STUDIES ON ENDEMIC PNEUMONIA OF THE ALBINO RAT

IV. Development of a Rat Colony Free from Respiratory Infections

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An earlier attempt to develop an albino rat colony free from infections of the respiratory tract succeeded only in the elimination of pleuropneumonia-like organisms (PPLO) and *Streptobacillus moniliformis* (1). Mature rats from this colony continued to develop the progressive inflammatory reaction of the lung which is here referred to as endemic pneumonia but is also known as rodent bronchiectasis. It was later shown that a virus-like agent, commonly acquired by maternal transfer at birth, was regularly recoverable from the affected lungs of these rats (2, 3). The etiological significance of this agent was left in doubt, however, due to the lack of suitable animals for test purposes. In 1948 a second attempt was made to establish an infection-free colony. The subsequent history of this colony, together with the methods employed in its development and maintenance is outlined in the present paper.

In 1931 Nelson and Gowen reported the establishment of an albino rat colony free from otitis media (1). Young rats from a single pair of breeders with normal lungs and middle ears constituted the nucleus from which the colony was developed. Brother and sister matings were subsequently made for 7 generations, the animals being housed in a special isolation unit. The 2 original breeders were taken from a colony, then maintained by Dr. John Gowen at the Rockefeller Institute in Princeton, New Jersey, in which there was a high rate of pneumonia and otitis in the adult rats. On weaning of the young, the parents of each generation were killed and autopsied. After the 7th generation was reared inbreeding was discontinued and the colony enlarged.

The outcome of this selection procedure was the nearly complete elimination of middle ear infection. In the first series of autopsies which included 50 young rats (3 to 4.5 months old) and 50 adult rats (upwards of 12 months old) no cases of otitis media were observed whereas the rate of this infection in contemporary rats from the original colony was 57 per cent. Since then additional autopsies have indicated an occasional probably non-specific case of middle ear involvement; but the over-all rate has been under 1 per cent. The incidence of pneumonia was not similiarly reduced. No cases were observed in young rats; but in adults the rate was 52 per cent as compared with 78 per cent in the original colony.

Streptobacillus moniliformis (*Actinobacillus muris*) was often recovered in cultures from the infected middle ears (43 per cent of 80 cultures) and the pneumonic lungs (35 per cent of 40 cultures) of adult rats from the original colony (4). The marked decline of otitis media in the selected rats was accompanied by the complete elimination of this organism from the respiratory tract. It has never been observed in the many cultures made from the lungs and nasal passages of these rats during the 20 years that the colony has been in existence.

In 1931 coccobacilliform bodies (PPLO) had not been recognized as the primary cause of otitis media in rats. As noted elsewhere it is highly probable that these organisms were present in animals from the original colony and that infectious catarrh coexisted with endemic pneumonia (2). For the past 10 years repeated cultural and microscopic examination of pneumonic lung tissue and nasal washings has failed to reveal this agent in rats from the selected colony.

These results were essentially duplicated by the findings of King who attempted to eliminate labyrinthitis from the inbred rat colony of the Wistar Institute by the use of captive, gray Norway females as foster mothers (5). Bacteriological studies, however, were not included in her work. The term "labyrinthitis" was used to denote the inflammatory reaction of the inner ear which invariably accompanies the well known condition of "twisting." In the Wistar colony the morbidity rate of this disease had increased from about 10 per cent in adults of the 50th generation to 40 per cent in those of the 75th. It had been noted, however, that labyrinthitis rarely occurred in gray Norway rats. Only 6 cases were observed in 3000 adults housed in the same room with the inbred albinos.

Litters of newborn rats were removed from normal appearing albino mothers and reared by lactating, gray, Norway females. This process was repeated a number of times, the young reared by foster mothers acting as the breeding stock for the succeeding generation. Ultimately all rats in the colony were descendants of individuals reared in this way. This procedure which seemingly eliminated labyrinthitis (no cases were observed during a 4 year period) failed to reduce the high incidence of pneumonia in mature animals.

