

## Respiratory disease in a colony of rats

### I. The natural disease

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#### SUMMARY

An epidemic of acute respiratory disease in a colony of CFE rats is described, the main laboratory findings are recorded and its aetiology discussed.

The epidemic showed that severe respiratory disease varying from peracute to chronic was associated with infection of the lungs with a mycoplasma but that mycoplasmas could be present in rats, even in the lungs, without signs of disease, thus suggesting that one or more other factors were involved. It is also evident that there are strain differences in the susceptibility of rats to this disease.

#### INTRODUCTION

A breeding colony of approximately 300 gnotobiotic CFE strain rats was established behind a barrier against infection in newly built quarters at Alconbury, England, in June 1966. The foundation stock was from germ-free animals that had received an enteric flora, consisting of a group N *Streptococcus*, a *Lactobacillus* and a *Bacteroides*.

By May 1968 eight additional micro-organisms had been identified in the colony, namely *Staphylococcus albus*, *Bacillus subtilis*, *Clostridium welchii*, *Escherichia coli*, *Bacterium aerogenes*, *Proteus* spp., *Penicillium* spp. and *Aspergillus* spp. There was no evidence of ectoparasites, helminths, protozoa, *Pasteurella*, *Pseudomonas*, mycoplasmas or viruses.

The general health of the colony was excellent until the summer of 1968 when an acute respiratory disease broke out; by September it had reached epidemic proportions. In this paper the disease and its attempted control are described and the possible aetiology discussed.

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## MATERIALS AND METHODS

*Species and strains*

*CFE rats.* In June 1966 the foundation stock was obtained from a colony at Carworth, U.S.A., which had been derived originally from the Sprague-Dawley strain and maintained as an outbred closed colony for some 25 years.

*CFHB rats.* From May to July 1968 a strain of Wistar origin was introduced by the 'dry' hysterectomy method.

*CFY rats.* From July 1968 to January 1969 a third strain was introduced by the 'wet' hysterectomy method. This strain was derived from rats obtained from the Charles River Breeding Laboratories, France.

*CFLP mice.* From April to August 1967 a colony of ICI Alderley Park strain 1 mice was established by the 'dry' hysterectomy method. The pups were initially fostered onto gnotobiotic mice of a different strain previously introduced into the 'red area'.

*Environment*

Each strain was housed separately as far as possible within the area behind the barrier, which was called the 'red area', and comprised some 5000 ft.<sup>2</sup> (465 m.<sup>2</sup>) of animal rooms and 2500 ft.<sup>2</sup> (232 m.<sup>2</sup>) of corridors, stores and other service areas.

Incoming air was filtered to exclude particles larger than 5  $\mu$ , heated to about 74° F. (23° C.) and humidified to about 60% R.H. Room air was changed 12 times/hr.

All materials entering the 'red area' were subjected to a decontamination process. The food was autoclaved at 105° C. for 10 min.; cages, general hardware and clothing at 120° C. for 10 min.; cage tops at 130° C. for 3 min.; bedding at 135° C. for 10 min. Other materials were passed through a 'dunk' tank containing a disinfectant,\* or were surface-sterilized by formalin in a gas lock.

Staff entering the 'red area' took a shower and changed into autoclaved clothes. No masks were worn. The hands and forearms were rinsed with 70% alcohol on entry and several times a day. Entry was prohibited to anyone having contact elsewhere with rats or mice during the previous 3 weeks; only very rarely were visitors allowed in.

*Husbandry*

The animals were housed in polypropylene cages which had galvanized iron tops and which stood on open shelves on metal racks. Sawdust and wood shavings with as low as possible a content of fine dust were used for bedding, and paper for nesting.

The feed was a high fat high protein diet.† Drinking water was acidified (pH 2.0–2.5) with hydrochloric acid. Breeding methods were intensive, but avoided closely related mating. Growth and reproduction rates were high.

\* Task: British Hydrological Corporation, Wimbledon.

† Carworth-Dixon diet. E. Dixon and Sons Ltd., Ware.

### *Hysterectomy*

The CFHB rats, the CFY rats and the CFLP mice were all introduced into the 'red area' by hysterectomy and fostering. The donor dam, judged to be within 24 hr. of full term, was killed by hyperextension of the neck and cervical fracture, immersed in warm freshly prepared 1% iodophore solution, pinned out on the operating board, and, under frequent drenching with the iodophore solution, the abdomen was opened by a long median incision. The *cervix uteri* was ligatured, cut immediately caudal to the ligature and the uterus was freed, placed in a screw-capped jar of iodophore solution and passed through the dunk tank into the 'red area'.

