Pneumocystis carinii : Experimental Pulmonary Infection in Rats*

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Pneumocystis carinii is a parasitic microorganism, which causes a fatal pneumonia in a variety of compromised patients such as infants and children with congenital immune deficiency, nutritionally deprived infants, and patients receiving immunosuppressive therapy for malignant disorders, especially leukemia and malignant lymphoma, or organ transplantation. Since Chagas found this organism in guinea pigs in 1909, it has been found in the lungs of various kinds of animals, those are rats, mice, rabbits, dogs, cats, monkeys, pigs, cattles, and so on, from all over the world.

Although the study of P. carinii infection and P. carinii itself has recently progressed, there are still many unsolved problems such as mode of infection, incubation period, life cycle and taxonomic position and so on. Experimental pulmonary pneumocystose was first demonstrated in rats by Weller (1955), in which one group of rats was inoculated with infected human lung tissue, and the other was not, then both were treated by cortisone and antibiotics. Since he found pulmonary pneumocystose not only in the inoculated rats but also in uninoculated rats by those treatment, he concluded that this pneumonia must be occured by

an activation of the latent pulmonary P. carinii infection. After that, this hypothesis was reexamined and made sure by himself (1956), Linhartová (1956, 1958), Goetz and Rentsch (1957) Pliess and Trode (1958), Ricken (1958), and Frenkel et al. (1966). In Japan, on the other hand, Higuchi et al. (1972), Nagai and Kamata (1974), and Yoshida et al. (1974) reported the similar results in animal experiments.

In the present study, an experiment was designed using rats to solve the following problems which are also important in the human P. carinii pneumonia : histopathological change of the lungs, development of the organism, incubation period, infection route, source of infection, and the presence or absence of systemic infection.

Materials and Methods

The Wistar strain albino rats used in this experiment were obtained from an animal dealer in Kyoto City. Those rats were then breeded under conventional condition in an animal house of our Medical School, keeping 3-5 rats in one wire cage. The study is composed of two experiments.

Experiment 1 consisted of 38 male rats with initial body weight ranging 110 g to 205 g. Of those rats, 3 were killed on the day when they were just sent to us from the animal dealer, and examined the lungs for P. carinii latent infection. Others were then devided into two groups. One group

consisting of 30 rats was administered 25 mg of cortisone acetate twice a week for seven weeks, and another group (5 rats) was breeded without cortisone treatment as a control. In order to prevent the bacterial infection, 0.05% solution of tetracycline hydrochloride was given to both groups as drinking water every day. The rats which died in the course of experiment were examined as soon as possible, and the survived rats were all sacrificed on the 49th day of the experiment, and examined for *P. carinii*.

Experiment 2 consisted of 62 young male rats with initial body weight ranging 55 g to 85 g. Of those rats, 5 were killed and examined on the day of their arrival from the animal dealer. After one month just breeding in our animal house without any treatment, 5 rats were killed and examined for *P. carinii* to reveal the infection in animal house. After that, the cortisone treatment with tetracycline hydrochloride began to 48 rats by the same manner as mentioned above. Other 4 rats were breeded without cortisone as a control group. Every week, 5 to 7 rats which were dead or killed, were examined. At the end of 9 weeks of investigation, the rest of the treated rats and control rats were all killed and examined for *P. carinii*. During the experiment, body weights of the rats were individually checked once weekly.

At autopsy of the rats, the lungs, liver, spleen and kidney were divided into 2 portions, one was fixed in 10% formalin solution, and the other stored in deepfreezer ($-80C$) for later use in counting the number of cysts by cyst concentration method (Ikai *et al.* 1977). At the same time the imprint smears of the lung tissue were also made and stained by Giemsa, Gomori's methenamine silver nitrate, and modified Toluidine blue 0. The lung sections which were prepared as 7μ thick, were stained by Hematoxyline-eosin, Gomori's methenamine silver nitrate, modified Toluidine blue 0, and Gram (Weigert's modification). When *P. carinii* was not found in the smears and 7μ thick lung sections, 100 pieces of serial 10μ thick lung sections with Toluidine blue 0 stain were further examined.

In order to know the presence or absence of the systemic *P. carinii* infection in rats, the liver, spleen and kidney of the rats whose lungs were heavily infected with *P. carinii*, were examined by the following manner : 100 serial pieces of each 10μ thick sections, and cyst concentration method both stained by Toluidine blue 0.

The density of *P. carinii* and the extent of the pneumonia in histopathological lung sections were evaluated by classifying into following four categories : Grade 1—only a few cysts adherent to the alveolar septal wall or free in the alveolar lumen without any inflammatory or cellular response. Grade 2—number of cysts increased with minimal septal inflammatory response and alveolar macrophages. Grade 3—number of cysts, septal inflammatory response and alveolar macrophages more increased, and honeycomb materials (mass of *P. carinii*) sporadically recognizable. Grade 4—honeycomb materials in almost all alveoli with tremendous number of *P. carinii* and rather reduced inflammatory response.

Results

I. Macroscopical and Histopathological Characteristics.

The macroscopic and microscopic investigations in the present study indicated a variety of pathological changes of *P. carinii* infection. As Hematoxyline-eosin stain usually does not reveal *P. carinii* cyst in the lung tissue, Toluidine blue 0 or Gomori's methenamine silver nitrate stain were always used at the same time.

Macroscopic characteristics : Some of the lungs of heavily infected with *P. carinii* were whitish gray in color, and solid, rubbery and liver like. They usually sank when they were put in the water. However, many of the lungs which were light infections showed almost normal appearance.

