THE ESTABLISHMENT OF AN ALBINO RAT COLONY FREE FROM MIDDLE EAR DISEASE

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Earlier experimental studies on middle ear disease in the albino rat were hampered by the high natural incidence of the condition, particularly among adult animals.[1] It was apparent that a population free from aural involvement was essential for interpreting precisely the etiological relationship of certain bacteria frequently isolated from the middle ear cavity under conditions of natural infection.[2] In addition to supplying uniform host material for experimental purposes, a colony free from middle ear disease would afford an opportunity for studying the relationship of that condition to the highly prevalent pneumonia of the albino rat. For these reasons an attempt was made to build up such a colony by the continued selection of rats from unaffected parents. The methods of procedure, together with observations on middle ear disease and pneumonia in the colony, are presented in the following sections.

Methods

The original breeders were selected from the large rat colony, developed by one of us (Gowen), which furnished the original data on the incidence of middle ear disease.[1] This colony comprises a number of lines which are preserved intact with no cross breeding.

Young breeders under 6 months of age chosen from several different lines were mated and placed in individual cages. The pregnant females were subsequently removed and isolated in a separate unit. The males meanwhile were killed and autopsied. The suckling rats were left with their mothers until old enough to wean. At this time the female parents were killed and examined. The young rats from a single pair of parents free from middle ear disease and pneumonia consti-

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

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

tuted the nucleus from which the selected colony was developed. They were born early in January, 1929. Brother and sister matings were subsequently made through seven generations. Several pairs of breeders from each generation were selected and segregated from the surplus stock of special rats. The breeders were all killed after their young were weaned and examined for middle ear disease and pneumonia.

From time to time small groups of rats were removed from the selected colony and killed. At autopsy, particular attention was paid to the condition of the upper respiratory tract, the middle ear, and the

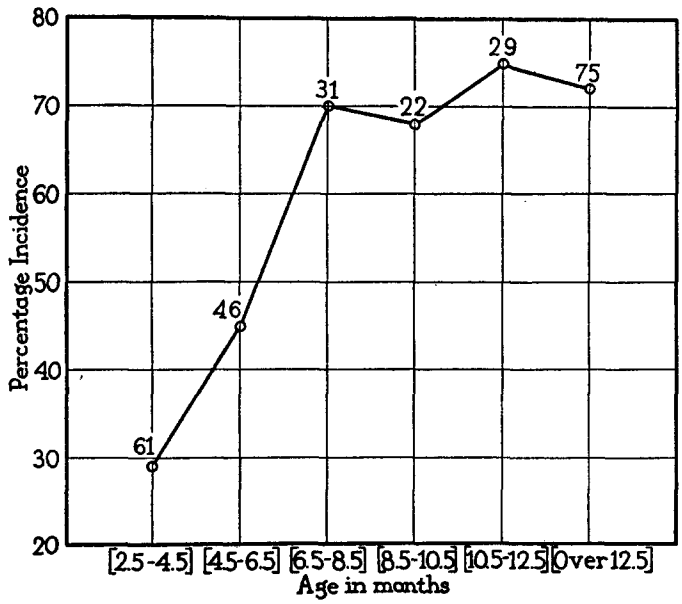

FIG. 1. The incidence of middle ear disease in stock rats of different ages. The number of rats in each age group is placed above the plotted morbidity rate.

lung. This series of autopsies now contains 120 individuals varying in age from 2 to 16 months and about equally divided as to sex. A few rats showed an increased mucus secretion in the nasopharynx or the nasal canals, but in general the upper respiratory tract was normal. The middle ear cavities throughout the entire series were normal in the gross. The tympanic membranes consistently gave the characteristic click upon puncture with the scissors point. Microscopic examination

of saline washings from the tympanic cavity, which was made in some instances, gave no indication of a cellular reaction. In no case was there an involvement of the tympanic bone, inner ear, or internal meatus. No rats were observed with the inclined position of the head or the rotary motion characteristic of the so called twister.

A similar postmortem examination of rats from the stock colony has been carried out since 1928. This series likewise included rats of all ages up to 2 years. Over half of the animals were contemporary with those of the selected population. This series now contains 264 rats, of which 152, or 57 per cent, showed an inflammatory involvement of one or both middle ear cavities.