At present the virus-like agent associated with endemic pneumonia can be identified only by its pathogenic activity on subnasal instillation in white mice (2). Infected mice, injected with lung and nasal suspensions, begin to chatter on about the 10th day. At autopsy on the 28th day they commonly show marked lung lesions (partial or complete consolidation of the right middle and of the azygous lobes) and otitis media. It may be added that middle ear involvement does not accompany the infection in rats. There may or may not be a frank rhinitis. In the presence of an exudate the nasal films show numerous leucocytes; but the extracellular and intracellular Gram-negative granules characteristic of PPLO are not demonstrable. Cultures from the lungs, middle ears, and nasal passages on horse serum–agar containing penicillin are generally sterile. A few bacterial colonies are sometimes observed and occasionally an overgrowth by a spreading organism but the small characteristic PPLO colonies are never encountered. If the injected mice are held for a longer period there is a slowly progressing morbidity without recovery.

The Establishment of the Second Selected Colony

The procedure employed in establishing the first selected colony was obviously inadequate for the elimination of the virus-like agent which may be present in the lungs of young female breeders in the absence of demonstrable lesions. In order to eradicate this agent it was essential that the newborn young be nursed by mothers whose lungs were not only lesion-free but also infection-free. The only known colony that might provide suitable breeders was the germ-free one maintained by Professor James A. Reyniers at the University of Notre Dame (6). Animals from this colony were not available. He called our attention, however, to a group of his rats which had been removed from their mothers by Cesarian section and subsequently raised by artificial feeding. It seemed likely that this procedure was adequate for the elimination of the virus-like agent and Professor Reyniers kindly placed a number of these rats

at our disposal. The original plan was to use the females as foster mothers for day old young removed from the first selected colony. Since the number of Notre Dame rats was limited it was more practical to breed them and start a colony from their young.

The second selected colony was begun in 1948 with a pair of females and one male from the artificially fed group of Professor Reyniers. It should be re-emphasized that these were not germ-free animals. These rats and their subsequent progeny were maintained under strict quarantine in an isolation unit. Two lines started by the 2 female breeders were continued for 5 successive generations by brother and sister matings. For the first 2 generations both parents were killed and autopsied shortly after the young were weaned. After the 5th generation cross-breeding between litters in each of the 2 lines was initiated and immediate examination of the respective mothers was discontinued.

The 2 selected colonies were housed in separate isolation quarters and were cared for by different attendants. Both groups were fed the same diet which was limited to commercial pellets and yellow corn. The young were weaned at the end of the 3rd week after birth. In the second colony the weaned rats were generally reduced in number to 5, this strain tending towards large litters, but were not separated by sex. Pregnant females in the first 5 generations were removed to individual cages prior to parturition. Beginning with the 6th generation, when brother and sister matings were discontinued, 4 to 6 breeding cages containing 4 females and 1 male were maintained. The young were left in these cages until weaned. At this time they were separated by sex into groups which numbered up to 10 individuals. The latter procedure was also carried out with the first selected colony. Both colonies were deliberately limited in size, the number of rats in each rarely exceeding 100. Both colonies were started at the department of Animal Pathology of the Rockefeller Institute in Princeton, New Jersey, and maintained there until September, 1951. At this time they were moved to the Institute's main laboratories in New York and again housed in separate isolation units. Shortly after this move was made line 2 of the second selected colony was discontinued.

At autopsy the lungs from all the adult rats were removed to Petri dishes and examined at low magnification with a dissecting microscope. The entire lung was then minced and a saline suspension (approximately 10 per cent) made with a glass tissue grinder. The middle ears and nasal passages were exposed and the latter aspirated with a capillary pipette. The nasal washings were filmed for Gram staining and a saline suspension made from them. The 2 suspensions were cultured on 30 per cent horse serum—heart infusion agar (pH 8) containing 2500 units of commercial penicillin (potassium G). The inoculated plates were sealed with scotch tape and incubated for 5 to 7 days at 37°C.