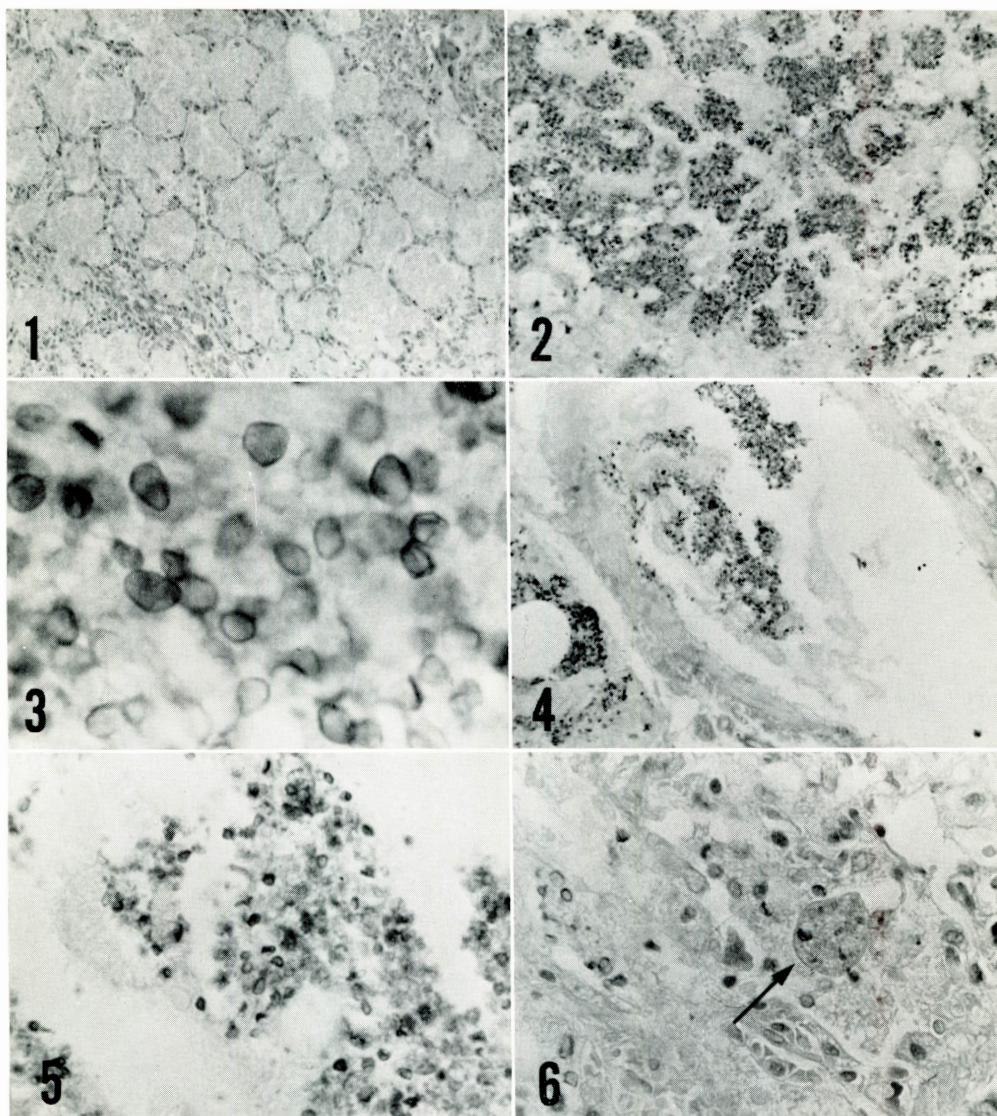Microscopic characteristics : The typical histopathological features of heavily infected

Fig. 1 Almost of all alveoli are occupied with typical honeycomb materials. Interstitial proliferation is less prominent (HE stain, 100 ×). Fig. 2 Tremendous number of cysts distributing in almost all alveoli (Toluidine blue 0 stain, 100×). Fig. 3 High power view of Fig. 2 (1000×). Fig. 4 Clump of cysts in the bronchi as well as in alveoli (Toluidine blue 0 stain, 100×). Fig. 5 High power view of Fig. 4 (400×). Fig. 6 The cysts phagocitized by alveolar macrophage (arrow) (GMS-HE stain, 400×).
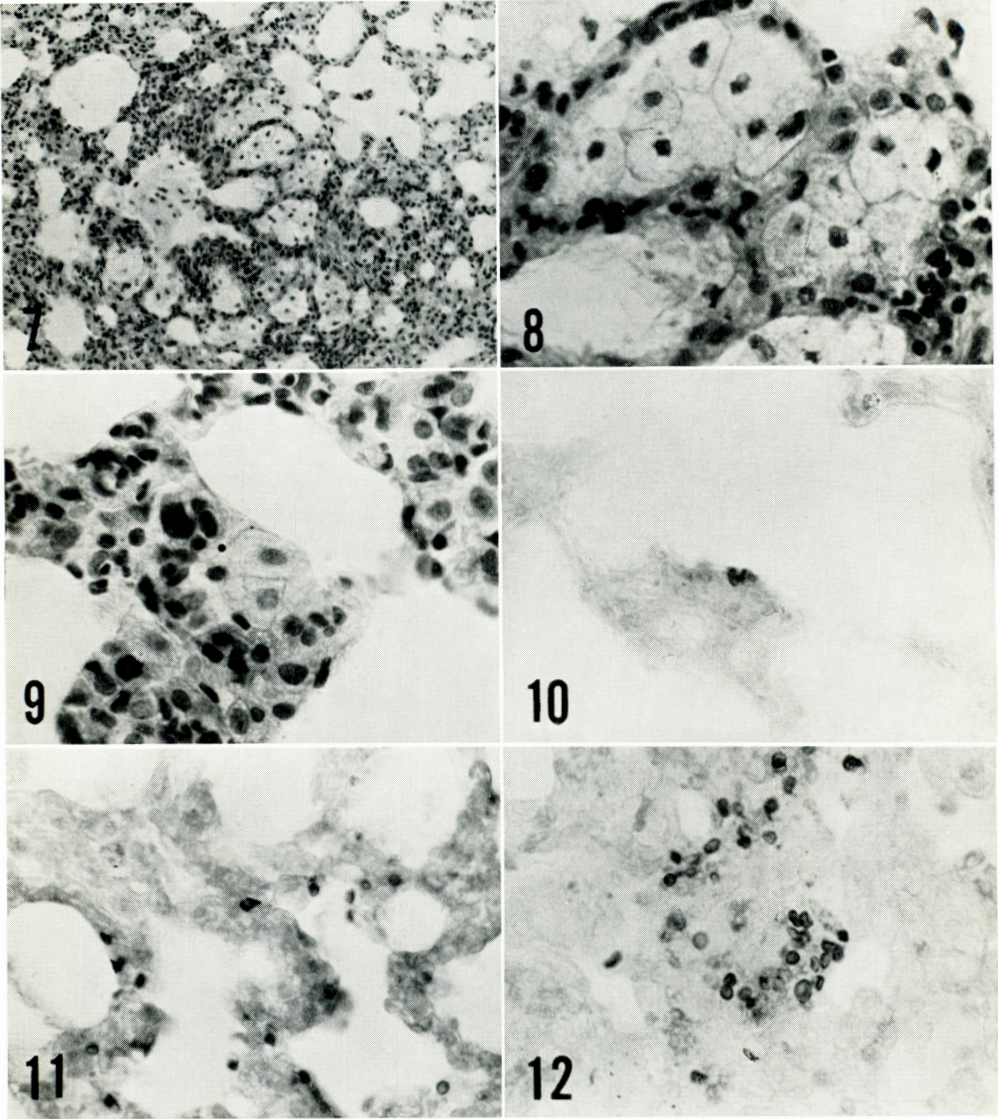
Fig. 7 Slight infiltration of alveolar septa and marked proliferation and shedding of foamy alveolar lining cells (HE stain, 100×). Fig. 8 High power view of Fig. 7 (400×). Fig. 9 Infiltration of small round cells and macrophages in alveolar wall (HE stain, 400×). Fig. 10 2 cysts attached to alveolar wall (Toluidine blue 0 stain, 400×). Fig. 11 Some cysts in or attached to alveolar wall (Toluidine blue 0 stain, 400×). Fig. 12 Clump of cysts only seen in limited some alveoli (Toluidine blue 0 stain, 400×).

