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Reservoirs of the Psittacosis Agent.*

By K. F. MEYER and B. EDDIE.

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A recent review (MEYER and EDDIE, 1951) postulated that any laboratory worker willing to carry out autopsies and inoculate through blind passages in mice suspensions of the spleen, liver, kidneys and cloacal content of different species of birds or the lungs of mammals may in time discover unknown hosts of microparasites morphologically and biologically indistinguishable from those of psittacosis. Scarcely had this statement been made when YORK and BAKER (1951), using a novel technique of inoculating intraperitoneally feces or a portion of intestines from apparently normal calves into guinea pigs, discovered a new member of this group. A second virus isolation was recently reported by BARWELL and BISHOP (1951), who credit STAMP et al. (1950, 1951) with the propagation of the virus of enzootic abortion in ewes in the embryonated egg. On primary inoculation, none of these viruses are pathogenic for mice no matter what route of administration is used. However, the calf virus, entirely latent in the ruminant, after adaptation through 20 serial passages may produce pneumonia fatal to mice. The ewe-abortion virus adapted to the yolk sac infects mice by the intranasal route. Heavily infected suspensions produce fatal infections (within 5 to 7 days) and areas of pneumonia. The infection is readily transmitted in series from mouse to mouse. The psittacosis-type viruses recently found in mammals contrast strongly with the agent in opossums (ROCA-GARCIA, 1949). This virus, secured from paralyzed marsupials, shares with the majority of the strains isolated from birds and man the property originally established by KRUMWIEDE et al. (1930)—pathogenicity for mice on primary inoculation. It follows then that the embryonated egg, the guinea pig and possibly other mammals can be used for the enrichment and primary isolation of the viruses of the psittacosis group. The statement made in the review must therefore be appropriately revised.

In fulfilment of the prediction, the vast distribution of these cytomicrobes is becoming constantly more apparent. Table 1 condenses the past records (MEYER, 1949; MEYER and EDDIE, 1951)

* Presented to Professor Walter Frei, director of the Institute of veterinary pathology Zurich, on the occasion of his seventieth birthday.
and brings up to date the list of birds and animals subject to spontaneous infections with the large viruses. The steadily increasing number of reservoirs and the extension of the host range are fully documented and need not be emphasized further here. The implications of the recent findings are thought provoking.

Epidemiologic inquiries have thus far traced 6 human infections to barnyard fowl (California and New Jersey). Chickens may harbor the virus in the spleen, liver or kidneys and may shed it through the cloacal content. KARRER et al. (1950), using the complement-fixation inhibition test on 183 chicken sera collected from hatcheries and farms in California, Michigan and Iowa, found that 24.2 per cent were positive in the presence of the psittacosis antigen. In fact, a flock on a small farm where the owner had contracted mild psittacosis (KARRER et al., 1950), furnished 50 per cent serologic reactors. Whenever an earnest effort was made, the virus was isolated if blind passages in mice were systematically carried out. The strains are of relatively low pathogenicity for mice and in this respect are similar to that originally isolated from a fowl in New Jersey in 1941 (MEYER and EDDIE, 1942).

The suspicion of turkeys as reservoirs of an ornithosis virus led to the examination of 48 sera from ranches where there was infectious sinusitis. The percentage of positive reactions was very low (1 per cent). However, quite recently an outbreak of 22 cases and 3 deaths of suspected ornithosis among 78 turkey dressers in Texas was inductively attributed to contact with the discharges from this bird (IRONS et al., 1951). The explosive outbreak strongly suggested that one flock of turkeys was the source of infection. The responsible virus has not yet been isolated, but the complement-fixation test with sera obtained from patients late in convalescence or several weeks after recovery from illness reacted in high titer with LGV ("Lygranum") and with psittacosis antigen. Important in the interpretation of these results is the fact that in the course of serial bleedings in one case the titer rose sharply during the second week of illness. The evidence which incriminates contact with turkeys as the cause for the unrecognized occupational illness or inapparent infection of Negro workers gives added weight to the warning extended by SMADEL (1943) : As proof that a certain illness is caused by a strain of a psittacosis virus, the following criteria must be met: (1) The virus must be isolated during the acute phase of the disease and (2) Complement-fixing antibodies should make their appearance during convalescence or the titer of these antibodies should rise during recovery.

