

THE REACTION OF THE ALBINO RAT TO THE INTRA-
AURAL ADMINISTRATION OF CERTAIN BACTERIA
ASSOCIATED WITH MIDDLE EAR DISEASE

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Observations on middle ear disease under natural conditions of infection in a colony of albino rats maintained on a balanced diet were previously reported.¹ Adult animals upwards of 1 year in age showed an incidence of 69 per cent, young animals 3 to 4 months in age an incidence of 32 per cent. The bacterial flora of the tympanic cavity was found to embrace a wide variety of microorganisms.² Three of these bacteria (*B. actinoides*, a group of streptococci, and a diphtheroid) were encountered sufficiently often with both age groups to suggest that they might be of etiological significance. The high incidence of isolation, however, was the only evidence favoring such a relationship. The particular bacteria might equally well be regarded as secondary invaders. The present paper is concerned with an experimental attempt to define the infective capacity of the three microorganisms for the rat by direct introduction into the tympanic cavity.

EXPERIMENTAL

A disease-free stock of rats was not available for the proposed experiments. Animals from the colony in which the infection was endemic and which had supplied the material for the original study were employed.³ Young rats between 2 and 3 months of age were chosen in preference to adult animals because of the lower incidence of natural infection. The rats used in any one experiment were generally picked from two or more litters and an approximate balance between

¹ Nelson, J. B., and Gowen, J. W., *J. Infect. Dis.*, 1930, **46**, 53.

² Nelson, J. B., *J. Infect. Dis.*, 1930, **46**, 64.

³ The writer is indebted to Dr. J. W. Gowen for supplying the rats used in these experiments.

males and females was maintained. The natural rate of infection was further reduced by selection at the time of injection. It was earlier observed that the passage of a needle through the tympanic membrane into a normal middle ear cavity could be both felt and heard. In the presence of exudate within the tympanum the membrane was dulled and afforded little resistance to the needle. The characteristic click heard upon puncture of the normal membrane was lacking. Rats which showed such an abnormality upon injection were discarded. This method of selection does not eliminate all cases of natural infection and it occasionally throws out a normal animal. Its application, however, was of unquestioned value in the reduction of affected individuals.

For injection the barrel of a tuberculin syringe fitted with a 24 gauge needle was employed. A short length of rubber hose with a mouthpiece of glass tubing was used instead of the usual plunger. The rats were anesthetized lightly with ether in a closed jar just prior to injection. The needle was passed through the opening of the external auditory meatus generally on the left side, the tympanic mem-

TABLE I
Bilateral Aural Examination Following the Injection of Sterile Medium into the Left Middle Ear

| Middle ear examined | Gross condition | | Isolation of bacteria | |
|---------------------|---------------------|----------------------|-----------------------|----------------------|
| | No. showing exudate | Percentage incidence | No. showing bacteria | Percentage incidence |
| Right side..... | 0 | 0 | 0 | 0 |
| Left side..... | 1 | 6 | 2 | 12 |

brane punctured, and a small amount of material gently blown into the middle ear cavity. The animals withstood the treatment well. With the experiments to be reported the rats were generally injected in groups of 8 or 16. They were killed 1 week later in most instances, the middle ear cavity exposed, and cultures made. The upper respiratory tract and lungs were examined as routine. Additional cultures were often made from the nasopharynx. Unsealed slanted agar (pH 7.6) enriched with 1 cc. of 50 per cent bovine serum bouillon at the base was used both for growing the bacteria employed in the experiments and for culturing after injection. This medium supports an abundant growth of the rat variety of *B. actinoides* and of the other less particular microorganisms. The fluid portion of the cultures, in undiluted state, was used for injection.

The direct introduction of bacteria into the middle ear cavity by way of the external auditory meatus appears to have had little application in animal experimentation. What effect the presence of foreign material might have in the small partially enclosed cavity of the

middle ear was not known. There was a possibility that the medium itself, rich in proteins, might call forth an inflammatory reaction on the part of the host. The first experiment conducted was a control on the method of procedure devised to test this possibility.

Sixteen young rats were injected under ether into the left middle ear cavity with small amounts of sterile 50 per cent bovine serum bouillon. The animals were killed after 1 week. They appeared normal in every way during this period. The middle ear cavities were opened, under precautions for asepsis, and cultures made. The gross pathological and the bacteriological findings are presented in Table I.

