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Association of PPLO infection and antibody response in rats and mice

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INTRODUCTION

Although naturally occurring infections with pleuropneumonia-like organisms (PPLO), such as bronchopneumonia and polyarthritis in rats and pneumonia and catarrh in mice, have been studied extensively (Klieneberger & Steabben, 1937, 1940; Nelson, 1937*a*, *b*, *c*; Findlay, Mackenzie, MacCallum & Klieneberger, 1939; Woglom & Warren, 1938; Collier, 1939; Edward, 1940, 1947; Sabin, 1941; Preston, 1942), the production of specific antibodies as a result of these infections has not been systematically investigated.

In recent years there has been increasing interest in PPLO infections of the human urogenital tract, in particular in non-gonococcal urethritis. Several workers (Melèn & Gotthardson, 1955; Stokes, 1955; Card, 1959; Klieneberger-Nobel, 1959) obtained cultural and serological results suggesting that PPLO were the infecting agents. However, the causal association of PPLO in the tissues and antibodies to PPLO in the blood has been questioned, especially in non-gonococcal urethritis (Freundt, 1953; Nicol & Edward, 1953).

It was therefore decided to study the immunological responses of rats and mice to PPLO infections to provide not only further information about the rat and mouse diseases but also to throw some light on the significance of PPLO antibodies found in man.

MATERIAL AND METHODS

PPLO strains

A total of fifteen strains of PPLO were used, four obtained from rats, ten from mice and one from a human patient. The following table gives the designations and sources of the strains.

Strains Kon and R107 were isolated from the lungs of rats with bronchopneumonia; Jasmin, a rat polyarthritis strain, was isolated from tumour material sent by Dr G. Jasmin of Montreal and strain R38 was isolated from pus in the nose of a rat with catarrh. Strain R38 is serologically distinct from the lung strains but related to Jasmin, although complement fixation tests indicate that Jasmin has antigens not present in R38. Nine of the mouse strains were newly isolated from mice belonging to two different stocks; further details about their isolation are given later. The tenth strain, DGE had been in culture for many years. Strain H34 was obtained from the genital tract of a human female.

Media

The solid medium was a heart infusion peptone agar base (1·5 % agar) containing Oxoid Yeast extract (0·5 %), desoxyribonucleic acid (20 μg./ml.), inactivated pooled human serum (20 %) and penicillin (50 units/ml.). This was similar to the medium described by Klieneberger-Nobel (1959) for the isolation of human genital PPLO, but the basal agar was less rich and staphylococcal filtrate was omitted. The addition of thallium acetate (1/4000) was useful in preventing the growth of contaminating bacteria in cultures made from mice. The liquid medium consisted of a tryptic-digest horse meat broth with the same supplements. All the strains grew better aerobically than anaerobically, so plates were incubated aerobically at 37° in a moist atmosphere and observed for 12–14 days before a negative result was recorded. Broth cultures were shaken during incubation.

Table 1. *Strains of PPLO*

Designation	Source	
Kon	Rat lung	
R 107	Rat lung	
Jasmin	Rat tumour	
R 38	Rat nose	
M 1	Mouse lung	Stock 'A' mice
M 2	Mouse lung	
MB	Mouse brain	
Peter	Mouse brain	
68 NP	Mouse nasopharynx	Stock 'B' mice
72 L	Mouse lung	
73 B	Mouse brain	
KSA	Mouse brain	
79 B	Mouse brain	
DGE	Mouse lung (originally isolated by Dr D. G. ff. Edward)	
H 34	Human genital tract	

Animals

The albino rats used belonged to a stock derived from breeding animals supplied by the Chester Beatty Research Institute. Newborn rats were removed aseptically by Caesarean section from two females on the point of delivery. Young rats less than 3 months old were infected with an inoculum prepared as follows. A piece of agar approximately 1 cm. square from a 24 or 48 hr. plate culture of PPLO was macerated in a sterile mortar and taken up in 1–2 ml. of liquid medium. Infected material such as pus or necrotic tissue from abscesses, was ground up in the same way and 0·5 ml. samples of such suspensions were injected subcutaneously at one side of the abdomen. Rats were killed with coal gas and blood was removed from the heart with a fine capillary pipette. Lesions and other tissues were excised aseptically, cut up finely and inoculated into appropriate media. Samples from the nose and nasopharynx were obtained after swabbing the muzzle with alcohol and removing the external nares; a sterile platinum wire was inserted first through the nares and then through the back of the buccal cavity behind the soft palate after cutting back through the angle of the jaw.