Mice were injected intranasally with lung and nasal suspensions from nearly all the adult rats of the first 5 generations. Prior to injection the mice were lightly anesthetized with ether and 0.05 cc. of the suspension was dropped on the nares. Young weaned mice of either sex weighing 12 to 13 gm. were used in groups of 3. It was possible to put 2 groups of 3 animals in each cage as it is now known that the virus-like agent is transmissible by direct contact in mice only after a period of several months. In most instances suspensions from individual rats were injected but occasionally a pool from 2 or more was used. The injected mice were held under observation for 3 to 5 weeks and inspected meanwhile for chattering. At autopsy the method of procedure was identical with that of the rat examination.

The Examination of the Original Notre Dame Breeders

Two female breeders (Nos. 1 and 2) were received from Professor Rèyniers in October, 1947, and 1 male (No. 3) together with 2 additional females (Nos. 4 and 5) in April, 1948. The first 3 rats were normal in appearance but females 4 and 5 showed rough coats and snuffled vigorously. Females 1 and 2 were mated with the male shortly after it was received and gave birth to young during the 7th and 8th weeks, respectively. These rats were killed and autopsied shortly after the second parturition, the females being approximately 6 months old. The 2 unmated females were also killed at this time.

At autopsy rat 1 showed bilateral otitis media. PPLO were observed in films from the middle ear exudate but the culture was overgrown by a spreading organism. The lungs and nasal passages were normal and the cultures negative. Rat 2 was normal throughout. PPLO were not recoverable on culture and were not seen in stained films. Neither PPLO nor the virus-like agent was demonstrable in mice injected with lung and nasal suspensions from these 2 rats. The male breeder, rat 3, which had been in contact with the females for a period of several weeks showed no involvement of the middle ear. There was no exudate in the nasal passages but leucocytes and PPLO were observed microscopically. The azygous lobe of the lung was completely consolidated and PPLO were obtained on culture. Since the pathogenic activity of the virus-like agent is usually masked by the presence of these organisms subnasal injections were not made in mice.

The unmated female breeder, No. 4, showed bilateral otitis media and rhinitis. PPLO were present in Gram-stained films and in cultures from both loci. *Streptobacillus moniliformis* was also isolated from the middle ear exudate. The lung was normal and culturally sterile. Carriage of the virus-like agent in the lung was indicated by a positive reaction in nasally injected mice. This strain was maintained for a number of passages in mice, being propagated in the absence of PPLO. The middle ears of rat 5 were normal. There was no definite rhinitis but leucocytes and PPLO were demonstrable in films. The lung was normal but a sparse growth of PPLO was obtained on culture. No injections were made in mice.

The Examination of Rats from the Five Subsequent Inbred Generations

The results of the autopsy findings on the original Notre Dame breeders were far from favorable for the establishment of a disease-free colony. PPLO infection was indicated in the male breeder and 3 of the female rats. In one of the latter it was accompanied by the presence of *Streptobacillus moniliformis* and in another by pulmonary carriage of the virus-like agent. The observation that the 2 mated females were free from infection with this agent was sufficiently encouraging, however, to warrant the continuation of breeding.

The breeding record of the second selected colony together with the number of litters reared in each generation during the period of brother and sister matings is presented in Table I. The mother of each litter in the 2 lines was killed and autopsied shortly after the young were weaned. In the first generation all the surplus rats, 5 in each line, were also autopsied. This examination included the male parent of the second generation. During the interval be-

tween June, 1948, and September, 1949, 35 litters were reared in the 2 lines of the colony. Exclusive of the 3 original breeders 43 rats were examined during this period. Thirty-three of these rats were female breeders which were 4 to 6 months old when they were killed. The lungs, middle ears, and nasal passages of all 43 rats were normal at atuopsy. Neither PPLO nor *Streptobacillus moniliformis* was recoverable at autopsy and the virus-like agent was not demonstrable on subnasal injection in mice.