TABLE I

The Incidence of Middle Ear Disease and Pneumonia in Adult and Young Rats from the Selected and Stock Colonies

	Adult rats					Young rats				
		Middle ear disease		Pneumonia			Middle ear disease		Pneumonia	
	No. of rats	No. of cases	Percentage incidence	No. of cases	Percentage incidence	No. of rats	No. of cases	Percentage incidence	No. of cases	Percentage incidence
Selected colony.....	50	0	0	26	52	50	0	0	0	0
Stock colony.....	50	36	72	39	78	50	15	30	0	0

It was previously shown that the incidence of middle ear disease in the albino rat increased significantly with age.[1] This is brought out in Fig. 1 which shows that the morbidity rate rises steadily to about 8 months and then remains nearly stationary at a high level.

Bearing in mind the effect of age on the incidence of middle ear disease, the rats removed from the special colony for autopsy were generally selected from two age groups, young rats 3 to 4½ months old and adult rats upwards of 1 year in age. The data on middle ear disease and pneumonia within these age limits in the selected and stock colonies are summarized in Table I. The series from the stock colony was composed of the last fifty animals in each age group examined at autopsy.

No cases of middle ear disease were found in the selected rats of either age group. The adult rats from the stock colony showed a high incidence, approximately 70 per cent. In the young rats from the same colony it was significantly lower, 30 per cent. Approximately 50 per cent of the adult selected rats showed pneumonic lesions in one or more lobes of the lung. The incidence of pneumonia in the adult stock rats was appreciably higher, 78 per cent. No cases of pneumonia were found among the young rats of either colony. In the past a few cases were observed in rats of this age, 3 per cent in a group of 70.[1]

It was of interest to know whether middle ear disease was present among the stock rats of the line from which the selected colony was developed. Inspection of the autopsy records showed that thirty-six rats from this line were examined. Sixteen, or 44 per cent, of these animals showed middle ear disease. The difference in the morbidity rates of this line and the stock group as a whole may be due in part to the chance inclusion of a greater number of young rats in the former. From these findings there is no indication that the rats of the selected line were more resistant to middle ear disease than the stock rats as a group.

The incidence of pneumonia in the adult selected rats was approximately a third less than that in the adult stock rats. The postmortem examinations suggested that in addition the disease was less advanced in the selected rats. The autopsy data were therefore analyzed to determine whether any significant difference in the extent or severity of the pneumonia could be brought out.

In order to compare the extent of the pneumonia in the adult rats of the two colonies, the number of affected lobes in individual cases was determined. The number of cases which showed involvement of two or more lobes was less among the selected rats but the difference was not significant. Thirteen, or 50 per cent, of the twenty-six adult selected rats affected with pneumonia showed involvement of two or more lobes, and twenty-three, or 58 per cent, of the thirty-eight adult stock rats. The two groups were then compared on the basis of the type of lesion most commonly encountered, with more significant results. Advanced lesions occurred approximately two and a half times as often among the adult stock rats. Twenty-five, or 63 per cent, of the selected adult rats displayed nodular lesions containing a

mucoid or purulent exudate, and only seven, or 23 per cent, of the affected rats from the selected colony were similarly affected.

Although the rats of the selected colony showed no evidence of middle ear disease, it was possible that carriers of the associated bacteria might be present. The obscure etiology of the condition made it impossible to employ direct methods for their detection. The institution of a vitamin-deficient diet was resorted to with the possibility that its debilitating effect on the rat might predispose to the development of middle ear disease in the presence of the infecting bacteria.

Twenty young rats from the selected colony, approximately 2 months of age, were placed on a diet deficient in vitamin D. The Steenbock rachitic ration containing 76 parts of yellow corn, 20 of wheat gluten, 3 of calcium carbonate, and 1 of sodium chloride was employed. The rats were housed in wire cages, two to four individuals to a cage, and were isolated in a unit separate from the main selected colony. The rachitic diet was maintained throughout their lives. Sixteen died between 4 and 6 months of age, and the other four were killed after 6½ months. All showed characteristic deformation of the hind legs and ribs. Postmortem examination was made in every case.

TABLE II

The Incidence of Middle Ear Disease in Rachitic Rats from the Selected and Stock Colonies and in Stock Rats on a Balanced Diet

	No. of rats	No. of cases	Percentage incidence
Rachitic selected rats	20	0	0
Rachitic stock rats	45	22	48
Normal stock rats	50	15	30

The experimental findings are summarized in Table II. For comparison, the incidence of middle ear disease in two other groups of rats is also shown. One group is composed of young stock rats from a number of different lines which were maintained on a similar rachitic diet. It represents one age group of a much larger series; in particular, rats which died between 3 and 4½ months of age. The other, taken from Table I, is composed of rats of the same age maintained on a balanced diet. It may be noted that the rachitic stock rats were kept in separate cages in the same room with the main colony. After the

institution of the special diet they never came in direct contact with other rats of the colony.