The two mouse stocks, A and B, were commercially available stocks of albino mice. Inoculation was either intracerebral or intranasal, both under ether anaesthetic. For the intracerebral, 0·03 ml. of a 24 hr. agar culture macerated in broth was injected; in one experiment the same amount of PPLO-infected brain tissue macerated in broth was inoculated. For the intranasal, 0·03–0·05 ml. of either macerated agar culture or lung suspension was instilled. Mice were killed with ether at various intervals after inoculation and blood was taken from the axilla after cutting the brachial vessels. The blood from two mice was usually pooled. However, when the lesions were obvious and the individual serum titre was of particular interest, individual samples were kept separate, though sometimes, even in these cases, the pooling of two samples was necessary to provide enough serum for the full tests. Swabs were taken from the nose and nasopharynx in the same way as from the rats, and tissues required for culture were removed and treated in the same way as material from rats.

Serological tests

The clotted blood samples were held at 4° C. overnight, the serum separated and stored at −25° C. without preservative. Antibody against antigens of various PPLO strains was estimated by the complement fixation test (CFT) of Card (1959). Sera were tested at 2-fold dilutions between 1/10 and 1/5120. At first both 'fresh' (F) and 'boiled' (B) antigens (Card, 1959) were used, but since rat sera reacted equally well with both F and B antigens only F antigen was used in the later experiments and F titres are quoted throughout. As controls, known positive and negative sera were included in each test. Rat sera were titrated against Kon, Jasmin, R 38 and H 34 antigens. Mouse sera were tested in the same way using F antigens of Peter, KSA and H 34.

For identification of PPLO isolates, the CFT was carried out with F antigens and rabbit antisera prepared against known strains. Only F antigens were used because B antigens gave lower titres than F with rabbit antisera and the reactions with B antigens were less specific. Homologous antigens were titrated along with the unknown antigens and all were simultaneously titrated against pre-inoculation rabbit serum as a control.

Titres are cited as the reciprocal of the serum dilution and sera not reacting at a dilution of 10 (< 10) are regarded as negative for antibody.

RESULTS

The naturally occurring lung infection in rats

The incidence of PPLO infection and the serum antibody in rats of different ages was determined from the examination of twelve newborn from two mothers, twenty-six 4–8-week-old and twenty-eight 8–20-month-old rats. Cultures in liquid and solid medium from the twelve newborn rats yielded no PPLO although both mothers had PPLO in the lung and nasopharynx and serum antibody against the rat lung strain Kon. It would seem that newborn rats are not infected either *in utero* or immediately *post-partum*.

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Five of the twenty-six 4–8-week-old rats had PPLO in the lung although none had any macroscopically visible lesions. Nasopharyngeal swabs were taken from only seven rats, but from five of these PPLO were cultured. These results suggest that although very few young rats have PPLO in the lung, nearly all of them already have the organism in the nasal passages.

Table 2. *Distribution of serum titres against rat lung PPLO antigen in rats of different ages*

Age (months)	No. of rats	Anti-Kon titre							Total no. with positive titre
		< 10	10	20	40	80	160	320	
1–2	26	18	0	3	4	1	0	0	8
8–12	17	3	1	2	7	3	0	1	14
12–20	11	0	0	0	3	2	5	1	11

In contrast to the young rats all twenty-eight of the 8–20-month-old rats had PPLO in the lungs and twenty-two of twenty-three in the nasopharynx. Clearly, the incidence of lung infection is much higher in aged than in young rats.

Two representative strains, one from the lung and one from the nasopharynx were of the same serological 'rat lung' type as represented by the strain Kon.