It was found to be important that the temperature of the iodophore solution should be 39–40 °C.

The donor dam's thorax was opened immediately after the removal of the uterus and before this was passed into the 'red area'. If there were macroscopic lesions on the lungs of the donor dam the uterus was rejected. In fact this happened on one occasion only.

When the jar containing the uterus in warm iodophore solution was received in the 'red area' one of two methods, called respectively 'dry' and 'wet', was employed. In the 'dry' method the uterus was removed from the iodophore solution, placed in a small dish and opened with scissors. The young with their attached placentas were removed and placed on a warm surface covered with a sterile paper towel. The pups were dried off and if necessary stimulated to breathe, and the placentas were removed. When the pups were bright pink in colour and moving actively they were given to the foster dam, which had had a litter 24–72hr. previously inside the 'red area'. Before fostering she had been removed from her cage, and her own pups taken away and subsequently killed with chloroform. The fosterling pups were placed in the nest with minimal disturbance. The dam was then returned to the cage and in the vast majority of cases adopted her fosterlings without trouble.

The 'wet' hysterectomy method differed from the above as follows: The uterus was opened up below the surface of the iodophore solution so that the pups were delivered in a bath of disinfectant; in this they were well rinsed before being removed from the solution and placed on the warm surface. Thus the maternal uterine tissue and its secretions never left the disinfectant solution and never came in contact with the air of the 'red area'.

### *Histopathology*

#### *Laboratory examinations*

Specimens were preserved in 10% buffered formalin and were referred to Dr L. Mawdesley-Thomas, the Department of Pathology, Huntingdon Research Centre, Alconbury, Huntingdon.

### *Bacteriology*

Most of the media were prepared by the methods of Cruickshank (1965). In the later part of this work liquid and solid media for the cultivation of mycoplasmas

were supplied by Dr P. Whittlestone, School of Veterinary Medicine, Cambridge. Their preparation is described elsewhere (Whittlestone, Lemcke & Olds, 1971).

Cultures were incubated at 37° C. and examined daily for at least a week. To reduce dehydration solid media were incubated in a plastic bag which contained a piece of moist paper. Liquid media were used in screw-capped bottles.

### *History of the epidemic*

For 2 years the general health of the CFE colony was excellent, although there were sporadic spontaneous haemorrhages especially in heavily pregnant females during the latter part of 1967. There was also some evidence of marginal hypovitaminosis E. These conditions were corrected by increasing the content of vitamins K and E in the diet.

In January 1967 there were no macroscopic lesions in the lungs of these rats and histological examination showed only minimal amounts of lymphoid tissue, chiefly in the bronchiolar bifurcations. By the end of 1967 the amount of lymphoid tissue had increased considerably although there were no frank lesions and no clinical or post-mortem signs of respiratory disease. In January 1968 forty CFE rats were sent from Alconbury to Carworth U.S.A. for a general microbial screen; no evidence was found of respiratory disease or of infection with mycoplasmas or viruses.

In June 1968 an acute respiratory syndrome appeared in CFE females being used as foster mothers for pups of the CFHB strain. The clinical signs were hunched posture, a dull staring coat, severe respiratory distress and readily audible râles. The animals lost weight, rapidly became moribund and were removed from the colony.

Shortly afterwards other CFE rats in this room became similarly affected. After several weeks cases began to occur in the other rooms and very quickly the incidence in these rooms rose. By September 1968 the epidemic was widespread throughout the whole CFE colony. As the epidemic developed clinical disease was seen in progressively younger animals until at the height of the epidemic (November–December 1968) rats as young as 10 days showed respiratory distress and died. Extensive areas of bronchopneumonia were found in their lungs on post-mortem examination.

### *Chemotherapy of the epidemic*

At its peak the disease was so severe that the whole colony seemed in danger of extinction. An attempt was therefore made to control the outbreak by using 1% oxytetracycline hydrochloride in the drinking water. Although the immediate results were encouraging, after several weeks treatment it was apparent that the disease was only being contained. This drug was therefore discontinued and 0.6% tylosin in the drinking water was tried instead. After some weeks of treatment preweaning mortalities fell to the pre-epidemic level; there was little evidence of pulmonary disease in adult rats, although some had otitis media from which mycoplasmas were cultured.

