COMPARATIVE STUDY OF MENINGOPNEUMONITIS VIRUS, PSITTACOSIS OF PIGEON ORIGIN, AND PSITTACOSIS OF PARROT ORIGIN

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In a previous paper (1), the recovery of a virus morphologically identical with psittacosis from pigeons on a thiamin-deficient diet was reported. This virus, injected intracranially into mice behaved like typical psittacosis, producing fatal meningoencephalitis with typical elementary bodies. The virus differed from typical psittacosis, however, in that intraperitoneal injection in mice failed to produce apparent illness. Cross immunity tests with typical psittacosis were not carried out. The conclusion was drawn largely on morphological grounds that the virus was closely related to, if not identical with that of psittacosis.

Francis and Magill (2), in 1938, described a virus recovered from ferrets inoculated with throat washings from human cases of an influenza-like respiratory infection. It was not clear whether this virus was derived from the inoculated human material or from the ferrets. In mice, it produced fatal meningitis after intracranial injection, and fatal pneumonitis after intranasal injection. After intraperitoneal inoculation, a fatal meningitis was produced in some mice. The hepatic necroses characteristic of psittacosis were not found. They were unable to identify their virus, and gave it a descriptive name—the virus of meningopneumonitis.

Eaton, Beck, and Pearson (3) have recently reported the recovery of a virus from four cases of human atypical pneumonia by intranasal injection into mice. This virus, in its properties, was practically identical with the virus of Francis and Magill. It showed immunological relationship but incomplete immunological identity with the latter virus and also with psittacosis. It produced a fatal illness in mice, transmissible by intracranial or intranasal inoculation, but like our pigeon virus, was apparently non-virulent in mice when injected intraperitoneally.

The experiments to be reported here were designed to test the hypothesis that these viruses might be related to the virus recovered from pigeons.

Material and Methods

Fully virulent typical psittacosis virus was kindly furnished by Dr. Karl F. Meyer. The meningopneumonitis virus of Francis and Magill was obtained by the courtesy of Dr. Eaton. Our virus of pigeon origin had been lost, but records of its behavior

in experimental animals and microscopic slides showing its morphological characteristics were available. Comparative studies in pigeons and mice, of the behavior and morphology of (1) typical psittacosis, (2) the meningopneumonitis virus, and (3) our pigeon virus, were carried out.

EXPERIMENTAL

Seven pigeons were injected intracranially with 0.06 cc. each of brain emulsion from two mice dying of typical psittacosis; three pigeons from the emulsion of one brain and four from the emulsion of the other. Six mice were injected intracranially with 0.03 cc. of each brain emulsion. The brain emulsified for injection into the group of four pigeons was proven by smears and sections to contain the characteristic elementary bodies of psittacosis in large numbers.

Nine pigeons were injected intracranially with 0.06 cc. each of tissue emulsions containing the meningopneumonitis virus. Twenty-two mice were injected intracranially with 0.03 cc. of these same emulsions.

Film preparations and paraffin sections were prepared from the brains of one or more animals of each group dying from the infections. The films were fixed in absolute methyl alcohol and stained by the Giemsa method. The tissues were fixed in Regaud's fluid and sections were stained by the Giemsa method.

RESULTS

The seven pigeons injected intracranially with typical psittacosis virus continued to eat well, held their heads up normally, and showed no evidence of illness during an observation period of 30 days. At the end of this period, they were killed. Autopsies showed no gross lesions, and microscopic sections of the brain and liver from two of them were entirely negative. Brain from one of these pigeons was emulsified and injected intracranially into six mice. These mice showed no evidence of illness. All mice injected intracranially with typical psittacosis virus died between the 3rd and 6th days after injection. Intracellular clusters of typical elementary bodies were found in large numbers in the brains of several of these mice.

The nine pigeons receiving intracranial injections of the meningopneumonitis virus of Francis and Magill all became ill on the 3rd or 4th days after injection. Failure to eat, ruffling of the feathers, and retraction of the neck were followed in a few days by inability to stand, staggering, or opisthotonos. Two pigeons died on the 7th day after inoculation. Three pigeons were killed in a moribund state between the 8th and 10th days after injection. The other four pigeons all suffered prolonged illness of variable severity. All mice injected with the meningopneumonitis virus died (with one exception) between the 3rd and 6th days after injection, with evidence of paralysis.