In an earlier study Klieneberger & Steabben (1940) found PPLO only rarely in the spleen and kidneys, suggesting that the lung infection remained localized. This was confirmed in the present work by negative cultures from the spleens of twenty-three of the oldest rats, which included all the most severely diseased.

The serological results are summarized in Table 2. Only eight of the twenty-six 1–2-month-old rats had serum antibody to the strain Kon, compared with twenty-five of the twenty-eight 8–20-month-old (seventeen 8–12 and eleven 12–20 months) rats. Titres were higher in the 12–20 month group than in 8–12 month rats, but never higher than 320.

At necropsy four of the 8–20-month-old rats had typical lesions of severe bronchopneumonia (Klieneberger & Steabben, 1937, 1940) with extensive consolidation and large abscesses containg pus, which was often caseous; the anti-Kon titres of these rats were 160 or 320.

Antibody to the rat polyarthritis antigen Jasmin was detected in very low titre in only two of the twenty-six young rats and in six of the twenty-eight 8–20 month rats. A latent or mild infection with this PPLO type may well have been present in these animals. Antibody to human genital PPLO was never found.

A comparison of the cultural and serological results shows that PPLO were cultured from six of the eight young rats which had antibody against Kon in their sera; the other two had low positive titres although PPLO was not isolated from them. No antibody was found in two young rats with PPLO in the nasopharynx but not in the lung; probably infection had not progressed sufficiently to evoke an antibody response.

Experimental infections in rats

Klieneberger-Nobel (1960) investigated two rat polyarthritis strains, Jasmin and Baxter. Subcutaneous inocula induced, at the site of inoculation, an encapsulated abscess which increased in size during the first 3–4 weeks and usually regressed slowly. Antibody titres rose to 2560 in the first 4–5 weeks after inoculation, and then declined gradually during the next 18 weeks. The titre in young control rats never reached 10. She also found that inocula of human genital PPLO failed to produce much reaction. At most, a small lump developed at the inoculation site, and, although PPLO remained alive in the lesion for about a fortnight, no appreciable antibody was produced.

This work was extended, using the freshly isolated rat lung strain R 107 and the nasal strain R 38.

Unlike Jasmin and Baxter, R 107 produced very little on subcutaneous inoculation into young rats examined 2–14 weeks later. Small encapsulated, PPLO-containing abscesses arose, but regressed within 6–8 weeks. The spleens were sterile and titres did not exceed 80. These results suggest that the rat lung strain was not very virulent even when freshly isolated, and produced infection only at the site of inoculation, just as the natural infection is usually restricted to the nasopharynx and lungs.

Table 3. *Cultural and serological results from rats inoculated with rat strain* R 38

Rat no.	Duration of experiment (days)	Culture from				Titres	
		Abscess	Spleen	Lymph nodes	Lung	Anti-R 38	Anti-Kon
1	8	+	+	NT	+	< 10	160
2	12	+	+	+	+	< 10	40
3	12	+	+	+	+	10	40
4	31	+†	−	NT	+‡	10	80
5	31	−*	+†	NT	+‡	320	160
6	42	−*	−	NT	+	320	40
7	42	+	−	NT	+	40	80
8	49	+†‡	−	NT	+‡	160	160

* Abscess regressed before necropsy.
† Culture tested by CFT and found to be type R 38.
‡ Culture tested by CFT and found to be type Kon.
NT, not tested.

The results of inoculating strain R 38 are given in Table 3. In all of eight rats abscesses were produced and regressed within 4–6 weeks in two of them. PPLO were isolated from the spleen or enlarged axillary or inguinal lymph nodes of four of the rats, indicating that at some stage the infection was systemic. In this respect R 38 resembled the polyarthritis strains, but high serum titres like those accompanying polyarthritis infections were not found. The highest titre observed was 320 in the two rats in which the abscesses had regressed. Thus, strain R 38 seemed less virulent than the polyarthritis PPLO. The results of CF tests suggest that Jasmin and R 38 are related but that Jasmin has antigens not possessed by

R38; the difference in virulence between the two strains may be associated with this antigenic difference.

