Activation and Transmission in Rats of Infection with *Pneumocystis*[1] (35798)

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Pneumocystis is the etiologic agent of a form of pneumonia seen principally in immunologically deficient individuals (1). The agent is probably a protozoan, although some disagree, and it has even been termed an artifact (2, 3). Cultivation of the organism has not been accomplished, and its study in animals may be complicated by the presence of a latent infection (4). Normal animals inoculated with *Pneumocystis* exhibit no symptoms; whereas in rats and rabbits treated with corticosteroids, *Pneumocystis* may appear spontaneously in the lungs of inoculated and of uninoculated animals (4, 5).

Pneumocystis from rats and man are similar tinctorially (6) and by electron microscopy (7), but the immunologic relationships are uncertain; Goetz (8) reported differences detectable by complement fixation.

Since human *Pneumocystis* is available only sporadically, corticosteroid-treated rats were investigated as sources of organisms for experiments on cultivation. An improved method for inducing overt infection was developed, employing oral administration of corticosteroids and antibiotics. Additionally, apparent transmission of *Pneumocystis carinii* to steroid-treated pathogen-free animals was accomplished.

Materials and Methods. Rats. Albino rats (200 g) of either sex were used. For experiments on activation of latent infections conventionally maintained Sprague-Dawley rats ("standard") were employed. For transmission experiments we used cesarean-section originated, barrier-sustained rats (COBS; CD Sprague-Dawley strain) from Charles River Breeding Laboratories, Inc., Wilmington, Mass.

Medication. Water requirements averaged 50 ml/rat/day, except during the second week of medication when consumption rose to 75–100 ml/day. Each pair of rats was supplied, via drinking bottle, with 200 ml/day of water containing 0.2 mg of dexamethasone (Azium, Veterinary Intravenous, Schering Corp., Bloomfield, N.J.) and 100 mg of tetracycline (Tetracycline-Vet Powder, Chas. Pfizer and Co., Inc., New York, N.Y.); medicated water was replaced daily.

Cages. Steroid-treated rats housed in wire-bottomed cages developed leg and foot lesions after 2 or 3 weeks. Therefore, medicated rats were kept on sawdust or litter in enameled pans (30 × 18 × 10 cm) covered with wire screen. The COBS rats were maintained until used in barrier shipping boxes into which air was circulated through glass-wool-covered ports. Autoclaved rat chow was supplied via a covered port; the metal tube of the water bottle was pushed into the box through a small hole.

Stains and quantitation. Imprint smears of cut lung surfaces were dried and stained with Giemsa's or modified toluidine blue (9). Tissues fixed in Zenker's–acetic acid were sectioned and stained with Giemsa's, toluidine blue, or methenamine silver (10). Infection with *Pneumocystis* was established by presence of the wafer-like purple cysts as stained with toluidine blue and/or the characteristic Giemsa-stained cyst which, when developed, contained eight bodies.

Intensity of infection was graded by estimating the number of blue-stained cysts per

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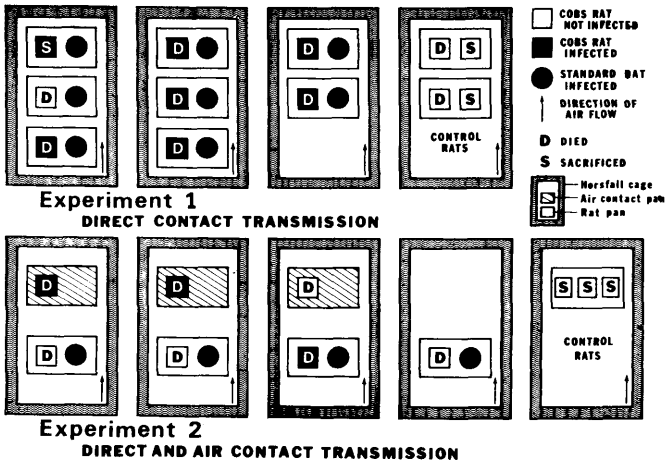

FIG. 1. Transmission experiments in Horsfall cages.

imprint and scored as follows (cysts/smear): very light, 1–14; light, 15–99; moderate, 100–500; and heavy, more than 500. A semiquantitative assessment of organisms per gram of rat lung was obtained by digesting 0.1 g of minced lung in 6 ml of 0.1% collagenase (General Biochem., Chagrin Falls, Ohio) at 37° after the method of Hinz and Syverton (11). After digestion for several hours with stirring, the material was filtered through gauze and centrifuged at 500*g* for 10 min; the supernatant fluid was discarded and Trowell's T-8 medium (12) was added to the sediment to a final volume of 1 ml. Serial tenfold dilutions of the suspension were made in T-8 medium, 1-μl samples were dried on slides, stained with toluidine blue, and the cysts were counted.