Sections of the brain of four pigeons dying of the disease or killed when moribund showed meningitis and encephalitis. In both smears and sections, many intracellular clusters of elementary bodies, giving the picture characteristic of infection with the psittacosis virus, were found. Sections of brain

from several mice injected with the virus showed a similar histological picture and entirely similar clusters of elementary bodies. Histologically, the meningoencephalitis produced in pigeons and mice with the meningopneumonitis virus showed a picture identical with that seen in pigeons and mice after intracranial inoculation of our virus of pigeon origin. The meningoencephalitis produced in mice was also indistinguishable histologically from that produced by the intracranial injection of typical psittacosis virus. A careful comparison of the intracellular clusters of elementary bodies found in typical psittacosis, in meningopneumonitis, and in our pigeon virus, showed apparently complete identity. (These structures are often called L.C.L. bodies.)

TABLE I

Comparative Pathogenicity of Psittacosis Viruses for Pigeons by the Intracerebral Route

Origin	No. of pigeons	Results
Pigeon (1)	2	2 died, 3rd day; L.C.L. bodies.
Pigeon (7754)	2	2 died, 3rd and 10th days; paralysed.
Pigeon (Haines)	2	2 died, 3rd and 15th days; paralysed.
Pigeon (Pas. 1868)	3	3 died, 3rd to 15th days; paralysed.
Pigeon (Yisalia 59)	2	2 died, 4th and 7th days; paralysed.
Pigeon (Br. lung)	4	2 died, 6th and 12th days; paralysed.
Parrot (King, 1939)	2	No illness. Killed 51st day.
Parrot (dwarf, 1938)	2	No illness. Killed 51st day.
Parakeet (2 Al)	4	1 died, 64th day. 3 killed, 142nd day.
Parakeet (6 BG)	6	1 died, 14th day. 3 killed, 37th to 55th days.
Parrot (Cohn M)	4	2 died, 5th and 6th days. 2 killed, 47th and 123rd days.
Parakeet (human lung, 1940)	6	No illness. Killed 102nd to 104th days.
Parrot (human sputum, 1941)	2	1 died, 7th day. 1 paralysed; recovered.
Parrot (human sputum, 1940)	2	No illness. Killed 29th day.
Parrot (human sputum, 1940)	3	No illness. Killed 29th day.

Dr. Karl F. Meyer kindly offered to test in greater detail the comparative pathogenicity of several strains of psittacosis virus of pigeon and of parrot origin when injected intracerebrally in pigeons. The results of this study are shown in Table I. Sixteen of the eighteen pigeons injected with viruses of pigeon origin died between the 3rd and 15th days, while only three early deaths, one late death, and one illness with recovery were noted among thirty-one pigeons injected with virus of parrot origin. In this group, the morbidity and mortality was confined to pigeons injected with recently isolated strains, while the meningopneumonitis virus had been carried in mice for several years. On the whole, therefore, the results which Dr. Meyer obtained confirm the belief that psittacosis viruses of pigeon origin show a significantly greater pathogenicity for pigeons when injected intracerebrally.

Certain other observations made by Dr. Meyer in the course of his studies should also be recorded. Contrary to our negative results obtained from one pigeon, Dr. Meyer on several occasions recovered virus from the organs of pigeons which showed no illness after the intracranial injection of strains of parrot origin; in one instance, as late as the 123rd day after injection. Complement fixation tests for psittacosis were also regularly positive in such clinically negative birds. These observations indicate that even when no apparent illness occurs, latent infection is commonly produced in pigeons by the injection of virus of parrot origin.

Another observation of interest was that two pigeons, even though proven to have latent infection by positive complement fixation tests, died of psittacosis on the 4th and 7th days after the intracranial injection of virus of pigeon origin.

DISCUSSION

The detailed morphological picture of cells containing clusters of L.C.L. bodies is believed to be unique, although the elementary bodies of lymphogranuloma venereum resemble them closely under certain conditions. The presence of this picture in pigeons and mice infected with the meningopneumonitis virus and with our pigeon virus is believed to be strong presumptive evidence that both of these viruses are strains of psittacosis. Close immunological relationship between the meningopneumonitis virus and typical psittacosis further supports this point of view, even though complete immunological identity does not obtain. Eaton, Beck, and Pearson (3) have found that solid immunity, even against homologous strains of virus in animals inoculated by the intranasal or intracerebral routes, is rather uncertain.

The similarities and differences between the four viruses under consideration are brought out in Table II. The ability to produce serious and usually fatal infection in pigeons with regularity after intracranial injection is shared by the meningopneumonitis virus and our pigeon virus[1] while typical, fully virulent psittacosis failed to produce evidence of illness in pigeons, except in a few instances when recently isolated strains were tested. The meningopneumonitis virus and our pigeon virus seem to have in common also a tendency toward "neurotropism." It has not been shown that these two viruses are identical, but their biological departure from typical psittacosis appears to be in the same direction in both cases.