Although these rats were only 2–3 months old, PPLO were found in the lung and antibodies to Kon were present in the sera of all eight. Three isolates from the lung were typed (see Table 3) and found to be indistinguishable from Kon. Two isolates from abscesses and one from the spleen were also typed to check that infections were not caused by endogenous PPLO; these were identical with the inoculated strain R38 and distinct from the rat lung type. One abscess contained the rat lung type as well as the R38 type, suggesting that PPLO which are normally localized in the lung can under some conditions spread to a site of trauma remote from their usual habitat.

Naturally occurring infections in mice

A natural infection of Stock A mice was discovered when brain suspensions from two series of mice, one used for the intracerebral passage of trachoma virus, the other a corresponding control series in which brain suspensions alone were passaged, proved to be heavily infected with PPLO. The representative strain isolated was designated 'MB'. Other untreated mice selected at random yielded three more strains, M1 and M2 from the lungs of two mice and Peter from the brain of a third. The four strains were typed serologically using rabbit sera prepared against M1 and MB. Five antigens from these four strains were tested because M2 produced two colonial variants, and all five reacted to the same titre or to within 25% of it. The strain DGE obtained from Dr Edward many years ago was also closely related. In view of this and because strain Peter behaved characteristically in mice, as shown in the next section, the type of PPLO to which these strains belong has been designated 'mouse lung'. It is very closely related serologically to the rat lung PPLO represented by the strain Kon. In CF tests with M1, MB and Kon antisera it was not possible to distingusish the mouse lung from the rat lung PPLO. Mouse lung PPLO usually had a very characteristic colony on primary culture. It was coarsely granular and, except when very young, was dark brown. The pigment obscures the typical PPLO colony structure with its dense centre and more transparent peripheral zone, which is seen only in young colonies. In later subcultures the pigment tends to be lost. The colony resembles that of the rat lung strain in which also the central zone is not clearly differentiated from the peripheral area, although it has more pigment than the rat lung strain on primary culture. When first isolated the growth of both strains in liquid media is finely granular, just visible with a hand lens by transmitted light. After several subcultures, the growth is more evenly turbid.

Four-month-old Stock B mice were originally obtained for the production of ascitic fluid by the intraperitoneal inoculation of Krebs ascites tumour cells. When killed to harvest the ascites cells and fluid some mice proved to be infected with PPLO. Of eleven mice, two had PPLO in the brain and three in the lung, although there were no obvious signs of disease. The colony of one brain isolate, KSA, differed from the mouse lung type in having a small discrete central zone clearly demarcated from the periphery. It was also distinct serologically from the mouse

lung type. In CF tests with antisera prepared against M1 and MB the titre with KSA was 80 compared with homologous titres of 5120 and 1280 for M1 and MB respectively.

As the incidence of lung infection and the amounts of serum antibody in rats had been found to increase with the age of the animals, it was thought that this might also happen in mice. Accordingly the presence of PPLO and PPLO antibodies in mice of different ages were investigated; the results are given in Table 4.

Table 4. *PPLO and antibody found in untreated mice of different ages*

Mouse Stock	Age of mice (weeks)	No. of mice examined	No. of mice with PPLO-positive cultures	No. of PPLO-positive cultures from			Titre against strain Peter
				Naso-pharynx	Lung	Brain	
A	3–4	16	0	0	0	0	< 10
	7–8	16	0	0	0	0	< 10
	12	5	4	NT	4	NT	160
B	4	16	6	6	3	4	< 10–10
	16	6	4	4	1	1	40–80
	24–32	17	17	15	1	7	80–320
	14	1*	1	1	1	0	640

* Developed pneumonia spontaneously. NT, not tested.