The world-wide distribution of an ornithosis virus in pigeon flocks is now fully recognized and reports of isolations of the agent
from England, Holland, France, and Switzerland have become common (MEYER and EDDIE, 1951; LÉPINE and SAUTTER, 1951). In a study at the Hooper Foundation of 524 pigeons from 5 states, the agent was isolated from 204. These birds are doubtless an important source of human infections.

Equally assuming ascendency of importance are the isolations of an ornithosis virus from *domestic ducks* (*Anas platyrhinchus*). A brief review of the essential facts appears justified. In 1942, EDDIE and FRANCIS reported definite, though low, complement-fixation reactions in 7 sera among a group of 24 collected from domestic ducks. The same reactions of 55 wild mallards were entirely negative. The first signal that ducks might be responsible for human infections flared in July 1945 when a woman who tended some mallard ducklings contracted clinical ornithosis. The virus was isolated from one of the ducklings and the course of the serologic reactions of the patient was typical. Shortly thereafter, Dr. WILLIAM WOLINS (1948) diagnosed 8 cases of pneumonitis among employees of the Long Island, New York duck farms. Serologic tests with psittacosis antigen were positive in dilutions of 1:16 to 1:256 and the isolation of strains of psittacosis virus from the tissues of 1 sea gull and 1 duck warranted further investigation. A joint survey then undertaken by the New York State Department of Health (through continuous assistance by Doctor KORNS) and the George Williams Hooper Foundation yielded the following significant results:

1. The organs of 44 of 123 sick and healthy ducks and sea gulls collected on 9 farms yielded, on intraperitoneal inoculation of mice, an ornithosis virus of moderate pathogenicity (Table 2).

2. The sera of 32 (40.2 per cent) of 65 duck sera tested in the complement-fixation inhibition test gave positive reactions in the presence of the psittacosis antigen.

3. The infection is largely inapparent, but may be activated by other concomitant bacterial infections: anatipestifer and fowl cholera (HILBERT and KISER, 1948). The same percentage (27 to 33 per cent) of isolations were successful from healthy, sick and dead ducks.

4. A survey of complement fixation to psittacosis antigen in sera from healthy individuals in contact with ducks on Long Island revealed 37.8 per cent of 45 persons with a titer of 1:16 or greater, in contrast to 3.4 per cent of 56 persons not exposed to ducks. In a more recent survey (1949, unpublished), the sera of 10 (17 per cent) of 60 duck handlers yielded complement-fixation reactions indicative of possible infection.
The indirect evidence that handling of ducks infected with an ornithosis virus may lead to occupational disease or to latent infection is quite convincing. However, until the virus is isolated from the sputum or blood of a patient, the proof for the existence of the duck-to-man infection chain is incomplete.

The presence of the duck-adapted strain in hatcheries is suggested by isolation of such a strain at the Hooper Foundation from young ducks obtained from a hatchery in Petaluma, California. Three of 11 apparently healthy ducks harbored the virus in the spleen, liver and kidney.

Finally, there is evidence that this latent virus infection in ducklings may seriously interfere with studies on the immunology of malaria. With specimens supplied by Mr. JAMES BURNS and Dr. H. R. JACOBS, Northwestern University, examinations were carried out at the Hooper Foundation. Not only did the tissues from ducklings originally secured from a hatchery in Michigan have gross lesions (exudate on the air saes and pericardium with microscopic findings of typical cytoparasites), but a virus weakly pathogenic for mice was isolated from them. The investigators suspected that an intercurrent disease was inducing resistance to duck malaria. Apparently the large amounts of parasitized blood subinoculated carried simultaneously the ornithosis virus. The "spontaneous" resistance of ducklings to Plasmodium lophurae is apparently induced by the latent or active virus infection. These agents have assumed the same interfering position in experiments as many other agents that give rise to intercurrent infections of other laboratory animals. Noteworthy is the fact that despite intimate and not too careful handling of these infected ducklings, the personnel of the building have not had clinical infections.