By March 1969 use of antibiotics had been substantially reduced. Mild clinical cases occurred and were being culled, but only occasional cases with severe respiratory involvement were seen.

### *Necropsy findings*

## RESULTS

On examination post-mortem some animals showed areas of red or grey hepatization in one or more lobes of the lung. Sometimes the pneumonic area consisted of discrete or confluent grey nodules often involving large areas of lung tissue. Frequently the trachea contained abundant mucus. Histologically there was evidence of an acute inflammatory reaction similar to that described by Mawdesley-Thomas (1968).

Most animals examined post-mortem had pus in the middle or inner ears. Sometimes the middle ear was so affected that the tympanic membrane was distended into the external auditory canal by yellow pus. More commonly, at necropsy no abnormality was found in the middle ear, but there was thick pus in the cavity of one or both cochleas, the lining membrane of which was thickened. Previously the animals so affected had no clinical signs of disturbance of the vestibular postural mechanisms. Except for a variable amount of mucus, no abnormality was found in the nasal cavity.

### *Virological examination*

Three rats examined by Dr R. D. Barry, Department of Pathology, Cambridge had clinical signs of acute respiratory disease. At necropsy the lungs of one appeared normal; the other two had some pulmonary consolidations.

Three tenfold dilutions of homogenate of each affected lung were inoculated into allantoic or amniotic cavities of chick eggs and into primary cultures of monkey kidney cells. Dr Barry reported that there was no evidence for the presence of Sendai virus or of any other virus detectable by the methods used. These included haemagglutination tests on allantoic and amniotic fluids, and haemadsorption tests and examination for cytopathic effect in monkey kidney cells; all tests were made 72 hr. after inoculation. Culture fluids from monkey kidney cells were passaged to fresh cultures which were similarly examined also.

### *Bacteriological examination*

Two advanced cases were killed with coal gas and examined for a possible bacterial cause. Their lungs showed typically affected areas, smears from which contained numerous polymorphonuclear neutrophils, but no recognizable bacteria. Tiny rods and cocci were found within neutrophils in Giemsa-stained smears.

The pneumonic areas of each lung were homogenized, one in saline and the other in peptone water. The following media were sown with each homogenate: for anaerobic incubation, Loeffler's serum, blood agar, heated blood agar and Albimi broth; for incubation in air + 5% CO<sub>2</sub>, the same media as well as serum penicillin agar and fluid thioglycollate medium. Eight mice (not from the CFLP

colony) were injected, some intraperitoneally and some subcutaneously, with 0.6 ml. of lung homogenate each.

After 6 days of incubation the blood agar plates showed tiny colonies surrounded by greyish zones of incomplete haemolysis. Amorphous films of growth were noticed on Loeffler's serum slopes. These were judged probably to be mycoplasmas, from the media on which they grew and the time of their appearance, from their minute colonies on enriched media, from their appearance by dark field microscopy, and from their failure to stain as recognizable bacteria by Gram's method. No eubacteria were recognized on any of the culture media.

Two of the three mice injected subcutaneously developed local swellings over the following 2-4 weeks. One was killed and the swelling was found to consist of an abscess containing semi-fluid pus composed virtually entirely of neutrophil polymorphs. A mycoplasma was grown in pure culture from the pus. The other affected mouse was observed for 3 months. The swelling was at its greatest size ( $10 \times 10 \times 3$  mm) at 2 months, but had disappeared by 3 months. The third mouse which received a subcutaneous injection developed no lesion. It had been injected with the homogenate which contained the fewest organisms.

Five other mice injected intraperitoneally showed no clinical abnormalities and no lesions were found when they were killed and examined 15 weeks after injection. Subsequently 51 CFE strain rats from the colony were examined and cultured. Where typical pulmonary lesions were found mycoplasmas were consistently isolated. On the other hand mycoplasmas were sometimes grown from sites which showed no macroscopic evidence of disease, but usually lighter growth was found in these cultures.

Two clinically normal CFY strain rats from the colony were examined for the presence of mycoplasmas. No macroscopic lesions were found post-mortem in either of the lungs or the internal ears, but mycoplasmas were grown from the lungs and the ears of both rats.