TABLE I

Breeding Record of the Second Selected Colony for the First Five Generations

No. of generation	Line 1		Line 2	
	Approximate date of birth	No. of litters reared	Approximate date of birth	No. of litters reared
1	June, 1948	1	June, 1948	1
2	Sept., 1948	3	Sept., 1948	1
3	Dec., 1948	3	Dec., 1948,-Jan., 1949	2
4	Jan.-Feb., 1949	5	Mar.-Apr., 1949	4
5	Jan.-Sept., 1949	9	June-July, 1949	6
Total.....		21		14

The Examination of Adult Rats from the Second Selected Colony

The pneumonic lesions produced by PPLO in albino rats may be observed at any age after weaning but the pulmonary reaction characteristic of endemic pneumonia rarely occurs before the 6th month. From this period on there is a progressive increase in the morbidity rate and the extent of the pneumonic process. Most of the selected rats which had been examined up to this time were below the critical age level for the macroscopic detection of endemic pneumonia. During the period between November, 1949, and May, 1951, additional observations were made on 39 mature rats from the second selected colony. These rats, 19 of which were males, varied in age from 8 to 15 months, 20 being at least 12 months old.

All the 39 adult rats were normal in appearance and showed no indication of persistent snuffling. All of them were also normal at autopsy. Low power microscopic examination of the lungs showed only small surface areas of anthracosis. There was no involvement of the middle ears or of the nasal passages in any of the rats. Nasal films showed no significant number of leucocytes and PPLO were never observed. All cultures were negative in respect to these organisms and also to *Streptobacillus moniliformis*. Neither pulmonary nor nasal carriage of the virus-like agent was revealed in any of the rats by the mouse test.

There has been a significant increase in the incidence of anthracosis since the rat colonies were moved to New York. In Princeton small spherical spots were often observed on the surface of lungs from older rats. These areas were commonly gray or light in color and presumably the result of dust inhalation. In some instances they were difficult to distinguish from the small early lesions of endemic pneumonia. In the present location of the rats these surface spots are almost invariably black and obviously due to carbon deposits.

The Examination of Adult Rats from the First Selected Colony

By way of comparison, additional observations were made on mature animals from the first selected colony. Nine groups of rats, totaling 27 in number, were examined during the interval between December, 1949, and March, 1951. Most of these rats which varied in age from 13 to 21 months had been cage mates since birth. They were killed and autopsied in lots of 2 to 4 individuals. The results of the group examinations are summarized in Table II.

Prior to autopsy intermittent snuffling was observed in 8 of the 9 groups of rats. Several individuals were thin but in general their appearance was normal for animals of advanced age. Inspection of the lungs indicated a definite pneumonic reaction in 14 of the 27 rats, an incidence of 51 per cent. This reaction was commonly limited to the partial consolidation of a single lobe, complete lobar consolidation of 2 lobes being observed in only 3 instances. All the rats showed normal middle ears. The volume of the washings removable from the nasal passages was significantly increased in 14 of the 27 animals. This material was quite different in nature from the mucopurulent exudate which characterizes the rhinitis of infectious catarrh, tending to a thick cellular consistency. Only 2 of the 27 nasal films failed to show leucocytes which were commonly well distributed in all microscopic fields. Extracellular and intracellular Gram-negative granules indicative of PPLO were regularly lacking.

Tests for the pulmonary and nasal carriage of the virus-like agent were not made in each instance, though at least one rat from each group was examined. Lung suspensions from 20 of these rats, 8 of which showed no lesions indicative of endemic pneumonia, were injected intranasally in mice. A positive reaction, diagnostic of the virus-like agent, was obtained from each of these suspensions. PPLO were not demonstrable in cultures from the rat lungs nor in those from the injected mice. Mice were also injected with nasal suspensions from 19 of the rats, 3 of which were from individuals with normal lungs. Thirteen of these tests were positive, indicative of the nasal carriage of the virus-like agent. Two were negative, the suspensions being from rats which had shown no leucocytes in the nasal films. Four were inconclusive due to the presence of diphtheroids which resulted in premature death of the injected mice.