Two new bird hosts for an ornithosis virus are of interest:

(a) In 1951 the Bureau of Animal Industry, United States Department of Agriculture, received from Hongkong a shipment of bleeding-heart doves (Galli columbia luzonica). Several of these birds died while under quarantine. At autopsy the extensive plastic purulent exudates on the air saes and pericardium associated with splenic and hepatic enlargement suggested psittacosis. The diagnosis was confirmed when enormous numbers of the cytoparasites were seen in smears prepared from the exudate and by isolation of the virus through mouse passage. The entire shipment was then submitted for detailed examination. Of 38 doves, 30 had lesions of acute ornithosis and smears were positive. The sera of 20 gave positive complement-fixation reactions in dilutions up to 1 : 256 in the presence of the psittacosis antigen. In 7 sera there was a prozone
negative reaction in dilutions up to 1:16. A psittacosis-type virus highly pathogenic for mice on intraperitoneal inoculation of a pool of liver, spleen and kidneys was isolated from all the 38 doves. In a 20 per cent emulsion of tissues, intraperitoneally injected viruses killed mice in from 3 to 19 days. Ever since TREMAIN (1938) isolated a psittacosis virus from a Bengalese finch (a hybrid between Aidemosyne malabarica and Uroloncha [Munia] striata) found dead among finches sent from China on the steamship Nankin, the ports of this Asiatic country have been suspected as disseminators of infected birds. As early as 1930, imported parakeets were incriminated, but conclusive proof was not obtainable because the same infection prevailed in locally bred and raised birds.

(b) Through the efforts of DR. MORRIS SCHAEFFER and R. E. KISSLING, Communicable Disease Center, Virus and Rickettsial Section, Montgomery, Alabama, attention has been called to a virus of the psittacosis group isolated by them from the blood of nestling snowy egrets (Egretta candissima candissima [Ginelin]) bled in June 1950 on a small uninhabited island in Barataria Bay, Louisiana. This strain, highly pathogenic for mice and guinea pigs by intraperitoneal route, on cursory study seems to be closely related to the well-known Louisiana strain (Borg) involved in the pneumonitis outbreak in the bayou country (LARSON and OLSON, 1946). The bayou episode is notorious for its unusually high mortality rate: of 17 known infections, all stemming from the primary fatal case, 7 (41 per cent) were fatal. The high pathogenicity for mammals, shared by the egret strain, paralleled the high human fatality rate. It seems quite plausible that these pathogenically similar viruses had a common source. It is interesting that on purely circumstantial grounds and in retrospect, a second epidemic was uncovered, also in the bayou country. Doctors who attended patients in both epidemics were convinced that the cases in the earlier outbreaks in 1936 and those in 1942 and 1943 were clinically the same. The infective agent in the first was even more lethal—in the total of 8 cases of "atypical pneumonia", 6 of the patients died.

All of these isolated observations amply attest to the possibility and probability that reservoirs of large viruses of the psittacosis group characterized by variable infectiousness and virulence are far more widely distributed than is generally appreciated.

On numerous occasions, ornithosis viruses have been isolated from finches, sparrows and canary species. Detailed studies have been made with a few strains. As a rule, they prove indistinguishable morphologically or biologically from parakeet or parrot strains. These findings are not surprising since these infections are as a rule contracted by exposure in aviaries or bird stores. The
pathogenicity of these strains for mice by every route of inoculation is high. Guinea pigs and pigeons are rarely susceptible. The strains are readily adapted to propagation in the embryonated egg.

**Viruses of the Psittacosis Group Isolated from Mammals.**

After a lag of several years, following the recognition of elementary bodies by pneumonia-producing viruses in apparently normal mice by GÖNNERT (1942), NIGG (1942) and NIGG and EATON (1944), four new mammalian reservoirs for these agents have been discovered: cats, calves, ewes and opossums. Several mouse pneumonitis virus strains have been carefully studied and, since these apparently do not cause disease in man, they serve as models for morphologic and antigenic studies.

The two viruses found in the family of Ruminants have entirely new tissue tropism. One has been carefully studied, but the other, although morphologically indistinguishable from the prototypes of the group, was placed in the group only by serologic tests a few months ago.