lungs with *P. carinii* when stained by Hematoxyline-eosin were as follows : almost all alveoli were occupied with so called honeycomb materials, and interstitial proliferation was less prominent with almost none of plasmacellular infiltration (Fig. 1). When the sections were stained by Toluidine blue 0 or Gomori's methenamine silver, tremendous number of the cysts were seen as a group in the alveoli (Fig. 2 and 3). In some bronchioles and bronchi the masses of the cysts were also found (Fig. 4 and 5). Those findings suggest that the cysts in the alveoli are discharged into the bronchi and finally appear in the sputum. Sometimes the features that the cysts were phagocitized by the alveolar macrophages were seen (Fig. 6).

Those findings of heavily infected lungs as mentioned above were rather less frequent, and the majority of the cases were medium size of infection. In such medium size of infections, marked proliferation and shedding of foamy alveolar lining cells in the alveoli and septal inflammatory changes were often seen (Fig. 7, 8 and 9).

The extent of *P. carinii* infection was rated according to four Grades as mentioned before. Grade 1 (Fig. 10) is characterized by only a few cysts exist in the alveoli, or adherent to the septal walls without any inflammatory or cellular response. These light infections were often overlooked in usual 7μ thick 3 sections. Therefore, examination of 100 serial sections of 10μ thick was added in such instances.

Grade 2 (Fig. 11) is characterized by an increase in number of the cysts with minimal septal inflammatory response and minimal appearance of the alveolar macrophages. Focally the septa were infiltrated with a few macrophages, lymphocytes, and occasionally plasmacells.

Grade 3 (Fig. 12) is the category that the number of cysts markedly increases compared with Grade 2, and the septal inflammatory response and macrophages also increase. Some of the alveoli are occupied with honeycomb materials.

Grade 4 (Fig. 1, 2, 3) shows signs of the last stage of the pneumonia. Almost all alveoli are occupied with honeycomb materials, and tremendous number of *P. carinii* cysts are seen in it. However, the inflammatory response of the host is rather reduced compared with Grade 3.

II. Experiment 1

Three out of 38 rats killed at the time they were just sent to us from an animal dealer showed all negative for *P. carinii* either by smear or 100 serial lung sections (Fig. 13). One rat which died on the 12th day of cortisone treatment was still negative for *P. carinii*. However, 2 rats died on the 18th and 19th day showed positive, one is Grade 1 and the other Grade 2 respectively. These results indicate that the period of first finding of *P. carinii* after initiation of cortisone treatment is much shorter than that of the past experiments. However, the cause of death of these rats seemed not due to *P. carinii* pneumonia but bacterial infection judging from histopathological findings of the lungs. The tremendous number of Gram-positive bacteria was found in lung sections by Gram stain Weigert's modification. On the 23rd day, one rat died also by bacterial pneumonia showed Grade 2 *P. carinii* infection. At the 5th week of cortisone treatment, 5 rats died and 3 sacrificed. One rat which died on the 34th day showed typical *P. carinii* pneumonia (Grade 4). However, other rats were diagnosed as Grade 2 (3 rats) and Grade 3 (4 rats). At the 6th week, 6 rats, 3 died and 3 sacrificed, were examined. Those were Grade 2 in 2 rats and Grade 3 in 4 rats respectively. At the 7th week, 2 rats died with remarkable *P. carinii* pneumonia (Grade 4). Other 10 rats which survived until end of the 7th week showed by the autopsy that one was Grade 4, 7 were Grade 3, and 2 were Grade 2.

Five control rats which were killed at the end of the 7th week, showed negative in 1 rat and Grade 1 in 4 rats. The fact that a certain amount of *P. carinii* was found even in the control normal rats indicates the infection in the animal house.

