Spontaneous Diseases and Their Control in Laboratory Animals

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THE NEED for laboratory animals that are free from spontaneous or naturally occurring diseases is being recognized by research workers throughout the United States. Laboratory animals with parasitic, bacterial, and viral diseases make poor experimental animals, produce inferior biological products, and markedly increase the cost of research. Moreover, diseased animals should not be used in research, especially in radiation, nutrition, and blood studies, because the normal physiological reactions or blood counts are not obtained from them. Researchers know that the results of experiments are reliable, trustworthy, and repeatable only if disease-free animals are used.

During the past 10 years, the Comparative Pathology Section and the Animal Production Section of the National Institutes of Health, Public Health Service, have investigated some of the diseases occurring naturally in mice, rats, guinea pigs, rabbits, and hamsters. The purpose of these investigations was to determine the diseases most injurious to these animals and to evaluate some of the methods of disease control and eradication.

External and Internal Parasites

External parasites cause or transmit some inflammatory skin disorders and parasitic and bacterial diseases in laboratory animals. Alopecia and dermatitis may be caused by the biting and sucking of lice, fleas, and bedbugs or by the burrowing of some species of mites. Eperythrozoonosis, transmitted by the mouse louse *Polyplax serrata*, produces a mild anemia in mice. It is caused by *Eperythrozoon coccoides*, a gram-negative, disk-shaped organism that occurs in the plasma and on the red blood cells. The rat louse *Polyplax spinulosa* transmits bartonellosis, caused by rod and coccoid-shaped organisms, *Bartonella muris*. They produce a severe anemia in splenectomized rats.

The intermediate or larval stage of the tapeworm *Hymenolepis nana* is transmitted to mice and rats by ingestion of fleas and meal beetles.

To eliminate these external parasites and control insectborne diseases, we used an insecticide consisting of 0.1 percent gamma benzene hexachloride, 2.0 percent methoxychlor, and 97.9 percent talc. The animal care workers at the National Institutes of Health have used more than a ton of this insecticide, and no deleterious effects due to the medicated powder have been reported.

Benzyl benzoate is effective in treating and controlling ear mange, or ear canker, a common parasitic infestation of rabbits. This inflammatory condition, usually confined to the external ear, is caused by a mite, *Psoroptes cuniculi*.

Diarrhea and death in young rabbits are frequently caused by two species of coccidia.

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Eimeria perforans causes intestinal coccidiosis and *Eimeria stiedae*, hepatic coccidiosis. We eliminated these two protozoan diseases from the rabbit colony by screening for infection through saltflotation fecal examinations and either removing the infected animals from the colony or giving them a saturated solution of sulfaguanidine in their drinking water for a 2-week period. Sanitation and the washing of all green vegetables are also important preventive measures. Since April 1955, after these procedures were adopted, only 12 positive coccidial fecal samples have been obtained in more than 1,400 examinations.

Tapeworms *H. nana* and *Hymenolepis diminuta* are the two common cestodes found in mice and rats. Treating groups of animals by adding 50 mg. of lead arsenate to 20 gm. of ground mouse feed and 100 mg. of the drug to 20 gm. of ground rat feed is effective. Culberton (*1*) reports that 10 mg. of atabrine by mouth for 2 successive days is also effective.

The oxyurids, or pinworms, of mice and rats are *Syphacia obvelata* and *Aspiculuris tetraptera*. These worms occur in the cecum and colon and produce a catarrhal enteritis when present in large numbers. A dose-rate per mouse of 40 mg. of piperazine adipate in 10 cc. of drinking water and a dose-rate per rat of 250 mg. in 50 cc. of water for 3 days for both mice and rats was found to be 95 percent effective in removing oxyurids from mature animals. Females and their litters should be re-treated in 30 days to remove pinworms from unweaned mice that were unable to drink the medicated water.

Although no critical anthelmintic tests have been run, we believe that the piperazine compounds would also be effective in removing the roundworm *Paraspidodera uncinata* from guinea pigs and the pinworm *Passalurus ambiguus* from rabbits.

The larval forms of the tapeworms of the dog and cat are occasionally encountered in laboratory animals. *Cysticercus fasciolaris*, the larval stage of *Taenia taeniaformis*, the tapeworm of the cat, appears in the livers of mice and rats. *Cysticercus pisiformis*, the larval stage of *Taenia pisiformis*, the tapeworm of the dog, is attached to the mesentery of rabbits.

Protozoa which may cause diarrhea and catarrhal enteritis are *Balantidium cavae*, a ciliated protozoan occasionally found in the colon of the guinea pig, and *Trichomonas* spp. and *Giardia* spp., frequently found in the cecum and colon of the hamster.

Salmonellosis

In the absence of an effective vaccine or therapeutic agent for the control of salmonellosis, research workers must rely on strict sanitation and general preventive measures to avoid the introduction and spread of this disease in laboratory animals. Prior to 1954, *Salmonella* spp. were frequently isolated from the organs and feces of the laboratory animals at the National Institutes of Health. In 1954, approximately 38,000 mice were screened and examined for salmonellae by bacteriological examination of composite fecal samples using the procedures outlined by Galton (*2*). In 1956 no salmonellae were isolated from the fecal samples of 2,800 mice, 1,000 rats, 800 guinea pigs, 1,200 rabbits, and 200 hamsters. During the same period in 1956 salmonellae were isolated from 25 monkeys (14.1 percent) of 177 in the colony. In 1957 salmonellosis was not observed in necropsies of more than 4,000 laboratory animals.