The right uninjected middle ear cavities were normal in the gross and sterile in every instance. One rat showed a scanty serous exudate in the left cavity which had received sterile medium. No growth was obtained in culture. The left tympanic cavities were normal in the gross with the other animals. In two instances, however, pure cultures of a non-hemolytic streptococcus were isolated.

In addition to the gross inspection of the middle ear a microscopical examination was also made.

After culturing, the cavity was rinsed with a small volume of saline and the washings examined in both fresh and dry preparations. Washings from the normal uninjected middle ear showed chiefly ciliated and unciliated epithelial cells together with erythrocytes and a very occasional leucocyte. The presence of the latter cells may be attributed to hemorrhage from superficial blood vessels ruptured during washing. Washings from the injected middle ear always showed, in addition, large mononuclear cells of the macrophage type. These were characterized by an abundance of large and small cytoplasmic vacuoles and granules. They varied considerably in number from rat to rat. In some instances there were ten or more cells per microscopic field. The contrast between unstained preparations from injected and uninjected middle ear cavities was very striking. The same cellular reaction was also observed following the intra-aural injection of sterile saline.

Injury to the middle ear as a result of the injection procedure calls forth a low grade inflammatory reaction demonstrable microscopically. The presence of exudate in one case and the isolation of bacteria in the absence of exudate in two instances cannot be definitely accounted for. Repetition of the experiment with smaller numbers of rats resulted in the same microscopic reaction unaccompanied by any gross

change. It may be that an occasional rat responds to the slight injury caused by injection with an inflammatory reaction sufficiently marked to be recognized in the gross. However, it can be safely said that the intra-aural injection of serum bouillon into an etherized rat is not followed by clinical middle ear disease in most instances.

Coincident with the introduction of sterile serum bouillon into the middle ear cavity was a slight outward leakage of the fluid through the punctured tympanic membrane. With the substitution of bacterial cultures for the medium it was important to know whether a detectable amount of culture remained in the cavity.

Three rats were injected into the right tympanum with small amounts of a 5 day old culture of *B. actinoides*. The animals were killed immediately and the middle ear cavities as well as the nasopharynx examined. A small amount of fluid was present in the right middle ears of two animals but absent in that of the third. Microscopic examination of saline washings showed the typical capsular aggregates of *B. actinoides* in each instance. Pure cultures of the organism were likewise obtained. The left uninjected middle ear cavities were normal in the gross and sterile. Washings failed to show the characteristic capsules. *B. actinoides* was not obtained in cultures from the nasopharynx. While the amount of fluid culture actually remaining in the tympanum after direct introduction was small it seemed sufficient for infective purposes. There was no indication of an inward flow of the material injected through the eustachian tube into the nasopharynx.

The results of these control experiments were sufficiently favorable to warrant proceeding with injection of the three bacteria. *B. actinoides* was employed in the first experiment.

Sixteen rats were injected into the left tympanic cavity with small amounts of a 4 day old culture of the organism. The culture was recently isolated from the middle ear of a naturally infected rat. The animals were kept under observation for a week and killed. They appeared normal during this time. The usual examinations were made at autopsy. The gross pathological and the bacteriological findings are presented in detail in Table II and summarized in Table III. The experiment was repeated at intervals with smaller groups of rats and different cultures of the same rodent variety of *B. actinoides*. The data for the total series of rats which received intra-aural injection of the organism are included in the latter table.

Three of the first 16 rats showed exudate in the right middle ear cavity. The exudate was sterile in one case, gave a pure growth of staphylococcus in one, and of a streptococcus in another. Twelve of the rats showed exudate in the left middle ear cavity. With the remainder the left tympanum was normal in the gross and

sterile. *B. actinoides* was isolated in pure culture from 9 of the 12 animals with middle ear exudate. No growth was obtained from one. Two others gave pure cultures of a streptococcus and a staphylococcus respectively. The rats of the additional series showed no gross involvement of the uninjected middle ear. The findings of the left middle ear were much the same as for the first group. The recovery rate of *B. actinoides* was somewhat greater with the second series.