The sixteen 3–4-week-old Stock A mice were taken from a fresh consignment and were found to be negative for PPLO both culturally and serologically. Four weeks later another sixteen from the same consignment, now 7–8 weeks old, were again negative. However, four of 5–12-week-old mice from another consignment of Stock A had PPLO in the lung and a corresponding titre of 160. There appeared to be a difference in the incidence of PPLO infection between different consignments of this stock. Whereas the consignment from which the 3–4- and 7–8-week-old mice were taken seemed entirely free from PPLO, others were clearly infected, e.g. those from which the strains MB, M1, M2 and Peter were obtained and those 12 weeks old. It is probable that some consignments of 3–4-week-old mice were already lightly infected when received from the dealer and that PPLO proliferated and antibodies were produced as the mice aged. The other possibility is that all the mice were at first free from PPLO but that some acquired the organisms later from other mice or rats on the premises. However, mice were never placed in direct contact with rats or mice of other stocks, and Nelson (1937*a*) found that direct contact was necessary for the transmission of PPLO respiratory infection in rodents. Whichever possibility is correct, it is important to note that a detectable PPLO infection in Stock A mice was accompanied by the presence of serum antibody against the mouse lung type.

Stock B mice of different ages were also examined (Table 4). Of sixteen mice 4 weeks old, six were infected with PPLO but had little or no antibody. At 16 weeks old, most of them were infected, PPLO being present in, e.g. four out of

six, with titres against lung PPLO of 40–80, although no lesions were visible. Mice 24–32 weeks old were all infected, with titres of 80 to 320. One 24-week-old mouse spontaneously developed active pneumonia with consolidation of the lung; a heavy growth of PPLO was obtained from the lung and the titre was 640. As can be seen from Table 4, of forty mice of different ages, twenty-six had PPLO in the nasopharynx, six in the lung and twelve in the brain. Sometimes PPLO were present in both nasopharynx and lung or in nasopharynx and brain; more rarely all three organs were infected. In every age group more mice carried PPLO in the nasopharynx than in the brain or lungs. Since strains 72L, 73B and 68NP, isolated respectively from the lung, brain and nasopharynx of three mice, were serologically identical and indistinguishable from others of the mouse lung type, the preponderance of positive cultures from the nasopharynx suggests that this is the primary site of infection and other organs are secondarily invaded. Edward (1947) found that a primary PPLO infection of the upper respiratory tract in mice could spread to the lung and cause pneumonia. The results show that PPLO are already present in some 4-week-old Stock B mice when they are received from the dealer. The incidence of PPLO infection and the amount of serum antibody both increase with the age of the mice.

Experimental infections in mice

It had been possible in only one naturally infected mouse to determine the serum titre when the lung infection had reached an advanced stage and the lung was consolidated. An attempt was made, therefore, to build up any existing PPLO infection and to produce pneumonia in 3–4-week-old Stock A mice by a series of blind passages of lung suspensions. Lung material was passaged intranasally six times using two mice at each passage. At necropsy none of the twelve mice had consolidated lungs, PPLO were not isolated from the nasopharynx or lung, nor were PPLO antibodies found in the serum (Table 5). Apparently this consignment was free from PPLO infection.

Lung infection was established and an antibody response produced in young mice of both stocks by nasal instillation first of strain Peter and, in subsequent passages, of suspensions of pneumonic lung. The results from necropsies performed 13–30 days after inoculation are summarized in Table 5.

Table 5. *Cultural and serological results from mice inoculated intranasally*

Mouse Stock	Inoculum	No. of mice	No. with PPLO-positive lung culture	No. with consolidations	Titre against strain Peter
A	Normal mouse lung	12	0	0	< 10
A	Strain Peter or pneumonic lung	18	15	9	160–320
B	Pneumonic lung	22	9	5	160–320, > 1280* 5120*

* Mouse with lung totally consolidated.

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Of eighteen Stock A mice inoculated with PPLO or infected lung, fifteen had the organisms in the lung and nine partial hepatization of the lung. In all of them the titre against the infecting strain was 160–320. In comparison with Stock A, fewer of the Stock B mice developed a lung infection; PPLO were isolated from the lungs of nine, and five of these had consolidations; in two mice the whole lung appeared hepatized. These two most severely affected animals had titres > 1280 and 5120. In the less severely infected mice the titres were 160–320—the same level as in the infected Stock A mice.