The body weights of experimental rats were markedly depressed by the cortisone treatment, that is, 156 g in average at the beginning of the experiment dropped down into 127 g 7 weeks later. On the contrary average body weights of control rats gained from 135 g to 242 g in this period. The process of body weight fluctuation was investigated more minutely in Experiment 2.

The possibility of systemic infection of *P. carinii* was studied by examining 100 serial sections of the liver, spleen and kidney of 4 rats which showed Grade 4 in lung sections. The results were all negative.
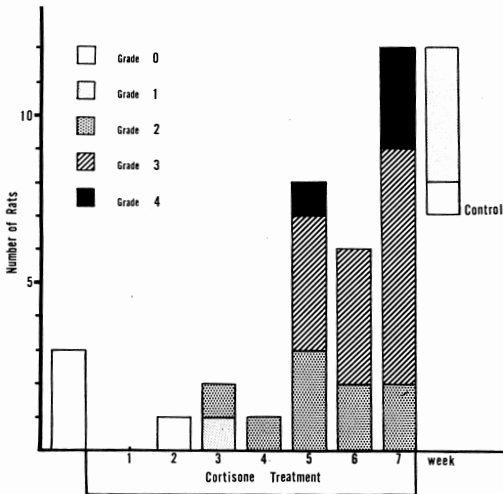

Fig. 13 Advance of *P. carinii* pneumonia experimentally produced in rats by successive cortisone treatment (Experiment 1).

III. Experiment 2

Experiment 2 is different from Experiment 1 in the following points: 1—The rats in Experiment 2 were 62 in number, and they were breeded for one month in a conventional animal house without any treatment before immunosuppressive administration which had immediately been started in Experiment 1. 2—Examination was performed weekly on 5–7 rats which were dead or sacrificed, and continued until end of the 9th week.

The body weights of rats in this experiment were ranging 55 g to 85 g (average 71 g) when they arrived here from the animal dealer. After one month breeding in the animal house, the body weights of those rats normally increased as ranging 180 g to 275 g (average 240 g). After that, the body weights of rats with cortisone treatment were not increased but rather diminished on the contrary the control rats normally gained the weight and showed 480 g in average at the 9th week as illustrated in Fig. 14.
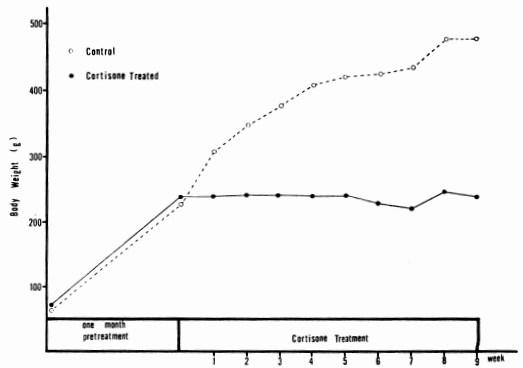

Fig. 14 Influence of successive cortisone treatment upon average body weights of rats. (Experiment 2).

The features of extent of *P. cainii* infection in Experiment 2 is shown in Fig. 15. Five rats which were immediately killed at their arrival to us from the dealer showed negative for *P. carinii* by any of lung smear, section, and cyst concentration method. However, 3 out of 5 rats which were examined after one month breeding without any treatment showed positive for *P. carinii*. The intensities of infection of those 3 rats were all classified as Grade 1. Those results suggest that the new infection of *P. carinii* is occurring among rats in the animal house. At the end of 1 and 2 weeks after initiation of the cortisone treatment, 5 rats were examined respectively. All the rats were infected with *P. carinii*, and 1 was Grade 2 and 4 were Grade 1 in each group.

Five rats sacrificed at 3 weeks and also 5 rats at 4 weeks alike showed increase in in-

tensity of *P. carinii* infection, that is, 4 rats were Grade 2 and 1 was Grade 1 in each group.

At 5 weeks, 6 rats were examined, in which 2 were dead on the 30th day of the treatment, and 4 were sacrificed. All were classified as Grade 2.

At 6 weeks, 2 rats died and 4 were sacrificed. The Grade 3 was first found in one rats, and the others were Grade 2. At 7 weeks, 7 rats were examined in which one was sacrificed, and 2 died on the 45th day, 3 on the 47th day, and one on the 48th day respectively. Four rats were Grade 3, and 3 were Grade 2. At 8 weeks, all of 4 rats were sacrificed in which 2 were Grade 3 and the other 2 were Grade 2. At 9 weeks, all of 5 rats which consist of one died and 4 sacrificed, were classified as Grade 3.

In the control group, one rats died on the 41st day, and the other 3 were sacrificed at the end of the 9th week. The intensity of *P. carinii* infection was classified as Grade 1 in 3 rats, and one was negative. It can be said from the facts that *P. carinii* may continue to exist but may not propagate so much in the normal rats.
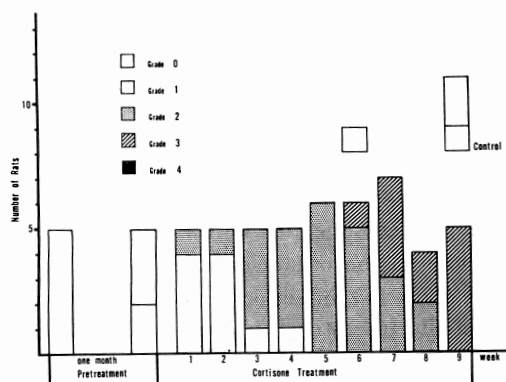

Fig. 15 Advance of *P. carinii* pneumonia experimentally produced in rats by successive cortisone treatment (Experiment 2).