The isolations reported by GÖNNERT (1942), NIGG (1942), KARR (1943), MOOSER (1943), HILLEMAN and GORDON (1944) and ANDREWES and GLOVER (1945) have dealt with mouse pneumonitis strains which readily attain a high virulence. When inoculated intranasally they kill mice within a few days, but they are not lethal when inoculated intracerebrally or intraperitoneally. The lungs of the intranasally infected mice are consolidated. Elementary bodies are found in lung smears, chiefly in mononuclear cells; in sections they are found in the alveolar endothelium and occasionally the epithelia of the bronchi (HORNUS, 1940; WEISS, 1949). These strains are nonpathogenic for birds. These strains appear to be specific antigenically and are related with the psittacosis agent only in the complement-fixation test. However, EDDIE and FRANCIS (1942) and DE BURGH et al. (1945) discovered, in the respiratory tract of mice, strains highly virulent for mice by every route of inoculation. They are not pathogenic for pigeons and are not susceptible to sulfonamides. In the toxin-neutralization test, they fall into the large group of viruses isolated from a variety of sources. Thus it must be recognized that the respiratory tract of mice may possibly harbor a variety of elementary-body pneumonitis viruses—all members of the psittacosis group. A similar state of affairs may exist for the pneumonitis viruses isolated from hamsters (NIGG and EATON, 1944; ST. JOHN and GORDON, 1947).

It is rather surprising that feline pneumonitis has not received
very much attention. All the studies on which far-reaching conclusions relative to the morphology and biology of the parasite are based were made with one of five strains originally isolated by Baker from cats in New Jersey and New York. This strain adapted to the embryonated egg more or less served as the prototype in the studies of Hamer and Rake (1947) and of Weiss (1949, 1950). Recently Dr. D. G. McKercher, University of California School of Veterinary Medicine, isolated several strains from sick cats in California by intranasal mouse passage of ocular and nasal washings. This strain produced moderately extensive pneumonia in the initial mouse-lung passages; on subsequent serial passage the lesions markedly diminished in size. Growth established in the yolk sac from the mouse lung fatally infected the embryo. On repeated passage, the pathogenicity for the embryo decreased. Nevertheless, complete lung consolidation developed in the mice inoculated. This strain is now fully stabilized and will be used for more elaborate studies. Doctor McKercher made another significant observation: the sera of 3 cats manifesting respiratory symptoms fixed complement in the presence of the feline pneumonitis virus of Baker and of his own strain. In this connection it is recalled that Thomas and Kolb (1943) obtained cross reactions in complement-fixation tests with mouse pneumonitis and psittacosis antisera in the presence of the feline pneumonitis antigen, thus demonstrating its relationship to the psittacosis group of viral agents.

The strains of the calf agent placed by York and Baker (1951) into the psittacosis-lymphogranuloma venereum group of viruses produce a febrile nonfatal fibrous peritonitis with focal necrosis in the liver on the 3rd or 4th day after intraperitoneal inoculation of guinea pigs. It is not transmissible by cage contact exposure and is not readily established in mice by intranasal instillation. After 20 successive passages, however, it did induce fatal complete consolidation of the lung. This adapted strain remains nonpathogenic for mice by intracerebral or intraperitoneal inoculation. The agent temporarily infects cats. On intracardiac injection it produces fever and anorexia and localizes in the spleen and liver for 1, but not for 2 or 4 weeks. The sera of 4 of 5 cats so infected yielded complement-fixing antibodies in dilutions of 1:4 to 1:16. The agent does not infect rabbits, swine or dogs. Possessing a specific tropism for the intestinal tract, in particular the cecum of calves, the mammalian microparasite exhibits several distinctive features which separate it from the other members of the group.

