. The organism was not encountered in any of the rats from the other 3 contact passages.

DISCUSSION

The transmission experiments just described indicate clearly that the original infection acquired by the brown hooded rats under natural conditions of exposure was a composite one. The bacterial flora included *Br. bronchiseptica* which was undoubtedly responsible for the early acute manifestations, a similar reaction being produced by that bacterium in pure culture. It persisted for 10 passages and was then lost, presumably by reason of dilution. The strains of *Br. bronchiseptica* that we have studied experimentally were pathogenic only when the concentration of bacterial cells per unit of inoculum was relatively high. With the disappearance of *Br. bronchiseptica* the acute manifestations ceased and were again observed only late in the series of passages when that microorganism was accidentally reintroduced. *A. muris* was also present in the initial passage and was maintained through the 12th when it also disappeared. In the absence of these two bacteria the injected rats continued to show a high rate of otitis media and rhinitis, accompanied by snuffling. Pneumonia was also observed but the rate was low. The syndrome which finally emerged is, we believe, sufficiently constant to constitute a disease entity and we propose to call it infectious catarrh.

Br. bronchiseptica in pure culture is incapable of reproducing the essential features of infectious catarrh, namely, otitis and a persistent rhinitis. It may, however, produce a transient inflammation of the nasal passages. This bacterium was present in the original outbreaks only as a secondary invader, contributing to the catarrhal syndrome certain acute manifestations which were atypical. *A. muris*, which we formerly believed to be of

some etiological significance, appears to be little more than a bacterial contaminant peculiar to the albino rat. It is certainly not the cause of infectious catarrh and though it may be carried along in transmission experiments both by nasal instillation and direct contact, it is probably not directly associated with any inflammatory state whether of the nasal tract, the middle ear, or the lung. Although *A. muris* evidently possesses some slight inherent pathogenicity as indicated by our earlier observations on intra-aural injection, it is apparently not invasive but behaves, rather, as a saprophyte which may accompany and complicate an inflammatory process initiated by some other agent.

The relation of the pneumonia observed in certain of the injected rats to the catarrhal syndrome is uncertain. Disregarding the passages in which *Br. bronchiseptica* was isolated from the lung, the incidence of pneumonia in 118 rats was 18 per cent, which exceeded only slightly the rate in uninjected rats of the same age, 11 per cent as indicated in Table I. In rats which were held under observation for a longer period, upwards of 3 months, there was no significant increase in the number of pneumonia cases, the rate being 20 per cent. In this connection it may be pointed out that the infected rats which were held in quarantine were less subject to exposure than those in the main colony where an increasing pneumonia rate with age was noted. The probability is that pneumonia may occasionally be a specific manifestation of infectious catarrh, but it is certainly not a characteristic one. It is evident that the pneumonia endemic in the selected colony bears no relation to infectious catarrh nor to the sporadic otitis media observed in rats from that group. It is also evident that pneumonia may occur in the rat in the absence of *A. muris*, since that bacterium has never been identified in cultures from the selected rats.

SUMMARY

A disease syndrome referred to as infectious catarrh, encountered under natural conditions of exposure in a rat colony, was transmitted to selected animals and maintained for 5 years by nasal instillation or contact.

During this period 37 passages were made in 156 rats, the rates of pneumonia, otitis media, and rhinitis being 28, 63, and 87 per cent, respectively.

After the 12th passage, *Brucella bronchiseptica* and *Actinobacillus muris* (*B. actinoides* var. *muris*), which were originally present, were no longer cultivable from infected rats.

By reason of the maintenance of infectious catarrh in the absence of the latter and also because of its non-invasiveness on nasal instillation, it is now believed that *Actinobacillus muris* is of no direct etiological significance.

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EXPLANATION OF PLATE 21

FIG. 1. Single colony of *Actinobacillus muris* from a 9 years old saline suspension of horse serum agar sediments. Unstained film spread between cover-glass and slide. × 640.

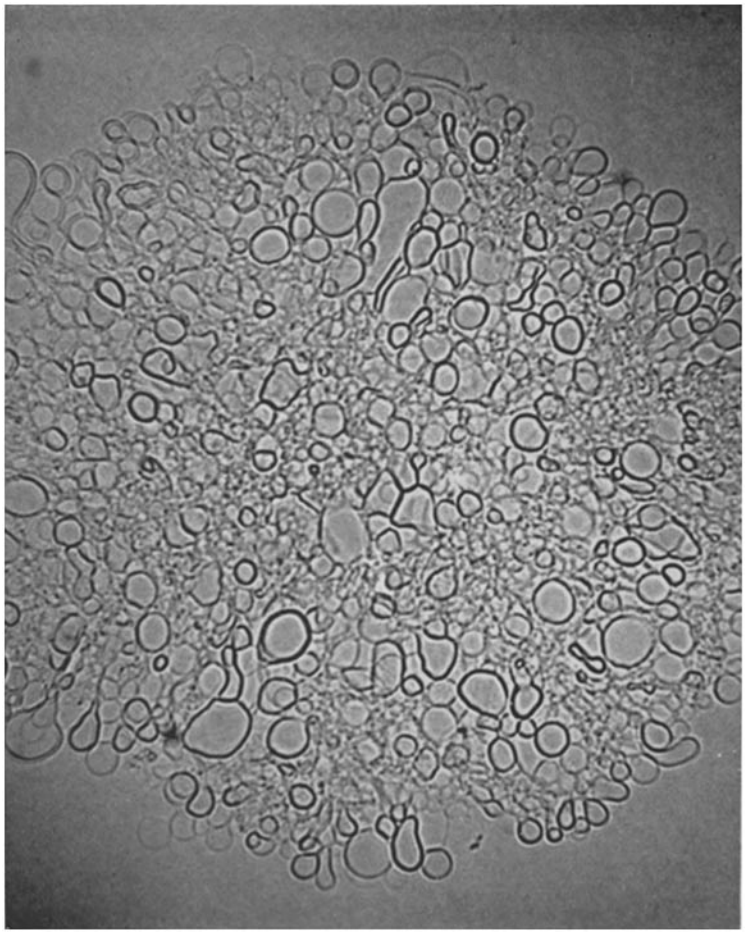

FIG. 1

Photographed by J. A. Carlile

(Nelson: Infectious catarrh of albino rat. I)