It is interesting to note that the intensities of *P. carinii* infection in Experiment 2 were limited within Grade 2 or Grade 3, and the cause of death was mostly not considered due to *P. carinii* pneumonia but due to

bacterial infections by histopathological examinations, on the contrary the typical *P. carinii* pneumonia classified as Grade 4 was often seen among rats examined at the latter half period in Experiment 1. As the reason of the differences between Experiment 1 and 2, it is considered that the rats in Experiment 2 might have received daily infection with *P. carinii* during the period for one month breeding before the cortisone treatment, and gained resistance to the organism. The resistance seemed to continue for a certain period even in the course of cortisone treatment.

When compared the intensity and the expanding of *P. carinii* infection in cortisone treated rats with those in control rats, it is evident that the latent infection was activated by the cortisone treatment as Weller (1955) already pointed out. In the present study, an attempt was made to analyse the course of expanding of the infection quantitatively by using cyst concentration method. As shown in Fig. 16, the average number of cysts per 1 g of the lungs increased, giving essentially a straight line on semilogarithmic graph, as 1.06×10^5 at 1 week after initiation of cortisone treatment, 1.83×10^5 at 2 weeks, 7.15×10^5 at 3 weeks, 1.10×10^6 at 4 weeks, 2.51×10^6 at 5 weeks, 1.16×10^7 at 6 weeks, 1.48×10^7 at 7 weeks, 4.29×10^7 at 8 weeks, and 4.90×10^7 at 9 weeks respectively.

In order to detect *P. carinii*, in Experiment 2, both of the lung section and cyst concentration method were used at the same time. The correlation between the grading by lung sections and the number of cysts per 1 g of the lungs is illustrated in Fig. 17. Actual number of cysts in Grade 1 are ranging $0-3.7 \times 10^5$ (average 7.4×10^4), Grade 2: $2.1 \times 10^5-2.8 \times 10^7$ (5.5×10^6), and Grade 3: $5.7 \times 10^5-1.1 \times 10^8$ (3.8×10^7) respectively. There were some cases which were diagnosed as Grade 1 by the examination of 100 serial lung sections in spite of negative by the cyst concentration method.

It has been achieved that the lungs are the only site for parasitizing of *P. carinii* although Zandanell (1954) and others found
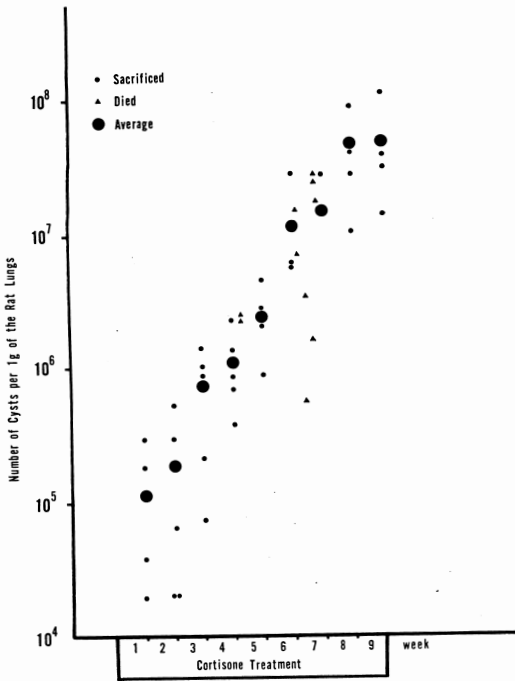
Fig. 16 Increase of the number of *P. carinii* cysts in 1 g of rat's lung counted by cyst concentration method in course of cortisone treatment (Experiment 2).

Fig. 17 Correlation between grading by lung sections and number of cysts per 1 g of the lungs (Experiment 2).

P. carinii in several organs other than the lungs in human cases. In this respect, the study was made to search for the cyst of *P. carinii* in the liver, spleen and kidney of 12 rats which were diagnosed as Grade 3 in Experiment 2. The examination was performed by using both histopathological sections and cyst concentration method, but no cyst was found from those organs.

Discussion

I. Histopathological characteristics.

At the age of *P. carinii* pneumonia was first known among prematures or marasmic orphans in Europe, strong interstitial plasma-cell infiltration of the lungs was noticed as the most characteristic feature of this pneumonia (Vaněk and Jírovec, 1952). This is the reason why this pneumonia has been called interstitial plasmacellular pneumonia for long time.

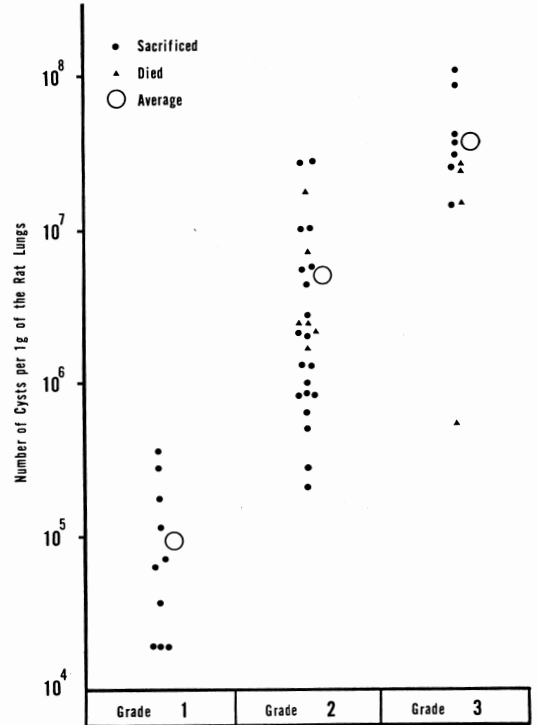On the other hand, Weller (1955), Linha-

rtová (1956, 1958), and Ricken (1958) stated that interstitial plasmacell infiltration was not conspicuous among experimental animals which were artificially produced *P. carinii* pneumonia by immunosuppressive treatment.