The presence of diphtheroids is one of the hazards in the performance of the mouse test. These organisms are carried in the nasal passages of some rats as part of the microbial flora and under normal circumstances they remain localized. In the mouse, however, these organisms are highly pathogenic and if transferred inadvertently, on nasal instillation, they

produce a massive pulmonary reaction which usually terminates fatally. Activity of the virus-like agent is completely masked.

TABLE II

The Autopsy Findings on Groups of Adult Rats from the First Selected Colony

No. of group	No. of rats per group	Sex	Age	No. with pulmonary reaction	No. with nasal reaction		No. with virus-like agent	
					Increased fluid	Leucocytes in film	Lung	Nasal passages
			mos.					
1	4	4 males	15	3	0	4	2+ 2 no test	2+ 2 no test
2	4	3 females 1 male	21	1	3	4	1+ 3 no test	1+ 3 no test
3	4	4 males	19	3	3	4	2+ 2 no test	4+
4	3	3 females	20	1	1	3	3+	3+
5	2	2 females	18	1	1	1	2+	1+ 1−
6	2	2 females	18	1	0	2	2+	2 no test
7	2	2 females	18	0	1	2	2+	2 inconclusive*
8	3	3 females	19	2	2	2	3+	1− 2 inconclusive
9	3	3 females	21	2	3	3	3+	2+ 1 no test
Totals.....	27	9 males 18 females		14	14	25	20+ 7 no test	13+ 2− 12 no test or inconclusive

* Secondary reaction with diphtheroids

The Examination of Young and Adult Rats from Unselected Colonies

To focus attention on the importance of the 2 selected colonies in the study of respiratory diseases of the albino rat and to emphasize the condition of the average colony in respect to these infections a few observations on rats obtained from outside sources are also included. Three groups of 5 rats from 3 different colonies were examined. The results of these examinations are shown in Table III.

The cultures that were made from the 15 rats were restricted to the middle ears in most instances. In group 2 the lungs were also cultured. Nasal films from all the rats were examined microscopically. Subnasal injections in mice were made only with lung suspensions from the rats of group 2.

The first group of rats was obtained from a large commercial colony and was composed of young females, 2 to 3 months old. Snuffling was noted in these rats as well as in those of the 2 other groups. At autopsy 4 of the group 1 rats showed otitis media, 5 rhinitis, and 1 pneumonia. PPLO were recovered from the 4 middle ear cultures, together with *Streptobacillus moniliformis* in one instance. All the nasal films showed leucocytes and the intracellular Gram-granules of PPLO.

TABLE III

Autopsy Findings on Rats of Varying Age from Three Unselected Colonies

No. of colony	Class of rat	No. of rat	Pulmonary reaction	Middle ear reaction	Nasal reaction	Bacteria identified in the middle ear or nasal passages
1	Young stock	1	—	BME*+	+	PPLO and *S. moniliformis*
		2	—	"	+	"
		3	1 lobe+	LME+	+	"
		4	—	BME−	+	"
		5	—	BME+	+	"
2	Young breeder	6	—	BME+	+	PPLO and *S. moniliformis*
		7	—	"	+	"
		8	—	"	+	" " " "
		9	—	"	+	"
		10	—	"	+	" " " "
3	Old breeder	11	3 lobes+	BME+	+	PPLO and *S. moniliformis*
		12	—	"	−	"
		13	1 lobe+	"	+	" " " "
		14	3 lobes+	"	+	" " " "
		15	2 lobes+	"	+	"

* BME = both middle ears; LME = left middle ear.

The second group was obtained from a small colony maintained for general laboratory use at an institution in New York. These rats were young female breeders about 6 months old. At autopsy all 5 of them showed otitis media and rhinitis. PPLO were isolated from each of the middle ear cultures, together with the streptobacillus in 3, and were also observed microscopically in nasal films.