Transmission experiments. In a preliminary experiment seven COBS rats, maintained in barrier cages as received, were treated with dexamethasone and tetracycline. Three to 8 weeks later they were killed and examined.

In transmission experiments we used a Horsfall-type cage (13) consisting of an airtight metal box (60 × 54 × 45 cm) with an inlet air filter impermeable to bacteria (Cambridge Filter Co., Syracuse, N.Y.) in the front and an outlet operated by vacuum exhaust in the rear. The cage, except for the filter, was autoclaved before use. Enameled pans, litter, food, and water bottles were autoclaved and placed in the cage. Thereafter, rats, pans, and water bottles were handled only by washed hands.

We investigated transmission of *Pneumocystis* from standard rats to COBS rats in 2 experiments. In the first experiment, 12 COBS rats received in the same shipping box were randomly assigned. Each of 8 of the COBS rats was placed in a separate pan together with a standard rat which had been treated with corticosteroids for 2, 4, or 6 weeks; the 8 pans in this "direct contact" study were placed in three Horsfall cages as shown in Fig. 1. The remaining 4 COBS rats were placed in two pans in a fourth Horsfall cage as controls. All animals were offered 100 mg of tetracycline and 0.2 mg of dexamethasone/200 ml of water daily. Lungs from animals dying spontaneously were examined for *Pneumocystis*, as were those of animals killed when the experiment ended after 5 weeks. In the second experiment with Horsfall cages, 10 COBS rats from one shipment were divided into three groups as diagrammed in Fig. 1: 3 controls, housed in a single pan in a separate Horsfall cage; 4 in individual pans each with a standard rat which had been medicated for 2.5 weeks; and 3 alone in pans in air contact, but not in physical contact, with standard medicated animals. Each "air contact" rat pan was placed in a Horsfall cage that also contained a pan housing a medicated standard rat and

a "direct contact" COBS rat. The air contact pan was physically separate from the direct contact one. Food and water supplies for the air contact animals, as contrasted to direct contact animals, were replenished at different times with different utensils.

Results. Activation of Pneumocystis *in standard rats by oral medication.* After 4 weeks of medication with oral dexamethasone and tetracycline, the weight of 7 rats averaged 170 g, while untreated controls averaged 340 g. Mortality was followed in 20 treated rats; 4 died during the first month, approximately two-thirds of the rats by 7 weeks, and 16 had succumbed by 2 months. The relationship between duration of medication and intensity of infection was assessed by lung imprint smears from 40 rats sacrificed at various times (Table I). Light infections were present by the third week of treatment, and moderate or heavy infections by the fifth week. Lungs from 3 rats medicated for 5 to 7 weeks yielded 2×10^6 to 4×10^7 cysts/g of tissue. An imprint smear of infected lung from a rat treated for 7.5 weeks is shown in Fig. 2.

Transmission experiments. The combined results of the two experiments employing Horsfall cages in which 12 COBS rats were placed individually in direct contact with standard rats are as follows. Four direct contact COBS rats that survived 3 weeks or longer all developed light or moderate infections with *Pneumocystis* (Table II and Fig. 1). None of 7 control COBS rats developed detectable *Pneumocystis* infections, although 5 of the 7 survived for 4 weeks. In addition, none of the 7 COBS rats that received steroids in a preliminary experiment had developed patent infections when killed and examined after 3 to 8 weeks. Two of 3 COBS rats in "air contact" (Fig. 1) with standard

TABLE I. Relationship Between Duration of Steroid Treatment and Intensity of Infection with *Pneumocystis* in 40 Standard Rats.