The exudate in the left middle ear cavity was commonly purulent in nature. Microscopically it regularly showed large numbers of polynuclear leucocytes with a few large mononuclear cells. Washings from the injected middle ear in the

TABLE II

Bilateral Aural Examination Following the Injection of B. actinoides into the Left Middle Ear

| No. of rat | Gross condition of middle ear | | Culture from middle ear | |
|------------|-------------------------------|---------|-------------------------|----------------------|
| | Right | Left | Right | Left |
| 1 | Normal | Exudate | Sterile | <i>B. actinoides</i> |
| 2 | " | Normal | " | Sterile |
| 3 | " | " | " | " |
| 4 | " | Exudate | " | <i>B. actinoides</i> |
| 5 | Exudate | " | Staphylococcus | " |
| 6 | Normal | Normal | Sterile | Sterile |
| 7 | " | Exudate | " | <i>B. actinoides</i> |
| 8 | " | " | " | " |
| 9 | " | Normal | " | Sterile |
| 10 | " | Exudate | " | Staphylococcus |
| 11 | Exudate | " | " | Sterile |
| 12 | Normal | " | " | Streptococcus |
| 13 | Exudate | " | Streptococcus | <i>B. actinoides</i> |
| 14 | Normal | " | Sterile | " |
| 15 | " | " | " | " |
| 16 | " | " | " | " |

absence of gross exudate showed chiefly epithelial cells, erythrocytes and the large mononuclear cells previously mentioned. With affected rats the exudate occupied approximately half the space of the middle ear cavity in most instances. Two rats of the additional group showed no gross exudate although *B. actinoides* was recovered in pure culture. Saline washings of the middle ear in both instances showed numerous polynuclear leucocytes. Microscopic examination of exudate from the left cavities, either in stained films or hanging drop preparations, often revealed slender rods and filaments. Similar forms are characteristic of the development of *B. actinoides* under certain artificial conditions of growth. The

organism was recovered in pure culture from such cases. The capsules which develop in fluid cultures of *B. actinoides* were never seen in exudate preparations.

In some instances cultures were also made from other sites. Blood drawn by cardiac puncture was cultured from six injected rats. All of these showed exudate in the left middle ear cavity from which pure cultures of *B. actinoides* were obtained in five instances. The organism was not recovered from the blood in any case. Gross changes were rarely noted in the lungs following the intra-aural administration of *B. actinoides*. Small pieces of lung from rats which showed minute foci of involvement were cultured in a few instances. *B. actinoides* was never recovered. Pulmonary lesions are observed under natural conditions in a few rats of this age. The nasopharynx sometimes showed a considerable amount of mucus but never a purulent exudate. Nasopharyngeal cultures were made from five of the above mentioned rats. A mixed growth of *B. actinoides* and other bacteria, chiefly cocci, was obtained in each instance. None of these rats showed involvement of the opposite middle ear cavity.

TABLE III
Summarized Data on the Intra-Aural Injection of B. actinoides

| No. of rats | Middle ear examined | Gross condition | | Recovery of <i>B. actinoides</i> | |
|-------------|---------------------|---------------------|----------------------|----------------------------------|----------------------|
| | | No. showing exudate | Percentage incidence | No. of cultures isolated | Percentage incidence |
| 16 | Right side | 3 | 18 | 0 | 0 |
| | Left side | 12 | 75 | 9 | 56 |
| 30 | Right side | 3 | 10 | 0 | 0 |
| | Left side | 23 | 76 | 20 | 66 |

No extensive experiments were conducted to determine how long *B. actinoides* persists in the middle ear cavity of the rat following intra-aural administration. Two injected animals were held for 2 weeks and a third for 3 weeks. The exudate in the middle ear cavity which received *B. actinoides* was scanty with some indication of an attempt at organization. Pure cultures of the organism were obtained in two instances. One of the rats held for 2 weeks gave a mixed growth of *B. actinoides* and a diphtheroid.

The intra-aural injection of streptococci was carried out with two groups of eight rats each at different intervals.

Administration was into the left middle ear. The first group received small amounts of a pure culture of a non-hemolytic streptococcus. The second group

received a mixed suspension containing the same non-hemolytic strain and two strains of hemolytic streptococci. The three cultures were recently isolated from natural cases of middle ear disease. The undiluted fluid portion of 48 hour cultures was employed. The mixed suspension contained equivalent amounts of the three strains. The gross pathological and the bacteriological findings were almost identical for the two groups of rats. The summary for the entire series is presented in Table IV.