That year bacteriological examinations for *Salmonella* spp. were run on 780 samples taken from unopened bags of animal feed and 125 samples of sawdust used by the Animal Production Section. In 375 samples of mouse and rat feed *Salmonella kentucky* (identified by Dr. P. R. Edwards) was isolated from 1 sample; *Escherichia coli* from 7; coliform organisms from 77; paracolon bacilli from 14.

No salmonellae were isolated from 375 samples of the guinea pig and rabbit feed, but *E. coli* was found in 9 samples, coliform organisms in 6, paracolon bacilli in 1.

Organisms isolated from sawdust were *E. coli* from 25 samples, coliform types from 42, and paracolon bacilli from 8.

No organisms were found in samples of heated feed: 9 samples of dog feed, 6 of monkey biscuits, 3 of guinea pig pellets, and 12 of dog chow. These examinations showed that although salmonellae were isolated only once

from the unheated animal feed, fecal contaminated feed and sawdust are possible sources of salmonellosis in an animal colony.

Chronic Respiratory Disease

Chronic respiratory disease, the infection most injurious to laboratory rats, is prevalent in all rat colonies unless disease-free animals have been obtained from outside sources. Early lesions from the naturally occurring disease are seen in 3-month-old rats, and, as the animals get older, the lesions become more extensive.

In most rat colonies approximately 70 percent of the animals past 1 year show rhinitis, middle ear infections, bronchiectasis, and abscesses of the lung. Nelson and King (*3,4*) have reported that infectious catarrh and middle ear infection are caused by pleuropneumonia-like organisms (PPLO) and *Streptobacillus moniliformis*, and that the etiological agent of endemic pneumonia is a filtrable virus (*5*).

In October 1955, we began a long-term experiment to study methods of control and treatment of chronic respiratory disease in rats. Eighteen female and nine male rats were selected from three 60-day-old litters. These animals were divided into 3 groups, each containing 6 females and 3 males. One group was offered chlortetracycline at the rate of 5 mg. per 20 gm. of feed daily; the second group, 5 mg. of sulfamerazine per 20 gm. of feed daily; and the third group, 20 gm. of unmedicated ground feed daily. Necropsies and histopathological examinations were conducted on the original 27 rats and the 5- to 11-month-old progeny through the fifth generation, a total of 175 animals.

At necropsy, the animals in each group were examined for exudates in the nasal passages and middle ears and for involvement of lung lobes. Exudates from the nasal passages, middle ears,

Treatment of chronic respiratory disease in rats

Groups of animals	Number animals	Duration of exposure (months)	Number of animals with lesions		
			Peribronchial infiltration	Bronchiectasis	Areas of consolidation in lungs
Original breeders:					
Control	9	8	5	2	2
Chlortetracycline	9	9	7	0	0
Sulfamerazine	9	8½	7	1	1
F1 generation:					
Control	11	7	9	0	0
Chlortetracycline	12	9	10	0	0
Sulfamerazine	16	9½	3	0	0
F2 generation:					
Control	21	7	9	0	0
Chlortetracycline	13	7	5	0	0
Sulfamerazine	14	7	2	0	0
F3 generation:					
Control	5	6	1	0	0
Chlortetracycline	9	9	1	0	0
Sulfamerazine	5	11	0	0	0
F4 generation:					
Control	7	7	6	0	0
Chlortetracycline	6	6	6	0	0
Sulfamerazine	4	7½	0	0	0
F5 generation:					
Control	7	7½	[1]	0	0
Chlortetracycline	4	7½	[1]	0	0
Sulfamerazine	14	7½	[1]	0	0

[1] Microscopic results not completed.

NOTE: The control group received 20 grams of unmedicated feed daily; the second group received a daily dose of 5 mg. of chlortetracycline per 20 grams of feed, and the third group 5 mg. of sulfamerazine.

and from lung lesions were collected, gram-stained films of exudates were made, serum agar plates were inoculated, and disease-free mice were inoculated with the suspension of the exudates, according to the procedures outlined by Nelson (*5*).

The table shows the results of the necropsy findings from the 27 original animals and their 148 progeny. Each group of the original breeders showed very little improvement from the treatment. The control group contained 2 rats with areas of consolidation and bronchiectasis. In the sulfamerazine group, 1 animal showed bronchiectasis and consolidation of the lung, and the chlortetracycline group contained 7 rats with small areas of bronchiectasis.

The 6- to 11-month old F3 and F4 generation animals, with 9 to 15 rats in each group of the combined generations, showed remarkable improvement. There were no abscesses or areas of consolidation in the lungs of any of the animals. However, in the control and chlortetracycline groups, exudates were present in the nasal passages of 12 rats and in the middle ears of 15 rats. PPLO were isolated from the exudates of the nasal passages of 1 rat and from exudates of the middle ears of 5 in these two groups. No exudates were present in the nasal passages or in the middle ears of the rats fed the sulfamerazine.