Dutz *et al.* (1973) considered that those findings were due to the difference of host response to the organism, and he distingui-shed *P. carinii* infections into following two types. 1. Interstitial plasmacellular pneumonia which occurs only in prematures or marasmic infants between 10 to 24 weeks of life. 2. Hypoergic pneumocystose which may occur at any age and is associated with congenital immunodeficiency or diseases of reticuloendothelial system or immunosuppressive therapy. In the former type, *P. carinii* may spread rapidly throughout the alveoli of all pulmonary lobes, and the capsular antigen of *P. carinii* cyst elicits a massive plasmacell infiltration in the alveolar

septa. On the other hand, low host response to the organism occurs in the case of latter type.

In the present animal experiment, 4 rats produced severe *P. carinii* infection by cortisone treatment. The histopathological examination by Hematoxyline-eosin stain revealed massive honeycomb materials occupying almost all alveoli. However, the interstitial inflammatory response was weak with almost none of plasmacell infiltration. Those type 2 features are usually seen at autopsy of *P. carinii* pneumonia patients who have received strong immunosuppressive therapy against their malignant basic diseases.

In the severe cases of *P. carinii* infection in rats, considerable amount of *P. carinii* was found in bronchioles and in bronchi as a mass. Those results support the possibility of finding the organism from sputum (Bachman, 1953; Le Tan Vinh *et al.*, 1963; Yoshida *et al.*, 1977) or from hypopharyngeal materials (Erchul *et al.*, 1962; Catar, 1968).

II. Propagation of *P. carinii* in experimental animals

Among 100 rats in the present Experiment 1 and 2, 78 rats were treated with cortisone in which 77 (98.7%) were found being infected with *P. carinii*. Particularly 48 rats of Experiment 2 which were breeded for one month before cortisone treatment, were all positive (100%) for *P. carinii*. Those positive rates are extremely high compared with those of Weller (1955, 1956) 59.5%, Pliess and Trode (1958) 56%, Linhartová (1956, 1958) 65%, Goetz and Rentsch (1957) 75%, Ricken (1958) 87% and Nagai and Kamata (1974) 65%. This difference seems to come from the detecting method of the organism in my opinion. Most of the past studies used Giemsa stain for the lung smears, and Hematoxyline-eosin, PAS and Gomori's methenamine silver stain for the lung sections. The former 3 techniques among 4 mentioned above are not so efficient in detecting the organism particularly in the light infection. Gomori's methenamine silver

stain is good for detection but is complicated in procedure, so it takes much time for making preparations. In the present study, *P. carinii* was seeked by examining 100 pieces of 10μ thick serial lung sections, and cyst concentration method was also used at the same time. Furthermore, Toluidine blue 0 stain (modified by Chalvardjian and Grawe, 1963) was mainly utilized in this series of experiment. This method is much simple compared with Gomori's technique, and exhibits the cyst walls in beautiful purple color with clear contrast to the background (Ogino *et al.*, 1977). The use of those technique might be the reason why so high infection rates were gained in this study.

III. Mode of infection

The mode of infection of man and animal with *P. carinii* is the subject of much controversy for many years. Although Pavlica (1962), Post *et at.* (1964), and Bazaz *et al.* (1970) reported possibility of intra-uterin transmission, many people believe, at present, that naso-tracheal inhalation of the cysts is the main route of invasion of this parasite. In this respect, Hendley and Weller (1971) reported the following experiment. They used two groups of cesarean-section originated barrier-sustained rats. Both groups were treated with dexamethasone and tetracycline. The rats of one group were contacted directly or through air with *P. carinii* infected rats, and those of another group were kept in barrier cage. As the result, the infection was only seen in the former group.

The infection of man with *P. carinii* was also seen in hospital, orphanage, and family. Ruskin and Remington (1967) resported two patients who hospitalized in the same room showed signs of *P. carinii* pneumonia almost coincidently. Watanabe *et al.* (1965) reported family case of this pneumonia, and Lim and Moon (1960), Redman (1975) reported epidemics in the orphanage.

In the present study on rats, 10 rats (71%) out of 14 untreated rats became positive for

P. carinii by just breeding in an animal house for 4 to 13 weeks. This fact also supports the hypothesis mentioned above.

IV. Incubation period

Incubation period means the term to the onset of P. carinii pneumonia from initiation of immunosuppressive therapy. In this respect, Bachmann (1954) stated 40 to 50 days, Gajdusek (1957) 3 to 11 weeks, and Kučera (1967) 16 to 100 days, all in clinical cases. In the present animal experiment, the incubation time was considered within 5 weeks since heavy infections were seen among rats, examined at 5 weeks cortisone treatment.

V. Resistance to the growth of P. carinii

It is commonly believed that P. carinii is distributing widely among man and animals as a saprophyte. In other words, host animals may have resistance to the organism when their immune systems are normal. In the present investigation, the grades of P. carinii infection were obviously much lower in rats which were breeded for one month before cortisone treatment than in rats immediately started the treatment. It seems that the normal rats gained the resistance by receiving small amount of P. carinii every day, and the resistance continued for a certain period even in the course of the cortisone treatment.

VI. Systemic infection

The problem of systemic infection of P. carinii is important connected with the mode of infection like diaplacental infection. In clinical field, Pavlica (1962) found massive infection of P. carinii in the lungs of a stillborn fully developed foetus, and Zandanell (1954), Jarnum et al. (1968), Awen and Baltzen(1971), LeGolvan and Heiderberger (1973) and Rahimi (1974) found the organism disseminating in many organs other than the lungs. However, the success in animals was only made by Walker (1912) who found the cysts in the spleen of guinea pig. Weller (1954), Linhartová (1956) and Ricken (1958) examined the liver, kidney and spleen of many rats experimentally produced P. carinii

pneumonia, but they could not find the organism. The present investigation also showed negative for P. carinii in the liver, kidney and spleen in spite of many sections were examined and cyst concentration method was used at the same time.