Two rats of the first group of eight showed exudate in the right uninjected tympanum. The right cavities were normal with all the rats of the second group.

TABLE IV

Bilateral Aural Examination Following the Injection of Streptococci into the Left Middle Ear

| Middle ear examined | Gross condition | | Isolation of streptococci | |
|---------------------|---------------------|----------------------|---------------------------|----------------------|
| | No. showing exudate | Percentage incidence | No. of cultures isolated | Percentage incidence |
| Right side..... | 2 | 12 | 0 | 0 |
| Left side..... | 12 | 75 | 2 | 12 |

TABLE V

Bilateral Aural Examination Following the Injection of a Diphtheroid into the Left Middle Ear

| Middle ear examined | Gross condition | | Isolation of diphtheroid | |
|---------------------|---------------------|----------------------|--------------------------|----------------------|
| | No. showing exudate | Percentage incidence | No. of cultures isolated | Percentage incidence |
| Right side..... | 0 | 0 | 0 | 0 |
| Left side..... | 3 | 18 | 0 | 0 |

Right middle ear cultures from the entire series were sterile. Six rats of each group showed exudate in the left injected tympanum. A pure culture of a non-hemolytic streptococcus was isolated from the affected middle ear of one rat from the first group. One rat of the second group gave a pure culture of a hemolytic streptococcus and another of a staphylococcus. Left middle ear cultures from the remaining rats were sterile. Nasopharyngeal cultures were not made as streptococci are commonly found on the nasopharyngeal membrane as a part of the normal bacterial flora.

The exudate present in the injected middle ear cavities was commonly scanty in amount and either serous or mucoid in nature. Microscopic examination of

the exudates showed both polynuclear leucocytes and large mononuclear cells as well as epithelial cells and erythrocytes. There was considerable variation in the number and the proportion of the exudative cells from rat to rat. They were never very numerous, at least in comparison with the thicker purulent material commonly found after the injection of *B. actinoides*. Washings from the left middle ear cavity in the absence of gross exudate showed fewer exudative cells, particularly polynuclears.

The intra-aural injection of the diphtheroid was also carried out with two groups of eight rats at different intervals.

One strain of the organism, of recent isolation, was employed throughout. Small amounts of the undiluted fluid portions of 48 hour old cultures were injected into the left middle ear cavities. The animals were killed and autopsied a week later. The gross pathological and the bacteriological findings are summarized in Table V.

The right middle ear cavities were normal and sterile in every instance. Three rats showed exudate in the left injected tympanum. Two of these were from the first group of eight, one from the second. The left cavities of the remaining rats showed no gross involvement. A pure culture of staphylococcus was isolated from the left tympanum of the first affected rat from Group 1. Cultures from the other two were sterile. A pure streptococcus culture was also isolated from the left middle ear of one rat in the absence of exudate. Nasopharyngeal cultures were also made from the eight rats of the second group. The diphtheroid was recovered in no instance.

Exudate present in the left middle ear of the first affected rat was definitely purulent, rich in polynuclear leucocytes. With the other animals which showed gross involvement the exudate was scantier and mucoid. Exudative cells were less numerous. Microscopic examination of the saline washings from the left tympanum, in the absence of exudate, showed large mononuclear cells in addition to epithelial cells and erythrocytes. The cells first mentioned varied considerably in number from rat to rat but were never more numerous than in washings made after the injection of sterile medium.

DISCUSSION

The bacteria employed for the experiments (*B. actinoides*, a group of streptococci, and a diphtheroid) were most frequently encountered among a group of nine microorganisms isolated during a study of middle ear disease under natural conditions of infection. The present experiments demonstrate a significant difference in their infective capacity for the albino rat.

Approximately 75 per cent of the rats injected with *B. actinoides*

showed an inflammatory reaction in the middle ear cavity. In most instances this was visible as a purulent exudate, tending to simulate clinical middle ear disease in adult rats. *B. actinoides* was recovered unilaterally in pure culture from about 60 per cent of the animals injected. An occasional rat showed no gross exudate but microscopic examination revealed the presence of polynuclear leucocytes, and *B. actinoides* was recovered in cultures. Twenty-five per cent of the rats showed no specific response to the injected microorganism. In a few instances *B. actinoides* was not recovered from the middle ear in the presence of a gross exudative reaction.