PPLO were not isolated from the lungs of all the mice inoculated with PPLO although all had positive serum titres. This failure may be due in part to the growth on four plates, seeded with lung material, of bacteria that inhibit PPLO. The PPLO-negative lung cultures in the remaining inoculated mice were not contaminated in this way so that the infection was probably confined to the nasopharynx although it had given rise to detectable antibody. As PPLO, when present in the upper respiratory tract can produce a catarrhal condition in mice (Edward, 1947) such an antibody response is feasible. However, it was only in mice with extensive pneumonia affecting the whole lung that the highest titres were found.

Mice which developed pneumonia became very thin and usually made a characteristic chattering noise, but some remained lively even when large parts of the lung were consolidated. Edward (1940) and Nelson (1937*a*) both reported a low death rate in mice with PPLO pneumonia. Nelson (1937*a*) never observed abscesses in infected mice, but the lungs of two animals in the present series contained yellow nodules which exuded sticky pus when cut.

In only two mice did conjunctivitis accompany the pneumonia, but PPLO were not isolated from the eyes. Nelson (1950) reported on a PPLO conjunctivitis which sometimes accompanied PPLO catarrhal infections in mice, but in the present investigations the incidence was too low to determine the aetiology.

The strain Peter was also injected intracerebrally into six 3–4-week-old Stock A mice. No signs of neural damage were observed, but at necropsy 9 days after injection small abcesses were present on the skull of two of the mice at the point of needle entry and PPLO were isolated from the brains of all the mice as well as from the abscesses. Abscess material re-inoculated intracerebrally into other mice produced no lesions and PPLO were not found in the brains 21 days later. This PPLO serotype was apparently not neurotoxic. These results agree with those of Edward (1940) who found that intracerebral inoculation of mouse lung PPLO was without effect.

In contrast, strain KSA damaged the brain when introduced intracerebrally; some mice died within 24 hr. and the rest within 72 hr. Of eight mice thus inoculated, three exhibited the characteristic 'rolling' described by Findlay, Klieneberger, MacCallum & Mackenzie (1938). It was not possible to obtain blood from any of these mice for CFT. Another brain strain, 79B, with a colonial form distinct from that of the mouse lung PPLO and more like that of KSA, was isolated from untreated Stock B mice and inoculated intracerebrally into eight mice; five died within 72 hr., one exhibiting 'rolling'. Two of the three survivors developed a pronounced hydrocephalus and the characteristic excitability associated with

'rolling disease'. At necropsy 26 days after inoculation, PPLO were still present in the brain of all three mice; the serum titre of the two with hydrocephalus was 40 and of the third 20 against strain KSA but < 10 against strain Peter. Eight control mice given sterile agar emulsion remained healthy throughout and no lesions were observed at necropsy.

These results confirm the conclusion from CF tests that KSA is distinct from the more frequently isolated mouse lung PPLO, and suggest that it is the same as the L5 of Findlay *et al.* (1938) and the Type A of Sabin (1941), the causative agent of rolling disease. The serological characters of strain 79B were not determined, but the symptoms it produced, associated with antibodies to KSA, which appeared *only* in mice infected with strain 79B, suggest that it is very similar to KSA and to the original L5 strain.

DISCUSSION

Cultural results from the study of rat bronchiectasis suggest that newborn rats are free from PPLO infection, but that they become infected in the first weeks of life, presumably from the mothers. In young rats the infection is usually confined to the nasopharynx and at this stage serum antibody is either absent or detectable only at low titre. As the rats age the lungs are invaded and eventually the lesions of bronchopneumonia may develop. Higher serum titres are also found as the rats age, the highest being associated with severe bronchopneumonia. Thus the changes in serum antibody levels demonstrated by CFT reflect the progressive increase of a chronic infection in rats.

There is rarely any cultural evidence that the infection becomes systemic in the chronic form of rat bronchiectasis or when the rat lung PPLO is inoculated subcutaneously. This is in contrast to infections with rat polyarthritis and related strains such as R38 which are generalized at some stage, as shown by the presence of the PPLO in the lymph nodes, spleen and blood. The comparatively low serum titres, not exceeding 320, which accompany bronchiectasis also suggest a more localized infection. In contrast Klieneberger-Nobel (1960) found serum titres of 2560 in polyarthritis infections.