For comparison, the Columbian opossum virus isolated from the blood of marsupials suffering from a paralytic-convulsive disease is highly pathogenic for mice by intracerebral and intranasal, but not
by intraperitoneal inoculation (Roca-Garcia, 1949). The opossum virus is pantropic—virus inoculated intraperitoneally may be found in the central nervous system and intracerebral inoculation leads to latent localization of the agent in the liver and spleen. This viscerotropism associated with neurotropism separates the agent from the rodent pneumonia viruses. Nonpathogenicity for guinea pigs separates it from the calf virus. Significant are the observations that the meningopneumonitis virus of Francis and Magill protected mice against the opossum virus on intracerebral inoculation, while mice injected intraperitoneally with opossum agent acquired no immunity against the meningopneumonitis virus. These tests further establish the well-known fact that the psittacosis agent, probably of avian (pigeon) origin, possesses a strong immunogenic and a broad antigenic pattern. It protects well against all representatives of the group except certain strains of psittacine and rodent pneumonia origin.

Finally, it must be emphasized that none of the recently isolated mammalian psittacosis viruses are associated with pneumonia in the spontaneous disease. Particularly intriguing is the placentotropism of the agent responsible for necrosis of the fetal membrane of sheep which leads to abortion or premature lambing (Stamp et al., 1950; McEwen et al., 1951). This characteristic is retained and placental infections have been produced in guinea pigs and in a cow. The egg-adapted agent causes fatal pneumonia in mice on intranasal inoculation; the intracerebral and intraperitoneal administration does not cause fatal infection. The relationship of these agents to the members of the group is as yet unknown except for the fact that sera of infected sheep fix complement in the presence of heated psittacosis and homologous virus. One naturally awaits with interest detailed comparative studies of the sheep virus.

One obviously wonders how many of these viruses are pathogenic for man. Agents having characteristics in common with psittacosis cytoparasites have been isolated from the lung tissues or sputum in cases of human pneumonia. They all stain by the method of Castaneda with formol methylene blue and hence are known as “Castaneda positive”. They form basophilic cytoplasmic colonies unlike typical virus inclusions which have an affinity for the acid dyes—eosin, phyloxins, acid fuchsins and light green. They are all virulent for parakeets and are readily adapted to the yolk sac of embryonated eggs. Antigens prepared from these yolk-sac cultures give specific complement-fixation reactions with sera derived from infected psittacine birds or pigeons. Many of these human pneumonia viruses are highly contagious and have been responsible for human-to-human infections (Meiklejohn et al.,

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1945: Olson and Larson, 1945). Fortunately these agents are susceptible to some of the antibiotics (penicillin, chloramphenicol, aureomycin and terramycin) and thus differ from all the other agents causing atypical pneumonia. Attempts have been made to classify many of the human pneumonitis strains by research techniques such as the pathogenicity tests, sensitivity to sulfonamide drugs, cross-immunity tests, toxin-antitoxin neutralization tests and neutralization of viral infectivity-protection tests (Meyer and Eddie, 1951). Thus far these comparative studies have merely proven that these strains have a great deal in common with the psittacosis virus. They do not differ strikingly from this classical infective agent. No convincing evidence has, however, accrued to prove conclusively that the mammalian strains in the large family of psittacosis viruses have caused human infections. As interest in this group becomes more universal and the range of reservoir hosts is defined, widening of the infective chain may reasonably be anticipated.

### Table 1.

Classes and Orders of Birds and Mammals in which Viruses of the Psittacosis Group Have Been Found.

#### Class: Aves.

**Orders:**

- **Procellariiformes:** (1) Fulmar (L)
- **Ciconiiformes:** (1) Egret (Egretta candissima candissima [Gmelin])
- **Anseriformes:** (1) Domestic mallard (L)
- **Galliformes:** (2) Common fowl, turkey (Meleagris gallopavo)
- **Charadriiformes:** (2) Willet and American herring gull
- **Columbiformes:** (3) Tame pigeon, ring turtle dove and bleeding-heart dove (Gallus columba cruenta [Gmelin])
- **Psittaciformes:** (31) Parrots, parakeets and parrotlets (See Meyer, 1948)
- **Passeriformes:** (14) Finches and sparrows
- **Fringillidae:** (7) Finches, canary and titmouse

#### Class: Mammalia.

**Orders:**

- **Primates:** Man
- **Ungulatae:** Ruminants (2) Calf and sheep
- **Carnivores:** (1) Domestic cat
- **Rodents:** Muridae (2) Laboratory mouse, hamster
- **Marsupiaux:** (2) Opossum (Didelphis paraguayensis and Caluromys laniger)

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1 Figures in parentheses indicate the number of species thus far found infected.
TABLE 2.