Seventy-five per cent of the rats likewise showed an inflammatory reaction in the middle ear cavity following the injection of streptococci. This was visible grossly as a mucoid or serous exudate which tended to simulate clinical middle ear disease in young rats. Streptococci were rarely isolated from the middle ear at autopsy. The incidence of recovery was about 10 per cent. Twenty-five per cent of the rats showed no specific reaction to the presence of streptococci in the middle ear. In this case, as with the rats of the preceding series, the microscopic reaction could not be distinguished from that following the injection of sterile medium.

Less than 20 per cent of the rats showed gross inflammatory changes in the middle ear after injection of the diphtheroid. The microscopic reaction in the absence of exudate was characterized by the presence of large mononuclear cells and could not be differentiated from the reaction caused by the introduction of serum bouillon. The diphtheroid was never recovered in cultures.

The rats not only showed group differences in their response to the three induced infections but they also displayed individual differences. This latter variability was more apparent with *B. actinoides* than with the other organisms. It was to have been expected in view of the history of the rat colony. The litters of young rats were random selections from a population in which middle ear disease was endemic. The chances were in favor of the inclusion of rats with native resistance reenforced by an acquired immunity of maternal transfer. Furthermore natural infection to the extent of approximately 30 per cent could be expected. This rate was materially reduced by selection at the time of injection. The actual reduction could not be determined

but the low incidence of unilateral middle ear disease, about 10 per cent, for the 32 rats of the first and last series may be taken as a rough index.

B. actinoides and the streptococci may be regarded as potentially capable of inducing middle ear disease in the albino rat. The route of infection was highly artificial in the experiments here described. The findings merely demonstrate the capabilities of the several bacteria upon direct introduction into the middle ear. The natural mode of entrance is probably through the eustachian tube by way of the upper respiratory tract.

B. actinoides appears to be particularly well adapted for development in the middle ear of the rat. It resists for a considerable period of time the marked inflammatory reaction with which the host opposes it. The infective capacity of the streptococcus, insofar as the present strains are concerned, is decidedly less. It is evidently able to establish itself in the middle ear but in most instances quickly succumbs to the defense of the host. The diphtheroid shows little evidence of pathogenicity for the rat. Elimination of the organism is generally effected without calling forth any specific inflammatory reaction. The several exceptions of the present experiments prevent assumption of a complete lack of pathogenicity. It is highly probable, however, that the diphtheroid is a secondary invader in middle ear disease and of no etiological significance.

An analogy between the experimental findings and the findings with natural middle ear disease may be pointed out. Middle ear exudate with naturally infected young rats is often serous or mucoid in nature and frequently sterile. In a previous study 44 per cent of the middle ear cultures from affected young rats were sterile, 12 per cent from adults. Middle ear exudate following the injection of streptococci is of a similar nature and frequently sterile. With naturally infected adult rats the middle ear exudate is often purulent. A fair number of them yield *B. actinoides* in pure or mixed culture (44 per cent). The injection of *B. actinoides* is commonly attended by a purulent aural exudate from which the organism is often recovered (65 per cent). There is a suggestion from this analogy, applicable to the present rat population, that streptococci may be of chief significance in the causation of middle ear disease in young rats, and *B. actinoides*

in adults. The more strictly parasitic nature of *B. actinoides*, implying the necessity of contact for dissemination, as contrasted with the saprophytic tendency of the streptococci which are found in the upper respiratory tract of normal young rats lends some support to this suggestion.

SUMMARY

The infective capacity of three bacteria commonly encountered during a study of natural middle ear disease in a rat colony has been determined by direct intra-aural injection in young rats. One week after the introduction of *B. actinoides* 75 per cent of the rats showed a purulent exudate in the injected middle ear cavity and 65 per cent yielded pure cultures of the organism. With hemolytic and non-hemolytic streptococci 75 per cent showed a serous or mucoid exudate and 12 per cent yielded the organism in culture. With a diphtheroid 18 per cent showed a gross reaction in the middle ear which was sterile in every case.

The experimental findings are discussed in relation to the etiology of middle ear disease.