#### DISCUSSION

These results suggested that the mycoplasma might be a cause of the disease, but that other factors were involved in producing the lesions.

The disease in the colony at Carworth Europe was first noticed after the introduction of hysterectomy-derived stock. The possible congenital transmission of the organisms is consistent with the findings of Graham (1963), who isolated *Mycoplasma pulmonis* from the ovaries or uterus of 23 of 77 female rats and produced genital lesions in female mice following intraperitoneal injection of cultures. He suggested that caesarean derivation might not eliminate mycoplasmas from the young of mothers with genital infection.

The genital tracts of three old female CFE strain breeders were examined and from the uterus of one pus was expressed which yielded abundant growth of a mycoplasma. On another occasion pus was found in the fallopian tube of a CFE rat from Carworth Europe which had been kept for some weeks in another laboratory. This too yielded a mycoplasma and Dr R. Lemeke confirmed that this was

serologically identical with that obtained from the respiratory tracts of the CFE colony in the course of the epidemic.

Although not enough animals have been examined to assess the frequency of genital carriage, it is clear that the mycoplasma isolated in this epidemic may be found in the female genital tract. This suggests that the dry hysterectomy method should not be relied upon to exclude mycoplasma from a barrier protected area, since contaminated uterine secretions would be exposed within the clean area. One might hope that the wet hysterectomy method would eliminate this hazard.

Mawdesley-Thomas (1968) brought forward histological evidence of an outbreak of acute bronchopneumonia in CFE strain rats which had been housed in the same animal rooms as clinically normal rats of the same origin as the CFY strain.

In May 1969 12 rats were obtained from the same source as that referred to by Mawdesley-Thomas, and were examined for the presence of mycoplasma immediately on arrival. They came by air and road in filter boxes and never had any contact with the Carworth Europe colony or rats coming from that colony.

At necropsy they showed no obvious clinical abnormalities, and no lesions that could be attributed to mycoplasma infection were found in the lungs, but in each of two females yellow pus was found in the middle ear. Mycoplasmas were grown from all 12 of these rats, from the inner ears of 11 and from the lungs of three. By serological and cultural tests the mycoplasma grown from these rats was identical with that grown from Carworth Europe rats (Whittlestone *et al.* 1971).

The disease in the CFE rat colony at Alconbury was clinically of an acute, even peracute, nature, and the histological picture was identical with that described by Mawdesley-Thomas (1968). Previously, mycoplasma-induced respiratory disease in rats has been referred to as 'chronic respiratory disease' (CRD). The relationship of mycoplasma to respiratory disease in rats, whether acute or chronic, has been discussed by Bell & Elmes (1969) and by Lane-Petter (1970).

Whatever the relationship between this organism and the disease it would appear to be preferable to discontinue using the phrase 'chronic respiratory disease' when referring to the syndrome, and to call it 'murine respiratory complex'.

Apparently healthy rats can carry mycoplasmas, particularly in their inner ears, without clinical signs of respiratory disease or macroscopic lung lesions.

Nelson (1963) associated the CRD syndrome with two agents, *M. pulmonis* and a virus. Tyrrell & Coid (1970) have reported acute respiratory disease in rats caused by Sendai virus. At the time of the outbreak of the disease in the CFE colony raised antibody titres to Sendai virus had been detected, and clinical disease attributable to this virus had been seen in the CFLP mouse colony housed in the same building. The possibility of its presence in the rats cannot be ruled out.

Gases such as sulphur dioxide, carbon monoxide and ammonia (Dalhamn & Reid, 1965) can produce marked changes in the lungs of rats. At the time of the outbreak a fault in the ventilation plant had allowed fumes, probably containing sulphur dioxide and carbon monoxide, to enter the 'red area' from the heating system. There was an appreciable ammoniacal smell in the animal rooms as the

result of a high stocking density of animals. These irritant gases could have played a part in lowering the resistance of the host to the mycoplasma. At the time of the outbreak sawdust and wood shavings were being used as bedding material and these produce dust, sometimes in considerable quantities.

It is possible that the severity of the disease was the result of the rat colony first being exposed to the mycoplasma at a time when the presence of other factors had lowered the host's resistance generally and in particular the resistance of the lungs. There does, however, seem to be a special strain susceptibility to this mycoplasma in CFE rats. Although the organism was found in the respiratory tracts of all three strains of rat, only in the CFE strain did it produce a severe epidemic of pulmonary disease.

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