Summary

Experimental pulmonary infection with Pneumocystis carinii was provoked in rats by giving cortisone acetate and tetracycline hydrochloride. The results obtained in two series of experiments are summarized as follows.

1. P. carinii infection was detected in 77 out of 78 rats treated by cortisone for 1 to 9 weeks. During the time, the number of cysts per 1 g of the lungs counted by cyst concentration method increased regularly, giving a straight line on semilogarithmic graph.

2. Light infection with P. carinii was also detected in 10 out of 14 normal rats which were breeded without any treatment. This fact suggests that the animal acquires the infection by contagion, possibly through air, in an animal house.

3. Severe P. carinii pneumonia was seen in rats of Experiment 1 on and after 5th week of cortisone treatment, whereas such severe case was not seen in Experiment 2 in which the rats had been breeded for one month before cortisone treatment. Probably, the rats in the latter group gained resistance to the organism by daily infection during the breeding time, and the resistance continued for a certain period even in the course of cortisone treatment followed by it.

4. The grade of P. carinii infection was classified into 4 categories judging from histopathological changes and number of cysts in the lungs. The feature of severe P. carinii pneumonia was characterized by honeycomb materials filling in almost all alveoli with tremendous number of the organism, and weak interstitial inflammatory response with almost none of plasmacell infiltration. This findings were similar to those of human P. carinii pneumonia occur-

red under immunosuppressive conditions.

5. In order to suppose the systemic infection of *P. carinii*, the liver, kidney and spleen of 16 rats whose lungs showed heavy infection were examined, but no organism was found from those organs.

Acknowledgement

References

1) Awen, C. F. and Baltzen, M. A. (1971) : Systemic dissemination of *Pneumocystis carinii* pneumonia. Can. Med. Ass. J., 104, 809–812.

2) Bachmann, K. (1953) : Über die Anwesenheit von *Pneumocystis carinii* bei der frühkindlichen, plasmacellulären, interstitiellen Pneumonie. Z. Kinderheilk., 73, 632–638.

3) Bachmann, K. (1954) : Zur Epidemiologie und Inkubation der frühkindlichen interstitiellen Pneumonie. Z. Kinderheilk., 74, 133 –140.

4) Bazaz, G. R., Manfredi, O. L. and Claps, A. A. (1970) : *Pneumocystis carinii* pneumonia in three fullterm siblings. J. Pediat., 76, 767–769.

5) Catar, G. (1968) : Some observations on *Pneumocystis carinii* and *Pneumocystis* pneumonia. Proc. 8th Internat. Congr. Trop. Med. Malaria (Teheran), 925.

6) Chagas, C. (1909) : Nova tripanozomiaze humana. Mem. Inst. Oswald Cruz, 1, 159– 218.

7) Chalvardjian, A. M. and Grawe, L. A. (1963) : A new procedure for the identification of *Pneumocystis carinii* cysts in tissue sections and smears. J. Clin. Path., 16, 383–384.

8) Dutz, W., Post, C., Kohout, E. and Aghmohammadi, A. (1973) : Cellular reaction to *Pneumocystis carinii*. Z. Kinderheilk., 114, 1–11.

9) Erchul, J. W., Williams, L. P. and Meighan, P. P. (1962) : *Pneumocystis carinii* in hypopharyngeal material. New. Engl. J. Med., 267, 926–927.

10) Frenkel, J. K., Good, J. T. and Shultz, J. A. (1966) : Latent *Pneumocystis* infection of rats, relapse and chemotherapy. Lab. Invest., 15, 1559–1577.

11) Gajdusek, D. C. (1957) : *Pneumocystis carinii*. Etiologic agent of interstitial plasma cell pneumonia of premature and young infants. Pediatrics, 19, 543–565.

12) Goetz, O. und Rentsch, L. (1957) : Weitere Untersuchungen zur experimentellen Rattenpneumocystose. Z. Kinderheilk., 79, 578– 585.

13) Hendley, J. O. and Weller, T. H. (1971) : Activation and transmission in rats of infection with *Pneumocystis*. Proc. Soc. Exp. Biol. Med., 137, 1401–1404.

14) Higuchi, H., Kameyama, K. and Kozima, K. (1972) : One human case of *Pneumocystis carinii* infection and experimental pneumocystose in rats. Jap. J. Thorac. Dis., 10, 515 (in Japanese).

15) Ikai, T., Yoshida, Y., Ogino, K., Takeuchi, S. and Yamada, M. (1977) : Studies on *Pneumocystis carinii* and *Pneumocystis carinii* pneumonia. II. Method for concentration and quantitation of *P. carinii* cysts. Jap. J. Parasit., 26, 314–322 (Japanese with English summary)

16) Jarnum, S., Rasmussen, E. F., Ohlsen, A. S. and Sφrensen, A. W. S. (1968) : Generalized *Pneumocystis carinii* infection with severe idiopathic hypoproteinemia. Ann. Intern. Med., 68, 138–145.

17) Kučera, K. (1967) : La pneumocystose en tant qu'anthropozoonose. Ann. Parasit. Hum. Comp., 42, 465–481.

18) Le Tan Vinh, Cochard, A. M., Vu-Trieu-Dong et Solonar, W. (1963) : Diagnostic "in vivo" de la pneumonie à "*Pneumocystis*". Arch. Franc. Pédiat., 20, 773–792.

19) LeGolvan, D. P. and Heidelberger, K. P. (1973) : Disseminated granulomatous *Pneumocystis carinii* pneumonia. Arch. Path., 95, 344–348.

20) Lim, S. K. and Moon, C. S. (1960) : Studies on *Pneumocystis carinii* pneumonia, II. Epidemiological and clinical studies of 80 cases. Jonghap Med., 6, 77–86.

21) Linhartová, A. (1956) : Experimentelle Pneumocystose bei Ratten. Z. Bakt. I. Abt. Orig., 167, 178–186.