<table>
<thead>
<tr>
<th>Date</th>
<th>Bird</th>
<th>Number tested</th>
<th>Virus isolated Number</th>
<th>Per cent</th>
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</thead>
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<tr>
<td>7/20/45</td>
<td>Pekin ducks</td>
<td>3</td>
<td>2</td>
<td>66.6</td>
</tr>
<tr>
<td></td>
<td>Sea gulls</td>
<td>2</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>9/14/45</td>
<td>Pekin ducks</td>
<td>15</td>
<td>8</td>
<td>53.3</td>
</tr>
<tr>
<td>10/31/45</td>
<td>Pekin ducks</td>
<td>97</td>
<td>31</td>
<td>31.95</td>
</tr>
<tr>
<td></td>
<td>Sea gulls</td>
<td>5</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Crow (black)</td>
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<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

References.


Es wurde festgestellt, daß bei Wirbeltieren die Infektionen, die durch intrazellulär sich entwickelnde und dem Psittacosisvirus morphologisch und in der antigenen Wirkung ähnliche Krankheitserreger verursacht werden, immer häufiger vorkommen. Abgesehen von der echten Psittacosisinfektion bei Vögeln, wurde das Geflügelvirus bei Hühnern, Tauben und Enten gefunden, und es besteht die Vermutung, daß dieser Krankheitserreger auch beim Truthahn vorkommt. Besonders ausführliche Untersuchungen wurden in Enten-Farmen durchgeführt. Es wird darauf aufmerksam gemacht, daß die Infektion meistens unauffällig verläuft. Man konnte indirekt feststellen, daß die Vögel die Quelle von Berufsinfektionen beim Personal derjenigen Anstalten darstellen, welches sich mit dem Töten und Untersuchen dieser Vögel befaßt. Latente Geflügel-

Zusammenfassung.


Résumé.

On a constaté un accroissement d’infections de vertébrés par des agents pathogènes intracellulaires qui sont, par leur morphologie et par leur action antigénique, voisins du virus de la psittacose. À part l’infection de psittacose vraie chez les oiseaux, le virus aviaire a été trouvé chez des poules, des pigeons et des canards, et il y a lieu de supposer qu’il se trouve également chez les din- dons. Des détails sont donnés sur des observations faites dans des élevages de canards. L’attention est attirée sur le fait que l’infection est le plus souvent in-apparente. On pouvait constater indirectement que dans des stations d’essais où l’on s’occupe à tuer et à analyser ces oiseaux, le personnel s’était infecté de cette source. Des infections aviaires latentes peuvent sérieusement entraver l’étude expérimentale de *Plasmodium lophurae*. On a isolé dernièrement chez des colombes poignardées et des aigrettes un virus qui ne se distingue en rien de celui des perruches et des pigeons. Ce virus est très toxique et virulent pour le cobaye après injection intrapéritonéale. Du point de vue immunologique il semble avoir une certaine ressemblance avec la souche Borg, cause de l’épidémie en Louisiane bayou en 1943. Très significatifs et importants sont aussi les isolements de l’agent pathogène intracellulaire à partir d’organes de mammifères et qui se sont avérés être voisins de la souche aviaire. Jusqu’à ce jour ces agents ont été trouvés chez la souris, le chat, l’opossum de Colombie, le veau et le mouton. Tous les agents de la psittacose originaires de mammifères sont non pathogènes pour les perruches et les oiseaux oryzivores. La relation entre ces agents et la pneumonie humaine n’est pas claire. Il est cependant connu que ces virus sont susceptibles vis-à-vis de certains antibiotiques (pénicilline, chloromycétine, auréomycine, terramycine). Ils stimulent tous la production d’anticorps qu’on peut démontrer par la réaction de fixation du complément, en utilisant le virus de la psittacose ou de la lymphogranulomatose. Il est possible qu’ils provoquent une infection latente chez l’homme.