No cases of middle ear disease occurred in the selected rats maintained on the rachitic diet. The rachitic stock rats, on the other hand, showed a high rate of middle ear disease, appreciably higher than the expected rate in stock rats of the same age group on a normal diet. Exposure of the rachitic rats to the infecting agents after they were placed on the special diet was solely by indirect contact, whereas the normally fed stock rats were directly exposed, or potentially so, through contact with their litter mates. The increased rate of middle ear disease in the former group suggests that a rachitic diet may predispose to the development of middle ear disease in a certain percentage of young rats which under normal conditions would not be affected.

Because of the negative evidence afforded by the above observations, one additional experiment was undertaken in the attempt to demonstrate the presence of carriers in the selected population. A prolonged state of overcrowding was chosen as an environmental factor operating against the defensive forces of the host and in favor of the development of such parasitic bacteria as might be present. Ten weaned, selected rats from two different litters were placed in a single cage (15 x 10 x 10 inches) and kept together for nearly a year. The animals were subsequently killed and autopsied. Examination of the middle ear cavities failed in every instance to show any indication of an inflammatory reaction.

DISCUSSION

Because of the high incidence of middle ear disease among the adult rats of the stock colony, it can be assumed that the total mass or dosage of associated bacteria, disseminated throughout the population, is large. The perpetuation of the condition from generation to generation is assured by the constant influx of younger susceptible rats.

The disappearance of middle ear disease from the selected colony was effected either by a prompt reduction of the associated bacteria to a non-infective level or by their complete elimination. Although it is possible that by chance a resistant line of rats was selected, the fact that rats from the same line as residents of the stock colony were affected with middle ear disease directly opposes such a view.

It is possible that the bacteria associated with middle ear disease may persist within the selected population, carried in the upper respiratory tract or elsewhere by a few rats. The subjection of selected rats to abnormal environmental conditions favoring the development and spread of infective bacteria failed, however, to bring out any cases of middle ear disease. Whether the present state of the colony with respect to the condition will be maintained in the future cannot be predicted. Unless the infecting agents are reintroduced in considerable numbers from the outside it may be supposed that the incidence of middle ear disease will be held at least to a low level.

The procedure followed in establishing the selected colony failed to eliminate pneumonia. The morbidity rate was lower than the normal rate for stock rats but was still high; approximately 50 per cent of the adults showed definite pneumonic areas in one or more lobes of the lung. The frequent development of pulmonary lesions without middle ear involvement strongly suggests that the primary inciting agents of the two infections are not identical. The group of selected rats maintained for a long period under conditions of overcrowding affords evidence against the unity of the infecting bacteria. 60 per cent of these rats showed pneumonia without a single case of middle ear disease. If the two conditions were caused by the same bacteria it would be necessary to assume either the rapid development of an acquired immunity following involvement of the lung, or the existence of a natural organ resistance in the selected rats. Neither assumption is in keeping with known facts. In a similar group of stock rats a nearly equal incidence of the two conditions would be expected. As previously noted, the strain of selected rats which remained in the stock colony was affected with middle ear disease.

Although the incidence of pneumonia was not markedly decreased in the selected rats, there was evidence that the pulmonary reaction was less advanced than that in the stock rats. It is suggested that the development of terminal lesions from the primary focus is accelerated or in part determined by the development of secondary invaders. Infection of the middle ear might well establish a locus for the spread of such bacteria. The removal of this locus from the selected rats may account for the retarded pneumonic reaction. In this connection it is of interest that *B. actinoides* variety *muris*, which was recovered at

times from the affected lungs of stock rats, was never isolated from the selected animals.

Each generation of young breeding rats which was removed from the main selected colony and kept under isolation was free from pneumonia. In the maintenance of rat colonies there is reason to believe that limiting the age of the breeding stock to approximately 6 months and removing old rats from the surplus stock would reduce the incidence of pneumonia to a low level.

SUMMARY

A special colony of albino rats was built up by selection and isolation from a population in which middle ear disease was highly prevalent. No cases of aural infection occurred in the selected group, whereas its precursor showed a crude incidence of 57 per cent. The subjection of selected rats to a rachitic diet and to overcrowding did not predispose to the development of middle ear disease.

The incidence of pneumonia was not similarly affected; thus, 52 per cent of the adult selected rats showed pulmonary lesions, and 78 per cent of the adult stock rats. There was, however, a significant reduction in the number of cases which showed advanced pulmonary lesions.

Certain theoretical considerations of middle ear disease and of pneumonia are discussed.