In Table III the lungs of these rats are listed as normal. They showed no lesions suggestive of infectious catarrh or endemic pneumonia but did show rather indefinite streaky areas of surface discoloration. Mice injected intra-

nasally with each of the 5 lung suspensions developed early signs of illness. Autopsies performed between the 10th and the 15th days revealed a diffuse reaction which involved all lobes and was accompanied by pulmonary edema. This reaction is characteristic of the previously reported wild rat pneumonia virus (7). A very scant growth of PPLO, less than 10 colonies in 2 instances, was also obtained from 3 of the 5 lung cultures.

The third group was obtained from a colony maintained for experimental use at an Eastern university. These 5 rats were mature female breeders, of uncertain age, but at least 12 months old. At autopsy 4 rats of this group showed well developed lung lesions. All 5 of them also showed otitis media and rhinitis. PPLO were demonstrable in the 5 middle ear cultures and also in the nasal films. *Streptobacillus monoliformis* was isolated from the middle ear exudate of 3.

DISCUSSION

It is of particular interest that the first selected colony of Nelson and Gowen (1) and probably also that of King (5) had been maintained for an extended period in the absence of epidemic otitis media (infectious catarrh). In the former colony *Streptobacillus moniliformis* was eliminated as well as the causal pleuropneumonia-like organisms. There seems little doubt that the use of relatively simple methods of selection and quarantine is adequate for restricting these infections which have long been prevalent in rat populations.

The subsequent history of these two colonies clearly indicated, however, that supplementary methods were required for the elimination of endemic pneumonia and, as concerns the colony of Nelson and Gowen, the associated virus-like agent. In establishing the second selected colony the artificially reared breeders obtained from Professor Reyniers were employed as a means of interrupting the usual postnatal cycle by which this agent is transmitted from mother to young. It was fully realized, however, that the etiological significance of the agent was still uncertain The second attempt was successful, resulting in the elimination of all 3 infections and of endemic pneumonia as well.

The factors responsible for the successful outcome of the second selection procedure cannot be precisely defined. Extrinsically, it is probable that the avoidance of infection by the artificial rearing of the original breeders and the strict isolation of their progeny were of major significance. Intrinsically, the genetic constitution of the rats must also be considered. The Notre Dame rats show significant differences in structure and behavior from those of the first highly infected colony. Their weight and bodily dimensions are greater at all ages after weaning. They are less inclined to play while immature and are less active as adults. The breeding record of the females has been consistently inferior to that of the Princeton rats. The 2 strains may also show differences

in the degree of their susceptibility to infection. The virus-like agent was recovered, however, from one of the original Notre Dame rats and pleuropneumonia-like organisms together with *Streptobacillus moniliformis* from several of them. Uncompleted experiments on the nasal instillation of infective materials, which have been in progress for over a year, indicate, moreover, that endemic pneumonia can be artificially induced in rats from the disease-free colony. These findings are suggestive of average susceptibility but are not sufficiently conclusive to warrant a final decision at this time. From the history of these particular rats it seems probable that the infective agents were acquired by contacts made after their birth and early development.

The observations on unselected rats afford some measure of the frequency with which pleuropneumonia-like organisms and *Streptobacillus moniliformis* are encountered in the average rat colony. The only unusual finding in this series of autopsies was the recovery of the causal virus of wild rat pneumonia. At present little is known concerning the distribution of this agent which is seemingly related to the grey lung virus of Andrewes and Glover (8).

SUMMARY

A colony of albino rats was developed from three breeders (not germ-free) originally delivered by Cesarian section and artificially reared in the laboratories of Professor James A Reyniers.

The colony has been maintained under quarantine since June, 1948, in the absence of any demonstrable involvement of the respiratory tract or of the middle ears.

At autopsy pleuropneumonia-like organisms, *Streptobacillus moniliformis*, and the virus-like agent associated with endemic pneumonia were not recoverable from the lungs or the nasal passages of 81 young and adult rats.

During the same time interval the morbidity rate of pneumonia in another rat colony known to be free from infectious catarrh was 51 per cent while the incidence of infection with the virus-like agent approached 100 per cent.

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