However, the isolation of both rat lung and R38 strains from a subcutaneous abscess produced by inoculating R38, indicates that under certain conditions the lung strain can be disseminated to other parts of the body. This is reminiscent of a report by Stokes (1955) on a patient who developed a post-operative empyema caused by PPLO. There was pyrexia lasting some days and a serum titre of 480. The PPLO responsible appeared to be of the human genital type and must presumably have been transferred to the pleural cavity via the blood stream from the genital tract.

In view of the fact that seven representative mouse strains from both stocks were serologically identical with a strain isolated by Dr Edward, the mice probably had the same type of respiratory infection as described by Edward (1940, 1947). The lung serotype must be distinct from the PPLO isolated from induced pneumonia by Sullivan & Dienes (1939), since Sabin (1941) reported that these were the same as his strain A, which was closely related to the L5 rolling disease strain.

The relationship of the mouse lung serotype to strains found by Nelson (1937*a*, *b*, *c*) in catarrhal infections and pneumonia of mice and to other isolated by Sabin (1941) from the brain, nose and lung of mice cannot now be established.

In mice, just as in rats, the incidence of infection with lung PPLO increased with the age of the animals; this was shown both by the increasing frequency of isolation of PPLO and higher serum titres. Although the rat and mouse lung PPLO are related serologically and the primary site of infection in both rats and mice seems to be the nasopharynx, a nasal infection in mice is not always followed by infection of the lung, as inevitably happens in rats. Involvement of the lung in mice with a nasal infection occurs much less regularly than in rats.

In both rats and mice there was a high correlation between the presence of PPLO demonstrable by culture and the occurrence in the blood of antibodies specific to the infecting strain. The level of the serum titre was related to the severity of the infection in both species.

The specificity of the antibody detected by CFT is evident from the results. Antibody to human genital PPLO was never found in rats or mice. Moreover, when an experimental infection with strain R 38 was superimposed on an existing lung infection in rats, antibodies to both R 38 and rat lung PPLO could be demonstrated. Whereas antibody to the more frequently isolated lung PPLO was repeatedly found in mice, antibody to strain KSA was present only in those intracerebrally inoculated with a related mouse brain strain.

These experiments demonstrate the immune response of rats and mice to various rat- and mouse-pathogenic PPLO which naturally infect them. Specific serum titres in these animals reflect the presence and severity of PPLO infection. As the PPLO are a closely related group of organisms, it seems permissible to regard these rat and mouse diseases as immunologically analogous to PPLO infections in other species.

If, then, antibody response in rats and mice signify infection it seems likely that antibody against human genital PPLO found in man is also indicative of past or present PPLO infection. It is true that high PPLO titres have been reported in human patients only infrequently, but when such titres occur it is usually when there has been an acute generalized infection (see e.g. Stokes, 1955). In contrast, the titres recorded by Card (1959) in patients with non-gonococcal urethritis seldom exceeded 80. More recently the author has examined sera from women with salpingitis; again the titres were usually low. However, these genital tract infection, like the rat lung disease tends to remain localized so that a strong antibody response is not to be expected.

SUMMARY

By means of a complement-fixation test of the sera of laboratory rats and mice, the immunological response of these animals to both naturally occurring and induced PPLO infections was determined, and the presence and extent of infection in the animals determined by culture.

PPLO antibodies specific for the infecting strain were demonstrable in rats and mice from which PPLO were isolated.

The amount of serum antibody rises with the extent and severity of the infection. Thus, young rats with PPLO infections confined to the nasopharynx had little or no antibody whereas the oldest rats with consolidated lungs had the highest titres. In mice too, the sera of those with pneumonia had the highest titres.

The comparatively low titres found in rat bronchiectasis together with the failure to isolate PPLO from the spleen and other organs, suggest that the chronic form of the disease remains localized. This is in contrast to infections with rat polyarthritis and related PPLO in which the organisms can be isolated from the lymph nodes and other organs and in which antibody is present in high titre.

In view of the high degree of correlation between the presence of antibodies to PPLO in the blood and the presence of PPLO in the tissue of rats and mice, it is suggested that specific antibody found in man is a significant indicator of PPLO infection.

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