The differences demonstrated between these viruses and typical psittacosis virus are apparently similar to, although quantitatively somewhat greater than, those which obtain between human and murine typhus. Such modifications,

[1] Four out of six pigeons injected intracranially with our pigeon virus died with meningoencephalitis and associated L.C.L. bodies. The other two became ill but recovered (previously unpublished observations).

which are of a rather permanent nature, are presumably due to prolonged residence in different species of animals, but are commonly believed to be reversible under suitable conditions.

Dr. Meyer, has seen several instances of proven psittacosis in man without contact with psittacine birds but with contact with doves and pigeons. Serologically, the existence of latent psittacosis infection was proven in seven out of nineteen doves and in a number of pigeons. It has not been demonstrated that human psittacosis acquired from pigeons shows any distinctive clinical features as compared with cases of parrot origin.

TABLE II

Comparison of Behavior and Morphology of Four Viruses

	Pigeons intracranial	Mice intracranial	Mice intraperitoneal
Typical psittacosis	Usually negative	Fatal meningoencephalitis. L.C.L. bodies*	Fatal with hepatic necrosis. L.C.L. bodies.* Brain negative (4)‡
Atypical psittacosis from pigeons (Pinkerton and Swank)	Fatal meningoencephalitis. L.C.L. bodies*	Fatal meningoencephalitis. L.C.L. bodies*	Negative
Meningopneumonitis virus (Francis and Magill)	Fatal meningoencephalitis. L.C.L. bodies*	Fatal meningoencephalitis. L.C.L. bodies*	Occasional fatal meningoencephalitis (2).‡ No hepatic necrosis.
Atypical pneumonia virus (Eaton, Beck, and Pearson)	Not tested	Fatal meningoencephalitis (3)‡	Negative (3)‡

* Intracellular clusters of coccoid elementary bodies of variable size, giving the unique and characteristic morphological picture of infection with the psittacosis virus.

‡ Observations not made by us, but taken from reports of other workers. Figures indicate references.

It has recently been suggested (5–7) that the virus of lymphogranuloma venereum may be closely related to psittacosis and to the virus of meningopneumonitis, since these viruses all have antigenic components in common, all produce meningitis, pneumonia, and granulomatous cutaneous lesions, and are all associated with the presence of elementary bodies in infected tissues. Our observations have shown complete morphological identity between the virus of typical psittacosis, the atypical "psittacosis" virus of pigeon origin, and the virus of meningopneumonitis, and for this reason we regard these viruses as closely related.

The morphological picture of lymphogranuloma venereum, as seen in smears and sections of the mouse brain, seemed to us to differ rather strikingly from that of psittacosis. The clusters of elementary bodies in the former appeared less compact, and the larger coccoid bodies, often appearing in mulberry form, which are a characteristic feature of psittacosis were not found in lymphogranuloma venereum. Rake and Jones (8), however, report that in the yolk sac of the developing chick embryo the larger forms are seen, and these workers stress the marked morphological similarity of lymphogranuloma venereum and psittacosis, under the conditions of their studies. The exact relationship of lymphogranuloma venereum to psittacosis requires further study.

SUMMARY

Comparative morphological, histological, and biological studies suggest a close relationship between the meningopneumonitis virus of Francis and Magill and a virus recovered from thiamin-deficient pigeons. Both of these viruses are morphologically identical with typical psittacosis, and it seems probable that they are biologically modified strains of psittacosis. They both differ from typical psittacosis in that they are regularly more pathogenic for the pigeon after intracranial injection, and fail to produce hepatic necrosis after intraperitoneal injection in mice. A virus recently isolated from human cases of atypical pneumonia by Eaton, Beck, and Pearson may also be closely related to these two viruses.

A number of psittacosis viruses of pigeon origin showed a similarly increased pathogenicity for pigeons by the intracerebral route, as compared with psittacosis viruses of parrot origin. The viruses of parrot origin, however, commonly produced latent infection in pigeons even when clinical illness was not evidenced.

For the isolation of psittacosis of pigeon origin from human sputum the intracranial injection of mice or pigeons may be essential, although it is probable that the intranasal injection of mice would be successful. The intraperitoneal injection of mice may give negative results.

The authors acknowledge with pleasure the generous cooperation of Dr. Karl F. Meyer, who carried out the detailed comparative study of several psittacosis viruses of pigeon and parrot origin.

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