In 10 mice inoculated with exudates from the control group of rats, 3 had exudates in the nasal passages and 3 had pneumonia. Of 10 mice inoculated with exudates from the chlortetracycline group, 5 had exudates in the nasal passages, and 3 had pneumonia. The 10 mice exposed to nasal passage and middle ear washings from the sulfamerazine group had no exudates in the nasal passages or middle ears and no gross or microscopic lesions in the lungs. Seven of these mice showed clumps of macrophages in some of the alveoli.

In the F5 generation, the 14 rats in the sulfamerazine group again showed no exudates or lung lesions, while exudates were seen and PPLO were cultured from the nasal passages of the control and chlortetracycline groups of animals.

Histopathological examinations of the lungs of mice that were inoculated with nasal and middle ear washings and emulsions of lung tissue from the three groups of F5 generation rats revealed bronchial pneumonia in each group of mice except those inoculated with washings or tissues from the sulfamerazine group of rats. No gross or microscopic evidence of toxicity or deleterious effects on any of the organs or on reproduction were seen in any of the animals.

In this experiment, sulfamerazine given daily in the feed eliminated rhinitis, middle ear infection, and endemic pneumonia in the 3d, 4th, and 5th generations of rats. Giving the drug at a daily dose rate of 5 mg. in 20 gm. of ground rat feed may be a good, practical way to obtain a rat colony free of chronic respiratory disease.

Other Bacterial and Viral Diseases

Endemic pneumonia, frequent in mouse colonies, is a contagious disease producing high morbidity and mortality. Preliminary tests indicate that the infection may be treated by offering 1.0 mg. of chlortetracycline daily in drinking water.

Epidemic diarrheal disease of suckling mice is of major concern to mouse breeders, since losses from it are as high as 70 percent and the production of mice is markedly reduced. Research workers are often forced to discontinue tests because of outbreaks of infant diarrhea. The disease occurs in mice 2- to 15-days old; older mice and nursing females are immune.

The symptoms, epidemiology, and transmissibility of the disease were reported in 1947 (*6*), and intranuclear and cytoplasmic inclusion bodies in the superficial epithelial cells of the small intestines have been described (*7*). Kraft transmitted infant diarrhea in suckling CFW mice by feeding crude and filtered intestinal contents from infected mice and noted cage-to-cage infections which she attributed to airborne or fly transmission (*8*).

In 1957, we infected 3- to 4-day-old mice through 10 serial passages with oral and intranasal suspensions of intestinal filtrates from mice with infant diarrhea. Ten serial transmissions of the infant diarrhea virus were also made on Chang liver tissue culture media. Infected Chang liver culture fluid from the second passage of the virus produced diarrhea in

7- to 10-day-old mice exposed intranasally. No chemotherapeutic treatment or other preventive measures have been effective in controlling this disease.

Snuffles and mucoid enteritis cause considerable losses of rabbits each year. Snuffles is a highly contagious respiratory disease, reported to be caused by *Pasteurella lepiseptica* and *Haemophilus bronchisepticus*. However, we have been unable to reproduce this disease experimentally in susceptible young rabbits. Chemotherapeutic treatments have been unsuccessful in the treatment of this disease.

Mucoid enteritis, which affects rabbits 5- to 7-weeks old, is responsible for losses up to 70 percent in some colonies. Its cause is unknown, but Templeton (*9*) has attributed it to hereditary or nutritional factors. He reported that chlortetracycline and vitamin B_{12} in feed reduced sickness and losses.

The disease most serious and injurious to guinea pigs is lymphadenitis, caused by streptococci type C. The guinea pig colonies of the Animal Production Section have been free of lymphadenitis since 1954. They were established and expanded from a small group of animals which were kept under strict methods of isolation and sanitation.

Salivary gland disease is a viral disease in guinea pigs recognized by the cytomegalic intranuclear inclusion bodies in the ductal epithelial cells of the submaxillary gland. We have been able to transmit this disease serially in young, pregnant guinea pigs and have produced death and abortion in the experimental animals. However, we have not been able to show that this disease occurring naturally is injurious or causes pregnancy toxemia.

Pregnancy toxemia is thought to be a viral disease of guinea pigs which is recognized by abortion during the latter part of the gestation period and death of the mother during abortion or a day or two after parturition. No treatment is known for it.

Diseases of unknown etiology occurring in laboratory animals are periarteritis in rats, calcium deficiency in guinea pigs, and amyloidosis in hamsters. No methods of control or treatment for these diseases have been reported.

Neoplasms are frequently seen in aged mice, rats, and guinea pigs, but seem to be infrequent in rabbits and hamsters.

Conclusion

The external and internal parasites and the bacterial and viral diseases cause enormous losses of laboratory animals and markedly increase the cost of research. These losses from diseases can be reduced considerably and the number of disease-free animals available for use in forming animal colonies can be markedly increased by good animal husbandry practices and sanitation, and by the development of effective methods of treatment and control.

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