Treatment (weeks)	No. of rats	Degree of infection			
		None	Light	Mod.	Heavy
>2	7		6	1	
3	14	1	5	8	
4	5			2	3
5	9		1	6	2
6	1			1	
7	4			1	3
Total	40	1	12	19	8

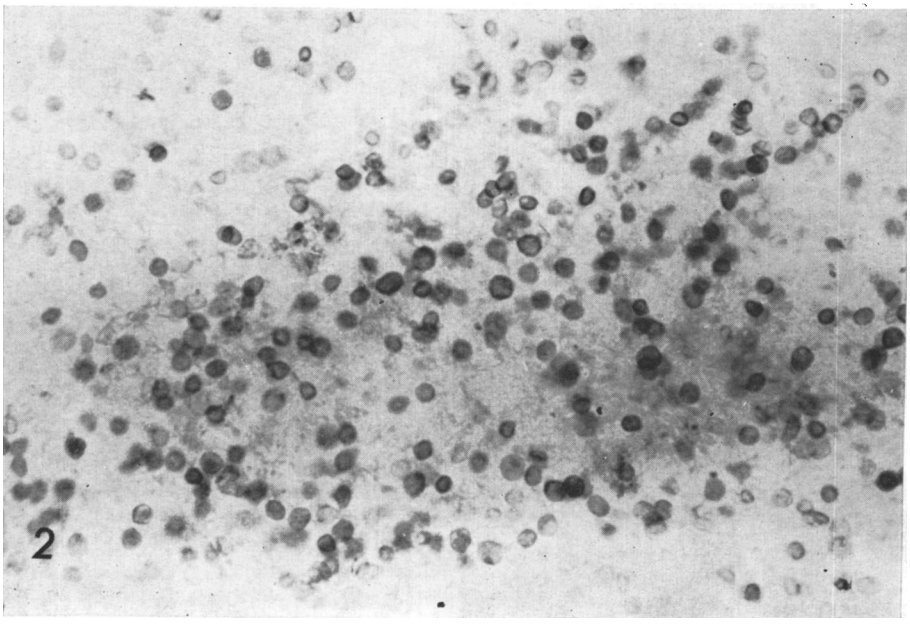

FIG. 2. Imprint smear of lung of rat treated for 7.5 weeks; heavy infection with *Pneumocystis* (toluidine blue; orig. 160×).

TABLE II. Infection with *Pneumocystis* in Corticosteroid-Treated COBS Rats in Direct Contact with Standard Rats.

Treatment and contact (weeks)	No. of COBS rats[a]	Intensity of infection			
		None	Very light	Light	Mod.
<3	6	2	4		
3	2			2	
4	1				1
5	1[b]			1	
Total	10	2	4	3	1

[a] Two rats not examined.

[b] Only rat sacrificed; all other direct contact COBS rats died spontaneously.

rats in Horsfall cages survived for 4 weeks, and a few cysts were seen in imprint smears from each.

Discussion. Oral administration of corticosteroid to rats was effective in activating latent infections with *Pneumocystis* and apparently enhanced susceptibility to acquired infection. Depending on water intake, 0.25 to 0.5 mg of dexamethasone/kg/day was provided. Since 0.75 mg of dexamethasone equals 25 mg of cortisone in terms of glucocorticoid activity (14), the regimen of oral dexamethasone contained about half as much glucocorticoid as did the parenteral regimen (30–40 mg/kg/day) of cortisone acetate employed by Frenkel *et al.* (4). Oral medication requires less manipulation of the animals than parenteral injection, although the dose per animal is less exact.

Corticosteroid treatment of rats and rabbits activates infection with *Pneumocystis* in uninoculated control animals (4, 5). Frenkel *et al.* (4) therefore used a strain of pathogen-free rats; it was observed that a spontaneous infection developed in one uninoculated animal, apparently the result of environmental transmission. In our studies with steroid-treated COBS rats, control animals failed to develop patent infections with *Pneumocystis*. However, 4 steroid-medicated pathogen-free COBS animals housed in direct contact with infected rats became infected. Further, two such animals, not in direct contact but exposed in a Horsfall cage via a common air supply, also acquired infection. Environmental transmission of infection appears to have been achieved. However, the experimental procedure is wasteful as many experimental animals are lost due to extraneous bacterial infections.

Summary. Corticosteroid-treated rats develop an overt infection with *Pneumocystis* and provide a ready source of organisms for study. A system of cortisonizing animals by the oral route was developed, utliizing daily provision of 200 ml of water containing 0.2 mg of dexamethasone and 100 mg of tetracycline to pairs of 200-g rats. The intensity of infection with *Pneumocystis* was related to length of time on treatment. Overt infection with *Pneumocystis* did not develop in a strain of pathogen-free rats in barrier cages while on the corticosteroid treatment schedule. However, transmission of the organism to pathogen-free treated animals was accomplished on contact exposure to infected rats.

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