22) Linhartová, A. (1958) : Weitere Beiträge zur experimentellen Lungen-pneumozystose. Zbl. Allg. Path., 98, 393–400.

23) Nagai, K. and Kamata, Y. (1974) : Experimental studies of *Pneumocystis carinii*

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pneumonia. The Saishin Igaku, 29, 399–407 (in Japanese).

24) Ogino, K., Yoshida, Y., Takeuchi, S., Ikai, T. and Yamada, M. (1977) : Studies on *Pneumocystis carinii* and *Pneumocystis carinii* pneumonia I. Evaluation on several kinds of staining method in the identification of *P. carinii*. Jap. J. Parasit., 26, 116–124 (in Japanese with English summary).

25) Pavlica, I. (1962) : Erste Beobachtung von angeborener Pneumozysten-pneumonie bei einem reifen ausgetragenen totogeborenen Kind. Zbl. Allg. Path., 103, 236–241.

26) Pliess, G. und Trode, H. (1958) : Experimentelle Pneumocystose. Frankf. Z. Path., 69, 231–246.

27) Post, C., Dutz, W. and Nasarian, I. (1964) : Endemic pneumocystosis in an orphanage in South Iran. Arch. Dis. Child., 39, 35–40.

28) Rahimi, S. A. (1974) : Disseminated *Pneumocystis carinii* in thymic alymphoplasia. Arch. Path., 97, 162–165.

29) Redman, J. C. (1975) : Mission to Saigon-An alert to PCP. J. A. M. A., 231, 1190–1191.

30) Ricken, D. (1958) : Histologische Untersuchungen bei experimenteller *Pneumocystis*-pneumonie. Virchows Arch. Path. Anat., 331, 713–728.

31) Ruskin, J. and Remington, J. S. (1967) : The compromised host and infection. I. *Pneumocystis carinii* pneumonia. J. A. M. A., 202, 1070–1074.

32) Vaněk, J. und Jírovec, O. (1952) : Parasitäre Pneumonie. Interstitielle Plasmazellenpne-

umonie der Frühgeborenen verursacht durch *Pneumocystis carinii*. Zbl. Bakt. I. Orig., 158, 120–127.

33) Walker, E. L. (1912) : The schizogony of *Trypanosoma evansi* in the spleen of the vertebrate host. Philip. J. Sci., 7, 53–62.

34) Watanabe, J. M., Chinchinian, H., Weitz, C. and McIlvanie, S. K. (1965) : *Pneumocystis carinii* pneumonia in a family. J. A. M. A., 193, 685–686.

35) Weller, R. (1955) : Zur Erzeugung von Pneumocystosen im Tierversuch. Z. Kinderheilk., 76, 366–378.

36) Weller, R. (1956) : Weitere Untersuchungen über experimentelle Ratten-pneumocystose im Hinblick auf die interstitielle Pneumonie der Frühgeborenen. Z. Kinderheilk., 78, 166–176.

37) Yoshida, Y., Ogino, K., Arizono, N., Kondo, K. and Matsuno, K. (1974) : Studies on *Pneumocystis carinii* and *Pneumocystis* pneumonia. (1) Appearance of this protozoa in cortisone treated rats. Jap. J. Parasit., 23, Supple., 23 (in Japanese).

38) Yoshida, Y., Ikai, T., Ogino, K., Takeuchi, S. and Yamada, M. (1977) : Studies on *Pneumocystis carinii* and *Pneumocystis* pneumonia (8) Cyst concentration method from sputum. proc. 33rd West. Local Meet., Jap. Soc. parasit. 19 (in Japanese).

39) Zandanell, E. (1954) : Pneumocystisbefund ausserhalb der Lunge bei interstitieller plasmazellulärer Pneumonie der Säuglinge und Frühgeburten. Zbl. Allg. Path., 92, 74–80.

Pneumocystis carinii：ラットにおける実験的 Pneumocystis 症

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副腎皮質ステロイドを長期大量投与することにより，ラットに Pneumocystis 症を発生させうることについては以前から多数の報告がある．今回の実験では，ヒトの P. carinii 肺炎に関連する諸問題，すなわちラットにおける P. carinii の感染率，肺内での増殖状態，感染ルート，感染源，感染肺の病理組織学的変化，全身感染の有無などをより明確にするため，この実験モデルを用いた．

実験1では38匹，実験2では62匹の計100匹のラットを用いたが，そのうちステロイドを投与した78匹のうち77匹（98.7％）に P. carinii 感染を見い出した．実験2では集 cyst 法を用いて肺1g 中の cyst 数を計測したところ，cyst は片対数グラフで経時的に直線的に増加していた．また無処置のまま同室内で飼育したラット14匹のうち10匹（71.4％）に P. carinii の軽度感染を認めた．このことは同室内で正常ラットに対しても感染があつたことを示唆している．

ラット購入後直ちにステロイド処理を開始した実験1では5週目に強度の P. carinii 肺炎を呈するものが存在したが，購入後1カ月間無処置のまま飼育した後にステロイド投与を開始した実験2ではこのような強度の感染は見られなかつた．このことは実験2のラットは，無処置の期間中に P. carinii の感染をうけ，これに対する抵抗を獲得し，それがステロイド投与開始後も或程度持続したのではないかと考えられる．

P. carinii 感染の強さを病理組織学的変化並びに cyst 数で4つの Grade に分類したが，最も強度の P. carinii 肺炎では，ほとんどの肺胞は典型的な honeycomb material で満たされていたが，間質への細胞浸潤はわずかで，形質細胞もほとんど見られなかつた．この所見は免疫不全下に発症した人体の本肺炎の所見とよく似ていた．さらにラットの重症例では気管支中に P. carinii の集塊が観察でき，これがヒトの症例の場合には，喀痰中に排出されるものと思われる．

P. carinii の全身感染の有無を知るため，肺に強度の感染を示した16匹のラットについて肝，腎，脾の組織を精査したが，病原体を見い出すことができなかつた．